



# Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk

## Workshop Summary Report

November 2013

### Prepared for

U.S. Environmental Protection Agency  
Office of Research and Development  
National Center for Environmental Assessment  
109 T.W. Alexander Drive  
Durham, NC 27711

### Prepared by

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713

### **Disclaimer**

Although the workshop described in this document was supported by the U.S. Environmental Protection Agency through EPA Contract No. EP-C-09-009, Work Assignment 2-62, 3-62, and 4-62 to ICF International, the views expressed here do not necessarily reflect the views of the Agency and no official endorsement should be inferred.

### **Acknowledgement**

This report was written by Geniece Lehmann of EPA's National Center for Environmental Assessment, and Bryan Luukinen and Cara Henning of ICF International.

This document was reviewed by internal EPA reviewers chosen for their expertise in relevant subject areas (e.g., toxicology, risk assessment, epidemiology, physiologically-based pharmacokinetic (PBPK) modeling, and public health): Lyle Burgoon, Erin Hines, Geniece Lehmann, Eva McLanahan, Connie Meacham, Anu Mudipalli, Linda Phillips, Christina Powers and John Vandenberg of EPA's National Center for Environmental Assessment; Satori Marchitti of EPA's National Exposure Research Laboratory; and Sheppard Martin of EPA's National Health and Environmental Effects Research Laboratory.

Appreciation is expressed to Audrey Turley, Penny Kellar, Whitney Kihlstrom, and Kristen Fedak of ICF International for research, technical writing and editing, and workshop planning, facilitation and support.



# Contents

<b>Figures.....</b>	<b>vi</b>
<b>Tables.....</b>	<b>vi</b>
<b>WORKSHOP PARTICIPANTS .....</b>	<b>xi</b>
<b>ABSTRACT .....</b>	<b>xii</b>
<b>1. BACKGROUND AND PURPOSE.....</b>	<b>1</b>
1.1. Overview Of Human Health Risk Assessment.....	1
1.2. Human Health Risk Assessment of Persistent, Bioaccumulative and Toxic Chemicals .....	4
<b>2. WORKSHOP PROCESS .....</b>	<b>7</b>
<b>3. CHEMICAL BREAKOUT GROUPS – Annotated HCB Briefing Packet .....</b>	<b>10</b>
3.1. Introduction .....	11
3.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	11
3.2.1. Mothers’ Exposure to HCB .....	12
3.2.2. Is HCB Sequestered in Breast Milk or Other Human Tissues? .....	14
3.2.3. How Much HCB Passes from Mother to Infant via Breast Milk? .....	15
3.3. Maternal Dose vs. Nursing Offspring Dose in Animals .....	16
3.3.1. Maternal Exposure and Elimination in Milk .....	16
3.4. Developmental Effects of HCB .....	19
3.4.1. Human Studies .....	19
3.4.2. Animal Studies.....	20
3.4.3. Exposure-Response Arrays for Developmental Effects .....	35
3.5. Human Equivalent Dose (HED) Estimation .....	37
3.5.1. Chemical-Specific Properties Affecting HED Estimation.....	39
3.5.2. Available Pharmacokinetic Data for HCB.....	40
3.5.3. Appropriate Dose Metric for Selected Point of Departure (POD) .....	45
3.5.4. Available Models for Estimating HCB Internal Dose.....	46
3.5.5. First-Order Single-Compartment Model (U.S. EPA, 1998).....	52
3.5.6. Biotransfer Method .....	65
3.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	69
3.7. Attachment A: Concentrations of HCB in Human Milk .....	73
<b>4. GLOBAL ISSUES, DATA GAPS AND UNCERTAINTIES.....</b>	<b>79</b>
4.1. Risk Communication .....	79
4.2. Critical Windows of Exposure .....	80
4.2.1. Toxicological Consequences of Prenatal and Postnatal PBT Chemical Exposures .....	80
4.2.2. Identifying Critical Windows of Exposure.....	84
4.2.3. Relative Magnitude of Prenatal and Postnatal PBT Chemical Exposures.....	86
4.3. Variability and Uncertainty in Modeling Data and Parameters .....	88
4.3.1. Variability in Species-Specific Parameters.....	88
4.3.2. Methods to Address Variability.....	89
4.3.3. Uncertainty in Species- and Chemical-Specific Parameters .....	90
4.3.4. Methods to Address Uncertainty .....	93
4.4. Metabolism.....	93
4.5. Mixtures.....	95
<b>5. STUDY DESIGN CONSIDERATIONS .....</b>	<b>98</b>
5.1. Human Studies.....	99
5.2. Animal Studies .....	100

<b>6. AVAILABLE APPROACHES.....</b>	<b>104</b>
6.1. PBPK Model Approach .....	107
6.2. Toxicokinetic Modeling Approaches.....	112
6.3. Combustor Emissions Model .....	116
6.4. Simple No-Elimination Method .....	118
6.5. Biotransfer Method .....	120
6.6. Monte Carlo Methods to Account for Variability .....	120
6.7. Selecting an Approach .....	122
<b>7. CONCLUSIONS AND FUTURE DIRECTIONS .....</b>	<b>125</b>
<b>8. REFERENCES .....</b>	<b>129</b>

## Appendices Contents

<b>Appendix A. Charge Questions for Workshop Participants .....</b>	<b>A-1</b>
<b>Appendix B. Original Briefing Packets, as Distributed to Workshop Attendees .....</b>	<b>B-1</b>
B.1. Chlordane Briefing Packet .....	B-1
B.2. DDT Briefing Packet .....	B-61
B.3. Hexachlorobenzene Briefing Packet .....	B-140
B.4. Mirex Briefing Packet.....	B-206
<b>Appendix C. Workshop Charge and Instructions for Attendees .....</b>	<b>C-1</b>
C.1. Charge for Chemical Breakout Groups .....	C-1
C.2. Common Issues and Data Gaps: Group Brainstorming and Approaches Breakout Groups .....	C-2

## Figures

Figure 3-1. Exposure-Response Array Showing All Developmental Effects, Sorted by Exposure Route, in Animals after Exposure to HCB .....	35
Figure 3-2. Exposure-Response Array Showing All Developmental Effects, Sorted by Effect Type, in Animals after Exposure to HCB .....	36
Figure 3-3. Process Diagram for Performing Cross-Species Extrapolation .....	38
Figure 3-4. Maternal and Infant POP Model (Verner et al., 2009) .....	50
Figure 3-5. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day HCB.....	60
Figure 3-6. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Mendoza et al., 1977) and an Administered Dose of 6.7 mg/kg-day HCB (POD).....	64
Figure 6-1. Decision Tree for Applying PBPK Approaches .....	109
Figure 6-2. Results from Exploratory Monte Carlo Analysis for HCB.....	122
Figure 6-3. Comparison of Time-Varying Body Burden Model and Simple Equation for HCB.....	124
Figure B-1. Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to Chlordane .....	B-24
Figure B-2. Exposure-Response Array Showing Developmental Effects in Animals after Gestational or Gestational and Lactational Exposure to Chlordane.....	B-25
Figure B-3. Process diagram for performing cross-species extrapolation .....	B-27
Figure B-4. Maternal and Infant POP Model (Verner et al., 2009) .....	B-35
Figure B-5. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day .....	B-41
Figure B-6. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Cassidy et al., 1994) and an Administered Dose of 0.1 mg/kg-day (POD) .....	B-44
Figure B-7. Total human milk levels of DDT (left) and p,p'-DDE (right), as µg/g milk fat, from 144 studies. ....	B-70
Figure B-8. Dietary Intake versus DDT Level in Blood.....	B-79
Figure B-9. Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to DDT Compounds .....	B-103
Figure B-10. Exposure-Response Array Showing All Developmental Effects in Male Pups after Gestational or Gestational and Lactational Exposure to DDT Compounds .....	B-104
Figure B-11. Process diagram for performing cross-species extrapolation .....	B-106
Figure B-12. Maternal and Infant POP Model (Verner et al., 2009) .....	B-114
Figure B-13. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day.....	B-122
Figure B-14. Mouse Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Palanza et al., 1999) and an Administered Dose of 0.02 mg/kg-day (POD) .....	B-126
Figure B-15. Exposure-Response Array Showing All Developmental Effects, Sorted by Exposure Route, in Animals after Exposure to HCB .....	B-166
Figure B-16. Exposure-Response Array Showing All Developmental Effects, Sorted by Effect Type, in Animals after Exposure to HCB .....	B-167
Figure B-17. Process Diagram for Performing Cross-Species Extrapolation .....	B-169
Figure B-18. Maternal and Infant POP Model (Verner et al., 2009) .....	B-177

Figure B-19. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day.....	B-184
Figure B-20. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Mendoza et al., 1977) and an Administered Dose of 6.7 mg/kg-day (POD) .....	B-187
Figure B-21. Compiled Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to Mirex .....	B-234
Figure B-22. Exposure-Response Array Showing Developmental Effects in Animals after Gestational Exposure to Mirex .....	B-235
Figure B-23. Exposure-Response Array Showing Developmental Effects in Animals after Combined Gestational and Lactational Exposure to Mirex .....	B-236
Figure B-24. Exposure-Response Array Showing Developmental Effects in Animals after Lactational Exposure to Mirex .....	B-237
Figure B-25. Process Diagram for Performing Cross-Species Extrapolation .....	B-239
Figure B-26. Maternal and Infant POP Model (Verner et al., 2009) .....	B-246
Figure B-27. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day.....	B-255
Figure B-28. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Gaines and Kimbrough, 1970) and an Administered Dose of 0.4 mg/kg-day (POD) .....	B-258

## Tables

Table 3-1. Intake of HCB as Estimated by FDA Total Diet Study, Select Years, 1982-1993 .....	13
Table 3-2. Concentration of HCB in Blood and Fat of Exposed Dams and Their Pups.....	18
Table 3-3. Mean HCB Concentrations (µg/g wet weight) in Serum and Milk of Dam and Infant Rhesus Monkeys, Dams Dosed with 60 mg/kg-day while Nursing.....	19
Table 3-4. Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene .....	23
Table 3-5. Available Pharmacokinetic Parameters for HCB in Humans and Rats.....	42
Table 3-6. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model (ICF, 2013a) .....	58
Table 3-7. Estimated Ratio of Infant Lactational Dose (ng/kg-day) to Average Daily Maternal Dose (ng/kg-day) for HCB Based on First-Order Model (ICF, 2013a).....	61
Table 3-8. Model Parameters and Their Values Used in First-Order Single-Compartment Model in Rat (ICF, 2013b) .....	63
Table 3-9. Estimated Ratio of Offspring Lactational Dose (mg/kg-day) to Average Daily Maternal Dose (mg/kg-day) for HCB in Rats Based on First-Order Model (ICF, 2013b) .....	65
Table 3-10. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	67
Table 3-11. Estimated Ratio of Infant Lactational Dose (ng/kg-day) to Average Daily Maternal Dose (ng/kg-day) for HCB Based on Biotransfer Method .....	67
Table 3-12. Potential Dose Metrics and Methods for HED Estimation.....	70
Table 3-13: Mean Levels of HCB in Breast Milk as Reported in IPCS (1997).....	73
Table 3-14. Mean Levels of HCB in Breast Milk as Reported by ATSDR (2002b) .....	77
Table 6-1. Model parameters used in Verner et al. (2013; 2009) simulations for HCB.....	111

Table 6-2. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB Using the PBPK Model Published by Verner et al. (2009) .....	112
Table 6-3. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB Using the Two-Compartment Model Published by Verner et al. (2013) .....	112
Table 6-4. Model Parameters and Their Values Used in the Human Time-Varying Body Burden Model (ICF, 2013a) after Workshop Participant Review .....	114
Table 6-5. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB Using the Time-Varying Body Burden Model (ICF, 2013a) .....	115
Table 6-6. Estimates of Human Equivalent Dose Using the Time-Varying Body Burden Model (ICF, 2013a, b) .....	115
Table 6-7. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB using the Combustor Emissions Model .....	117
Table 6-8. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB using the No-Elimination Method .....	120
Table 6-9. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB using the Biotransfer Method.....	120
Table B-1. Summary of Breast Milk Concentration Studies.....	B-7
Table B-2. Concentration of Oxychlordane in Breast Milk and Maternal Blood (Mes et al., 1984) .....	B-8
Table B-3. Concentrations of Chlordane in Human Tissues Other than Breast Milk.....	B-9
Table B-4. Developmental Effects from Gestational and/or Lactational Exposure to Chlordane .....	B-14
Table B-5. Available Pharmacokinetic Parameters for Chlordane in Humans and Rats .....	B-30
Table B-6. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model .....	B-40
Table B-7. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model.....	B-41
Table B-8. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat .....	B-43
Table B-9. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model.....	B-44
Table B-10. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-47
Table B-11. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method .....	B-47
Table B-12. Potential Dose Metrics and Methods for HED Estimation .....	B-48
Table Att. B.1-1. Concentrations of Chlordane in Human Milk (whole milk, ppb) .....	B-51
Table Att. B.1-2. Concentrations of Chlordane in Human Milk (milk fat, ppb).....	B-53
Table B-13. DDT in Breast Milk in Japan .....	B-71
Table B-14. DDT in Breast Milk in Canada .....	B-72
Table B-15. DDT in Breast Milk in Eastern Europe.....	B-73
Table B-16. DDT in Breast Milk in India .....	B-74
Table B-17. DDT in Breast Milk in China .....	B-76
Table B-18. Concentration of ΣDDT in Children’s Adipose Tissue Related to Breast Milk Consumption Patterns.....	B-77
Table B-19. Concentration of DDE in Various Human Tissues.....	B-78
Table B-20. Overview of Developmental Effects of DDT and/or DDE in Humans Reported in Epidemiological Literature .....	B-85
Table B-21. Developmental Effects from Gestational and/or Lactational Exposure to DDT Compounds <sup>a</sup> .....	B-94



Table B-22. Available Pharmacokinetic Parameters for DDT in Humans and Mice.....	B-111
Table B-23. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model .....	B-121
Table B-24. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-122
Table B-25. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Mouse .....	B-125
Table B-26. Estimated Mouse Pup-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-126
Table B-27. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-129
Table B-28. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method .....	B-130
Table B-29. Potential Dose Metrics and Methods for HED Estimation .....	B-131
Table B-30. Intake of HCB as Estimated by FDA Total Diet Study, Select Years, 1982-1994 .....	B-146
Table B-31. HCB Residues in Breast Milk and Blood During Lactation .....	B-148
Table B-32. HCB Concentrations in Mother's Milk and Fecal Excretion of HCB in the Breastfed Infant at 1 and 5 Months of Age .....	B-149
Table B-33. Concentration of HCB in Blood and Fat of Exposed Dams and Their Pups.....	B-151
Table B-34. Mean HCB Concentrations (µg/g wet weight) in Serum and Milk of Dam and Infant Rhesus Monkeys, Dams Dosed with 60 mg/kg-day while Nursing.....	B-152
Table B-35. Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene .....	B-155
Table B-36. Available Pharmacokinetic Parameters for HCB in Humans and Rats.....	B-172
Table B-37. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model .....	B-183
Table B-38. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-184
Table B-39. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat .....	B-186
Table B-40. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-187
Table B-41. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-190
Table B-42. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method .....	B-190
Table B-43. Potential Dose Metrics and Methods for HED Estimation .....	B-191
Table Att. B.3-1. Mean Levels of HCB in Breast Milk as Reported in IPCS (1997).....	B-194
Table Att. B.3-2. Mean Levels of HCB in Breast Milk as Reported by ATSDR (2002b) .....	B-197
Table B-44. Summary of Breast Milk Concentration Studies.....	B-214
Table B-45. Mirex in Maternal Blood in the Circumpolar North (µg/kg lipid) .....	B-215
Table B-46. Daily Intake of n-3 and n-6 Fatty Acids (FAs) and Mirex Compared by Two-tailed, Independent Samples T-test.....	B-216
Table B-47. Plasma n-3 Fatty Acids (% of total lipids) and Plasma Levels of Mirex (µg/kg plasma lipid) in Women from Greenland <sup>a</sup> .....	B-217
Table B-48. Mirex in Blood Plasma of Female Fish Eaters and Non-Fish Eaters <sup>a</sup> .....	B-218
Table B-49. Concentration of Mirex in Serum and Milk (1991-1993), from Greizerstein et. al. (1999) .....	B-219
Table B-50. Concentration of Mirex in Adipose Tissue and Blood Serum <sup>a</sup> .....	B-220
Table B-51. Developmental Effects from Gestational and/or Lactational Exposure to Mirex <sup>a</sup> .....	B-224
Table B-52. Available Pharmacokinetic Parameters for Mirex in Humans and Rats .....	B-241

Table B-53. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat and Monkey .....	B-248
Table B-54. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model .....	B-254
Table B-55. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model.....	B-255
Table B-56. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat .....	B-257
Table B-57. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model.....	B-258
Table B-58. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-261
Table B-59. Estimated Infant-to-maternal Dose Ratio Based on Biotransfer Method .....	B-261
Table B-60. Potential Dose Metrics and Methods for HED Estimation .....	B-262
Table Att. B.4-1. Concentration of Mirex in Human Milk (whole milk) .....	B-265
Table Att. B.4-2. Concentration of Mirex in Human Milk (milk fat).....	B-269

## WORKSHOP PARTICIPANTS

Sue Anne Assimon	U.S. Food and Drug Administration
John Barnett	West Virginia University School of Medicine
Jerry Campbell	The Hamner Institutes for Health Sciences
Matthew Davis	U.S. EPA
Hisham El-Masri	U.S. EPA
David Farrer	Oregon Health Authority
Suzanne Fenton	NIEHS
Paul Foster	NIEHS
Warren Foster	McMaster University
Bettina Francis	University of Illinois at Urbana-Champaign
Sami Haddad	University of Montreal
Wilfried Karmaus	University of South Carolina
Susan Knadle	California Environmental Protection Agency, OEHHA
Judy LaKind	LaKind Associates, LLC; University of Maryland School of Medicine
Matthew Longnecker	NIEHS
Mike Poulsen	Oregon Department of Environmental Quality
Walter Rogan	NIEHS
Sharon Sagiv	Boston University School of Public Health
Jane Ellen Simmons	U.S. EPA
Jeff Swartout	U.S. EPA
Cecilia Tan	U.S. EPA
Rogelio Tornero-Velez	U.S. EPA
Marc-Andre Verner	Harvard Medical School
Clement Welsh	ATSDR
Raymond Yang	Colorado State University

## **ABSTRACT**

Persistent, bioaccumulative and toxic (PBT) chemicals have the potential to accumulate within a woman's body lipids over the course of many years prior to pregnancy and subsequently to partition into human milk upon breastfeeding. As a result of this accumulation and partitioning, infant intake of PBT chemicals from breastfeeding may be much greater than average daily PBT chemical doses encountered by mothers. The developmental period is critical because it sets the stage for lifelong health. Because humans continue to develop postpartum, PBT chemical exposures during this period have the potential to reprogram the developmental trajectory. Thus, it is important to be able to accurately assess early life exposures to these chemicals. In many cases, current environmental risk assessment methods do not account for differences between maternal and infant PBT chemical exposures. In order to fully consider the breastfeeding pathway when assessing lifetime or infant lifestage risk from exposures to PBT chemicals, it is desirable to have methods to estimate a breastfed infant dose based on an average daily maternal dose. The appropriate choice of method may vary based on (1) how much information is known about a given PBT chemical regarding its toxicokinetic profile and its potential effects on developing organisms, and (2) the particular risk management application.

Because it is the policy of the U.S. EPA to consider risks to infants and children consistently and explicitly as a part of risk assessments generated during its decision making processes, including those for regulations, policies and site-specific plans, a workshop was convened to stimulate ideas and provide a forum for discussion regarding innovative approaches to the assessment of early life exposure to and potential health effects from PBT chemicals in breast milk and the challenges involved in developing such approaches. This document provides a summary of the workshop discussions, with emphasis on potential approaches for improving the risk assessment of PBT chemicals in breast milk, the data gaps, uncertainties and other issues identified, along with suggested solutions.

## 1. BACKGROUND AND PURPOSE

### 1.1. Overview Of Human Health Risk Assessment

The purpose of this workshop was to discuss possible methods to improve risk assessment of persistent, bioaccumulative and toxic (PBT) chemicals in breast milk. To form a solid foundation for these discussions, some basic principles of risk assessment were reviewed at the workshop and are summarized here.

U.S. EPA human health risk assessments generally follow the paradigm established by the National Academy of Sciences ([NRC, 1983](#)). This paradigm describes a group of interconnected processes for performing a risk assessment that include hazard identification, dose-response assessment, exposure assessment, and risk characterization. Information for hazard identification and dose-response assessment may come from studies of humans or of laboratory animals. Information from human studies is preferred for use in risk assessment. However, adequate human data are often unavailable, particularly for infants and children, and in the absence of adequate human data, laboratory animal data are used for hazard identification and dose-response assessment. EPA is also in the midst of reviewing its approaches to risk assessment in response to a more recent report and recommendations from the National Academy of Sciences ([NRC, 2009](#)).

In hazard identification, available data on biological endpoints are used to determine if a material is likely to pose a hazard to human health. These data are also used to define the type of potential hazard (e.g., does the material act as a developmental toxicant?) ([NRC, 1983](#)). Comprehensive hazard identification requires the investigation of a wide variety of potential health effects that might occur as a result of chemical exposure. Toxicological studies commonly investigate effects on endpoints such as survival, body weight, organ weights, organ and tissue histopathology, serum biochemistry, hematology, endocrine disruption, hepatic enzyme induction, and tumor formation. Until more recently, standard toxicological studies rarely covered all developmental endpoints of interest or multiple generation studies, which are relevant for examining PBT chemical exposure to infants via lactation.

In dose-response assessment, data from studies demonstrating a hazard from chemical exposure are used to estimate the amount of exposure that may produce that effect in humans. When relevant data are available, risk assessors calculate a quantitative dose-response relationship that allows for estimates of effects at low-dose exposures. Such calculations are often carried out by applying mathematical models to the data ([NRC, 1983](#)). Currently, methods for dose-response assessment differ for cancer and non-cancer effects. The methodological difference is based on differences in the dose-response relationships assumed for these effects at low doses. Unless there is sufficient evidence to the contrary, the dose response for cancer is assumed to be linear at low doses while the dose response for a non-cancer effect is assumed to include a threshold dose below which there are no significant health effects. For cancer assessment, the assumption of low-dose linearity means that even the lowest level of

exposure to a chemical is expected to pose a small, but finite, probability of generating a response, and that the probability increases with increasing average dose over a lifetime. The products of a cancer dose-response assessment are quantitative toxicity estimates or cancer potency values. In risk characterization, these can be combined with lifetime average daily dose estimates to quantify the probability of developing cancer as a result of exposure. Cancer risks less than 1 in 1,000,000 are generally considered negligible.

For non-cancer assessment, where the existence of a threshold dose is assumed, dose-response data are used to estimate a level of exposure that is not expected to cause adverse health effects. The dose-response point from which this safe level of exposure is derived is known as the point of departure (POD). Examples of values commonly selected for the POD include the no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) from the experimental data, and the benchmark dose lower confidence limit (BMDL). A benchmark dose (BMD) is a dose that, based on a dose-response curve modeled from the experimental data, produces a predetermined change in response rate of an adverse effect compared to background. The predetermined change in response rate used to determine the BMD is called the benchmark response (BMR). A BMDL is the statistical lower confidence limit (usually 95%) on the BMD. The BMD approach to POD derivation is preferred over the NOAEL/LOAEL approach because it allows for the identification of a POD based on a given response rate that is not constrained to the specific doses included in the experimental dataset ([U.S. EPA, 2012a](#)). The product of a non-cancer dose-response assessment is a reference value or an estimate of an exposure to a toxic chemical for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. Reference values typically derived by EPA for a particular toxicant are reference doses (RfDs) for oral exposure and reference concentrations (RfCs) for inhalation exposure.

When using the results of animal studies to estimate a safe level of exposure in humans, it is important to acknowledge that exposure conditions used in an animal study may not faithfully replicate an expected human exposure scenario. Chemical doses administered to animals are often dissimilar to doses that humans may be exposed to in the environment. Also, the timing and routes of exposure may differ. For example, animal studies may utilize gavage administration of a bolus dose once per day while humans would be exposed to small amounts of a chemical throughout the day (e.g., in food or water). These differences are magnified when adding the complexity of reproductive and developmental studies, where factors such as lactation and number of offspring differ between humans and animals. The use of animal data in human health risk assessment may also be impacted by differences in sensitivity to chemical exposure between humans and animals. In other words, the dose of a chemical that causes an effect in a laboratory animal may be different from the dose of that chemical that would cause a similar effect in humans. Differences in sensitivity to chemical exposure may result from species-dependent differences in toxicokinetics (i.e., absorption, distribution, metabolism, and excretion) or toxicodynamics (i.e., toxicological mode of action). In standard risk assessment practice, such differences in sensitivity may be addressed through the application of uncertainty factors. Toxicokinetic differences may also be addressed by calculating a human equivalent dose (HED). A HED is calculated

using toxicokinetic information to extrapolate from a dose administered in an animal study to a dose expected to induce the same magnitude of toxic effect in humans. The application of uncertainty factors is based on established conventions in the field of toxicological risk assessment for addressing uncertainty in a dataset, along with Agency and program-specific guidance developed with consideration of both science and policy issues. Since the appropriate application of these factors will differ across Agencies and programs according to their specific guidance, this component of dose-response assessment is considered to be beyond the scope of this workshop. When workshop participants considered the use of animal data in dose-response assessment, their primary focus was on the development of methods that would allow for the extrapolation of an animal dose to a quantitatively equivalent human dose, or HED.

If available, the use of a physiologically-based pharmacokinetic (PBPK) model is the preferred approach to use for species extrapolation. PBPK modeling uses mathematical equations and models to describe absorption, distribution, metabolism, and excretion of chemicals as they are introduced into the bodies of animals or humans. The models help to define the relationship between external and internal exposures in experimental animals and humans. The models are used to support extrapolation between species, across dose levels, and between exposure routes, and may predict an internal dose that would result from an external exposure. PBPK models are built using data on the physical and chemical properties of hazardous substances, physiological and metabolic parameters, and partition coefficients for various tissue compartments. Existing PBPK models range from simple to complex, depending on the number of compartments and parameters in the model, and may be specifically built for certain species or types of chemicals. The application of PBPK modeling to the dose-response assessment of PBT chemicals in breast milk is discussed in detail in [Section 6](#). In the absence of specific toxicokinetic information, EPA typically uses allometric scaling (body weight to  $\frac{3}{4}$  power) for derivation of HEDs ([U.S. EPA, 2011b](#)). However, allometric scaling may not be appropriate for developmental endpoints, which are a key focus of this workshop, because the empirical allometric scaling factor of 0.75 is based on adult body weights; this relationship does not necessarily hold across the growth curve for each species ([Feldman and McMahon, 1983](#); [Heusner, 1982](#)).

The exposure assessment portion of the risk assessment process seeks to provide an estimate of exposure to a chemical for a specific population within a particular context ([NRC, 1983](#)). Exposure assessment uses chemical concentrations measured or modeled in environmental media with information on human exposure factors (e.g., ingestion or inhalation rates, frequency, and duration of exposure, body weight, etc.). As examples, an exposure assessment might be conducted to determine exposure among adult males in primarily outdoor occupations from inhalation of air pollutants, exposure to a toxicant among subsistence-fishing Native American adults from ingestion of contaminated fish, or exposure among infants to chemicals in human milk. As described in [Section 1.2](#), because of their unique toxicokinetic properties, PBT chemicals bioaccumulate over time within the body lipids of women, subsequently resulting in daily breastfed infant exposures to PBT chemicals that are higher than daily maternal exposures to those chemicals. To date, this phenomenon is not fully addressed in many human health risk assessments for PBT chemicals.

Risk characterization is the integrating step in the risk assessment process that evaluates the assessment of effects of a chemical on human health and assessments of exposure to the chemical from multiple environmental media. It identifies human lifestages and groups at elevated risk, and describes the uncertainty and variability in these characterizations ([NRC, 1983](#)). To characterize the nature of the human risk of exposure to a chemical, the results of a dose-response assessment are integrated with the results of an exposure assessment. Therefore, a high-quality risk characterization depends on a solid foundation provided by hazard identification based on a comprehensive dataset, reliable dose-response data, and accurate exposure assessment.

## **1.2. Human Health Risk Assessment of Persistent, Bioaccumulative and Toxic Chemicals**

PBT chemicals tend to be well-absorbed following ingestion ([McLachlan, 1993](#)). Many are not subsequently metabolized after absorption in humans, but some break down into metabolites. These chemicals are generally highly lipophilic; thus, in humans and other animals, most PBT chemicals are stored in body lipids where they have long elimination half-lives. The excretion of PBT chemicals in urine occurs only to a limited extent ([Yang et al., 1978](#)); rather, the predominant elimination routes for these chemicals are fecal excretion and lactation ([Sonawane, 1995](#); [Yang et al., 1978](#)). The latter is found to be a very efficient elimination route for PBT chemicals because of the high lipid content of human milk.

Due to their slow rate of elimination in the absence of lactation, PBT chemicals have the potential to accumulate within a woman's lipids over the course of many years prior to pregnancy and child birth ([Arcus-Arth et al., 2005](#)). PBT chemicals partition from body lipids into breast milk because of its high lipid content; consequently, breast milk produced by exposed women may have a significant burden of PBT chemicals. Hence, this leads to the possibility that the exposure to PBT chemicals encountered by infants through ingestion of breast milk may be at higher levels over a shorter time period compared to maternal exposure, which occurs over the long-term prior to and during pregnancy and lactation ([Verner et al., 2009](#); [Rogan and Ragan, 1994](#); [Smith, 1987](#)). In addition, because of the relatively small size of a nursing infant, this high exposure may lead to PBT chemical levels in blood and tissues that far exceed those in the mother.

Because human infants continue to develop postpartum in significant ways, it is important to evaluate the potential health impacts of exposures to PBT chemicals at various dose levels during this critical period. As discussed in [Section 1.1](#), the foundational components of a human health risk assessment are hazard identification, dose-response assessment, and exposure assessment. The tendency for PBT chemicals to persist and accumulate in body lipids and to partition into breast milk presents unique challenges for risk assessment. These challenges are briefly outlined below for each component of the risk assessment process, including hazard identification, dose-response assessment, and exposure assessment.



There is a rich literature demonstrating that children are exposed to PBT chemicals during infancy via breastfeeding ([Lakind et al., 2004](#)); however, many human studies have also reported beneficial effects of breastfeeding relative to formula-feeding despite this exposure ([Mead, 2008](#)). In order to identify hazards posed by PBT chemical exposure in breastfed infants, toxicological endpoints relevant to that time period of exposure should be compared across populations of breastfed infants with varying levels of exposure ([Makris et al., 2008](#)). Whether infancy encompasses a window during which PBT chemical exposure may trigger developmental effects has been addressed in relatively few studies. Furthermore, for many toxicological endpoints, critical and/or sensitive windows of exposure have not been delineated; the potential impacts of early life exposure to environmental chemicals on these endpoints are not clear. Thus, identification of hazards to health or development associated with early life exposure to most PBT chemicals is limited by a lack of data.

Challenges to dose-response and human exposure assessment determinations for infants arise mainly from the accumulation of PBT chemicals in the body lipids of women prior to pregnancy and their subsequent partitioning to breast milk. As noted above, a human infant's exposure to PBT chemicals through ingestion of breast milk (hereafter, "infant (or offspring) lactational dose") may be substantially greater than the average daily exposure of his/her mother from contact with PBT chemicals in environmental media (hereafter "average daily maternal dose"). Although many studies have reported PBT chemical concentrations in human milk ([Lakind et al., 2004](#)), dose-response and human exposure models presently conducted for PBT chemicals often do not account for the nature or magnitude of the differences between infant lactational dose and average daily maternal dose of these chemicals. This particularly holds when (1) the dose-response assessment for toxicological endpoints relies on data derived from animal studies, or (2) the human exposure assessment relies on measures of PBT chemical concentrations in environmental media. These situations are further outlined and delineated as follows:

- (1) Several specific differences between humans and laboratory animals are important to consider when using experimental animal data for a dose-response assessment of developmental endpoints that could result from early life exposure to PBT chemicals:
  - a. **Differences in elimination half-life.** A PBT chemical's elimination half-life in humans may be dramatically longer than it is in laboratory animals ([Poiger and Schlatter, 1986](#)), and elimination half-life is a major factor in the bioaccumulation of a PBT chemical and its subsequent elimination in milk.
  - b. **Differences in exposure duration.** PBT chemicals may accumulate in a female's lipids throughout the period of time prior to pregnancy. Many reproductive and developmental toxicity studies in animals expose females to PBT chemicals for only a relatively short time (e.g., days or weeks) before and/or during gestation and/or lactation. In contrast, this period in humans may span decades.
  - c. **Differences in distribution to offspring.** Humans most often give birth to singletons while most laboratory animal species bear young in litters. As a result, in humans, the lactational dose is delivered to one infant while, in animals, it is divided among offspring.

As a result of these and other interspecies differences (e.g., differences in absorption and metabolism), lactational exposure to a PBT chemical in an animal study may be very different compared to lactational exposure for a human infant, even when the animal dam and the human mother are exposed to the same chemical at the same average daily dose. In assessing the developmental effects of PBT chemicals that occur in offspring, it is the dose of these chemicals to the offspring that is the most important for dose-response assessment. Yet, most studies report doses administered to animal dams and not the lactational doses ingested by their offspring. Thus, when animal data are used in the dose-response assessment of developmental endpoints resulting from lactational exposure to PBT chemicals, methods are needed to determine (1) the offspring lactational dose achieved in an animal study, and (2) the average daily maternal dose in humans that would deliver an infant lactational dose in humans of the same magnitude as the offspring lactational dose achieved in the animal study. The method employed to estimate these respective doses may vary depending on how much information is known about a given PBT chemical with regard to its toxicokinetic profile in humans and laboratory animals.

- (2) Several considerations are important when conducting an exposure assessment for PBT chemicals in breastfed human infants:
- a. **Lack of data.** Data for PBT chemical content of breast milk are often unavailable. As a result, it may not be possible to estimate exposure based on the concentration of the chemical of interest in breast milk.
  - b. **Unique exposure conditions.** Available data to support exposure assessment often consist of chemical concentrations in environmental media (e.g., food, water, soil, air), which are combined with data on the intake or contact rate of a population with one or more of these media to estimate the chemical exposure level of particular groups within the population (e.g., adult men, adult women, children of various ages). However, compared to other groups, breastfed infants are relatively sheltered from some environmental media (e.g., food, ingested water) while being uniquely exposed to breast milk ([Cohen Hubal et al., 2008](#)).
  - c. **Differences between maternal and breastfed infant exposures.** As discussed above, PBT chemical concentrations in breast milk may produce infant lactational doses that are higher than the average daily maternal doses encountered from contact with environmental media.

Given these considerations, in order to use environmental sampling data to estimate PBT chemical exposure in breastfed human infants, methods are needed to determine the relationship between the infant and maternal doses of exposure. U.S. EPA's *Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions* ([U.S. EPA, 1998](#)) offers some guidance to support the estimation of an infant lactational dose

that might result from a certain average daily maternal dose of a PBT chemical. However, improvements could be made to the approaches presented in this guidance document to better account for human variability, uncertainty, and the toxicokinetic profile of specific PBT chemicals.

## **2. WORKSHOP PROCESS**

Because it is the policy of the U.S. EPA to consider risks to infants and children consistently and explicitly as a part of risk assessments generated during its decision making process, a workshop was convened to stimulate ideas and provide a forum for discussion regarding innovative approaches to the assessment of early life exposure to and potential health effects from PBT chemicals in breast milk. A group of 24 experts was selected to attend the workshop, with individuals representing a variety of sectors (e.g., academia, state and federal government) and disciplines (e.g., toxicology, risk assessment, epidemiology, PBPK modeling, public health).

The focus of the workshop was on discussing and evaluating potential approaches to risk assessment for PBT chemicals from exposure through breast milk. Briefing packets were developed for a group of four PBT chemicals (i.e., mirex, hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT), and chlordane). These briefing packets summarized available information on the chemicals and potential approaches to assessing risks for children exposed to these chemicals in breast milk. The briefing packets were provided to workshop participants to facilitate their evaluations of the potential approaches prior to the workshop. The focus of the workshop was on development of risk assessment methods; these specific PBT chemicals were chosen as examples to support this process, to illustrate the range of data that may be available for chemicals that fall into this class, and to facilitate the identification of global data gaps. In general, these are considered legacy chemicals for which there is considerable, if not complete, information. For many other PBT chemicals, there is far less information available and fewer studies to review. The selection of these particular PBT chemicals for inclusion in the workshop materials should not be interpreted as an indication that U.S. EPA has any intention to pursue specific regulatory or policy objectives related to the presence of these specific chemicals in breast milk.

To facilitate discussion, workshop participants were asked to answer five charge questions prior to the workshop. These questions were developed to guide the participants in their review of the briefing packets and to help them focus on issues of most direct relevance to the subject matter of the workshop. The questions are summarized below and are included in their full form in [Appendix A](#). The original briefing packets, as distributed to workshop participants prior to the workshop, can be found in [Appendix B](#). Similar charge questions ([Appendix C](#)) were also used at the workshop to guide break-out group discussions and presentations.

1. Select an approach that could be used to estimate the quantitative relationship between infant dose of the PBT chemical from milk and continuous daily maternal dose for humans and any relevant laboratory species.

2. Using the quantitative relationship(s) identified in response to Charge Question 1, how could an extrapolation be performed from a dose that produces effects as a result of postnatal exposure in animal studies to a continuous daily maternal exposure that would be expected to produce the same effects in human infants exposed through breast milk?
3. What data gaps may exist that, if filled, could significantly improve the approaches discussed in Charge Question 1 for assessing risk to breastfeeding infants?
4. What problems, complications, or uncertainties exist with the approach identified in Charge Question 1? How might these problems affect your confidence in using the approach in risk assessment?
5. How could the approach discussed in the response to Charge Question 1 be adapted for application for estimating risk to breastfeeding infants to PBT chemicals in general?

What follows in the main body of this workshop report is an organized summary of the workshop discussions, with emphasis on potential approaches for improving the risk assessment of PBT chemicals in breast milk, the data gaps, uncertainties and other issues identified, and solutions suggested. In [Section 3](#) of this report, revised briefing materials for HCB are presented with annotations to highlight areas of interest that were discussed by workshop participants. The text of the briefing materials presented in [Section 3](#) has been revised to address comments provided by workshop participants and EPA reviewers following the workshop. HCB was chosen as the exemplar because (1) its toxicokinetic profile is relatively well-understood (e.g., validated PBPK models exist for HCB in both humans and laboratory animals), (2) its metabolism in both humans and laboratory animals is limited (see [Section 4.4](#) for a discussion of complications associated with metabolism, such as those observed for DDT), and (3) it exists in the environment as a single chemical (see [Section 4.5](#) for a discussion of complications associated with mixtures, such as chlordane). [Section 4](#) provides a synthesis of the global issues identified, including a number of data gaps and uncertainties that were found to be common to many PBT chemicals. [Section 5](#) describes the types of studies and data suggested by workshop participants to best support PBT chemical hazard identification and dose-response assessment. And, [Section 6](#) outlines a set of modeling approaches discussed by the workshop participants. These approaches may be used to extrapolate dose-response data from animal studies to an estimate of a maternal dose in humans that will limit fetal and/or infant exposure to a level of minimal risk. These approaches can be adapted to apply to any PBT chemical, whether it be one of the legacy chemicals addressed in the workshop briefing materials, other known PBT chemicals, or PBT chemicals that may emerge in the future. Workshop conclusions and future directions for risk assessment and research are discussed in [Section 7](#).

Data presented in this workshop report have been compiled from various sources, including government reports and information presented in the scientific literature. The data presented are the result of analyses by the individual study authors. However, in some cases, the U.S. EPA conducted additional analysis of published primary data to present results in a way that will be most useful for risk assessment in the context considered here. Studies presented in this workshop report were chosen

based on the following considerations: (1) soundness (adequacy of approach and minimal or defined bias); (2) applicability and utility (focus on lipophilic PBT chemicals and/or the developmental period of interest); (3) clarity and completeness (accessibility, reproducibility, and quality assurance); (4) variability and uncertainty (variability in the population and uncertainty in the results); and (5) evaluation and review (level of peer review and number and agreement of studies).

### 3. CHEMICAL BREAKOUT GROUPS – Annotated HCB Briefing Packet

*The following text is a revised, annotated version of the HCB Briefing Packet. This version has been annotated using text boxes that contain feedback from workshop participants on specific areas of interest within the briefing packet. For example, data gaps are provided in blue boxes, comments on approaches in orange boxes, comments on study design in red boxes, and other comments in green boxes. The original HCB Briefing Packet, as distributed to workshop participants prior to the workshop, can be found in [Appendix B](#).*



## Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk Workshop Briefing Packet: Hexachlorobenzene

#### Prepared for

National Center for Environmental Assessment  
U.S. EPA  
109 T.W. Alexander Drive  
Durham, NC 27711

#### Prepared by

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713

#### Notice

Although the research described in this briefing packet has been supported by the U. S. Environmental Protection Agency through EP-C-09-009 to ICF International, it does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

### **3.1. Introduction**

Hexachlorobenzene (HCB) is a synthetic compound that was widely used in the United States as a pesticide until 1965; use as a fungicide on seeds of onions, sorghum, wheat, and other grains continued until 1984. It was also used to make fireworks, ammunition, military pyrotechnic and ordinance materials, and synthetic rubber ([ATSDR, 2002b](#)). Commercial production of HCB in the U.S. for these direct uses ended in the late 1970s, but HCB is still formed (1) as a by-product of the manufacture of solvents, other chlorine-containing compounds and pesticides (e.g., trichloroethylene, vinyl chloride, pentachlorophenol, atrazine, lindane), (2) during the combustion of municipal waste, and (3) in the waste streams of chlor-alkali and wood-preserving plants ([ATSDR, 2002b](#)).

In this briefing packet, the available literature on gestational and lactational exposure to HCB was reviewed for key findings and data to help estimate the maternal dose and breastfeeding infant dose of HCB in humans. Selected references are summarized and evaluated in this briefing packet in five sections: maternal dose and breastfeeding infant dose of HCB in humans; maternal dose and nursing offspring dose in animals; developmental effects of HCB in humans and animals; human and animal kinetic models; and a final section, which discusses the application of kinetic models to derive an example human equivalent dose (HED).

These briefing packets are intended to stimulate ideas and provide material for discussion regarding innovative approaches to the assessment of risk to breastfeeding infants from HCB and other PBT chemicals in breast milk. The information in this and other briefing packets is provided to help workshop participants discuss and formulate answers to the workshop charge questions and does not reflect Agency opinion or policy regarding HCB. Areas where there exists uncertainty, data gaps, or where there is a lack of understanding in the current literature are highlighted to foster further discussion.

### **3.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans**

This section discusses how women of child-bearing age may be exposed to HCB; how their bodies sequester HCB in various tissues, and the extent and manner that HCB is eliminated through breast milk, leading to infant exposure. HCB exposure levels vary geographically and may be at least partially dependent on social factors (e.g., dietary choices) and environmental factors (e.g., the amount of residual HCB in the local environment). Concentrations of HCB have been reported in various human tissues, including breast milk, blood plasma, and adipose tissue.

In this briefing packet, measured concentrations of HCB in the diet or environmental media and their relationship with measured body burdens in mothers are presented. Studies are also described that inform the characterization of the relationship between the body burden of HCB in mothers and intake by breastfeeding infants. However, the relationships between maternal exposure to HCB (i.e., intake from environmental and dietary sources), maternal body burden, and infant dose in humans are not well described in the literature. Attempts have been made to draw connections between studies

showing intake and breast milk concentrations in similar geographic areas during similar time periods to illustrate relationships between exposure and body burden of HCB.

### 3.2.1. Mothers' Exposure to HCB

#### **Workshop Notes: Data Gaps**

Although the primary route of exposure to HCB is oral, inhalation is an exposure route of greater concern for some PBT chemicals, such as chlordane ([Appendix B.1](#)).

Workshop participants noted that it is important to understand how all routes of exposure may contribute to maternal body burden of a PBT chemical. This understanding requires kinetic data (e.g., absorption fractions) for each route, and these data are often unavailable.

Trace levels of HCB have been measured in nearly all people who have been tested ([ATSDR, 2002b](#)). While direct exposure to HCB is limited in the general population, indirect environmental exposure can occur because HCB released into soil, air, or water remains in the environment for a long time. The half-life of HCB in soil is 3-6 years; in surface waters it is 2.7-5.7 years; in ground water it is 5.3-11.4 years; and in air it is 0.63-6.28 years. Releases of HCB can be significant at or near sites where it is generated, such as industrial facilities ([ATSDR, 2002b](#)). HCB does not dissolve easily in water and has been detected at very low to trace levels in drinking water, surface water, and groundwater (below 0.1 parts per trillion) ([ATSDR, 2002b](#)). Due to its long half-life in air, HCB released into air can be transported over wide geographic areas. Inhalation or ingestion of dust particles or dermal contact with contaminated soil/sediment or dust are major routes of exposure, especially for individuals who live near industrial sites where HCB is unintentionally produced as a by-product or disposed of as hazardous waste ([ATSDR, 2002b](#)). Levels up to 440 ng/g have been detected in agricultural soils at sites in 37 states, and at 15 ng/g in lake sediment. Ambient air measurements have ranged from  $1 \times 10^{-4}$  ng/m<sup>3</sup> to 1.5 ng/m<sup>3</sup>, but indoor air concentrations in a pesticide production facility and industrial plant were much higher: 22,000 ng/m<sup>3</sup> and 150,000 ng/m<sup>3</sup>, respectively ([ATSDR, 2002b](#)). Environmental monitoring of HCB has been focused in the Great Lakes region as historic production of chlorobenzene products was high in this region. Lake sediment levels of HCB have been as high as 97 ng/g in Lake Ontario; mean ambient air levels were 0.03668 ng/m<sup>3</sup> in Villeroy, Quebec ([ATSDR, 2002b](#)).

It has been estimated that about 92% of total HCB intake comes from food, 7% from air, and 1% from drinking water ([IPCS, 1997](#)). Intake studies conducted in various countries (Canada, USA, Germany, Finland, Vietnam, Thailand, India, Japan, Australia, and the Netherlands) have estimated daily intake of HCB from food ranging from approximately 0.0004 to 0.0028 µg/kg-day ([IPCS, 1997](#)). The Fourth National Report on Human Exposure to Environmental Chemicals, published in 2009, did not include any data on HCB. Higher serum HCB levels have been associated with living near agricultural or industrial areas, but background levels in the human population have declined over the last 20 years. Recent



measurements of HCB in human tissues, including serum concentrations reported in the National Health and Nutrition Examination Survey (NHANES), have been near the lipid adjusted limit of detection.<sup>1</sup>

Generally, levels of HCB in foods, human adipose tissue, and breast milk declined from the 1970s to the mid-1990s (IPCS, 1997). These decreases have been attributed to the discontinued use of HCB in agriculture and industrial applications. Decreased levels of HCB in food were illustrated in the data from the U.S. FDA Total Diet Studies (FDA, 1994; Yess, 1992, 1991; FDA, 1990, 1989; Gunderson, 1988). Table 3-1 shows estimated HCB intakes as determined from the FDA Total Diet Studies for select years from 1982 through 1993.

**Table 3-1. Intake of HCB as Estimated by FDA Total Diet Study, Select Years, 1982-1993**

Year(s)	Frequency of Detection (%)	Intake for 14-16 year old males (µg/kg-day)	Intake for 60-65 year old females (µg/kg-day)	Intake for 25-30 yr old females (µg/kg-day)	References
1982-1984	9	0.0020	0.0011	0.0013	Gunderson (1988)
1988	7	0.0011	0.0006	NR	FDA (1989)
1989	5	0.0009	0.0005	NR	FDA (1990)
1990	4	0.0005	0.0002	NR	Yess (1991)
1991	2	0.0004	0.0002	NR	Yess (1992)
1991-1993	<2	NR	NR	NR	FDA (1994)

NR = Not reported.

Not only has the potential for human exposure to HCB varied over time, it also varies according to geographical location. Nakata et al. (2002) reported levels of HCB (and other chemicals) in 39 food items collected from Shanghai and Yixing, China in 2000 and 2001. The authors determined that the average daily intake of HCB in China, based on food consumption, was 0.046 µg/person-day. Assuming an average adult body weight of 60 kg, authors calculated that the intake was approximately 0.0007 µg/kg-day, indicating a similar exposure and intake level to the U.S. Total Diet Study a decade prior.

HCB exposure potential is also impacted by dietary habits. Several studies have investigated intake levels as a result of fish/seafood consumption. Dewailly et al. (1993) reported that the average HCB concentration in milk of Inuit women, whose traditional diet included marine mammal fat, was significantly higher than that of Caucasian women in neighboring regions, who did not consume marine mammal fat. Similarly, Ayotte et al. (1995) found that the levels of organochlorine compounds, including HCB, in the breast milk of Inuit and Caucasian women correlated with plasma phospholipid omega-3

<sup>1</sup> Data discussed here are described in the Biomonitoring Summary for Hexachlorobenzene, from the CDC National Biomonitoring Program. Information available at: [http://www.cdc.gov/biomonitoring/Hexachlorobenzene\\_BiomonitoringSummary.html](http://www.cdc.gov/biomonitoring/Hexachlorobenzene_BiomonitoringSummary.html)

fatty acid levels. This relationship suggested to these authors that consumption of marine organisms serves as a source of HCB exposure and intake. Newsome et al. (1995) also reported that higher breast milk levels of HCB were related to eating more than 100 g of fish weekly. More recently, Falco et al. (2008) estimated intake levels of HCB from marine fish for four age groups (children, adolescents, adults, and seniors) separated by sex. The HCB intake estimated for female adults was 13.3 ng/day, or  $2.4 \times 10^{-4}$  µg/kg-day (Falcó et al., 2008).

#### **Workshop Notes: Data Gaps**

In general, the studies estimating PBT chemical doses from food do not link these data to measured body burdens. Furthermore, because infant exposure to PBT chemicals through ingestion of breast milk is influenced by maternal exposure accumulated over many years prior to pregnancy, it would be useful to obtain accurate information on PBT chemical exposures from the environment among human females from birth through childbearing age. These data would be useful for validating models that predict the kinetic profile of these chemicals in humans. Presently, this represents a key data gap common to all PBT chemicals.

Furthermore, workshop participants stressed that dietary exposure estimates from different sources may be derived using very different methodologies. Thus, it is important to consider the source and nature of exposure estimates based on chemical content of foods and diets. For example, data from market basket dietary surveys (FDA, 1994) represent population or “per capita” consumption values based on cross-sectional exposure assessments; these typically represent chronic exposure estimates. On the other hand, data from duplicate diet studies represent exposure values for individuals, reflecting all food eaten by assessed individuals over the duration of the study; in general, these studies tend to be associated with higher levels of estimated intakes. Per capita consumption values represent both eaters and non-eaters of particular foods, while intake values derived from duplicate diets are based on individuals who actually consumed the food (eaters only). Some dietary exposure studies measure chemicals in foods prepared as they would typically be consumed (FDA, 1994), while others measure chemicals in unprepared (i.e., raw) food samples collected directly from food markets (Nakata et al., 2002). These types of differences in study design across dietary exposure studies need to be clearly delineated and described when evaluating, drawing conclusions about, and making comparisons of exposure estimate values for any environmental chemical, including PBT chemicals.

### **3.2.2. Is HCB Sequestered in Breast Milk or Other Human Tissues?**

Several studies were identified that measured HCB concentrations in human milk. In parallel with the decreases in HCB in food discussed in Section 3.2.1, most studies have shown a decrease in observed HCB concentrations in breast milk and/or neonates (Norén and Meironyté, 2000; Craan and Haines, 1998). The decrease in detected HCB levels has been attributed to the discontinued use of HCB in agriculture and industrial applications (Hardell et al., 2010; Lackmann, 2002; Vaz et al., 1993).

Findings from several epidemiological studies suggest that HCB levels decrease in breast milk as the number of children breastfed increases, with the percentage of decline ranging from approximately 11% to 50% ([Ennaceur et al., 2007](#); [Kunisue et al., 2006](#); [Kunisue et al., 2004](#); [Newsome et al., 1995](#); [Vaz et al., 1993](#); [Skaare and Polder, 1990](#)). However, recent data show relatively constant levels of HCB in breast milk in repeated measures taken from the same women over the duration of lactation ([Sjödin et al., 2005](#)). HCB levels in breast milk might not decrease during lactation due to current low-level dietary exposures or minimal elimination during breastfeeding. HCB levels in breast milk may also be related to maternal age and changes in body mass index. Ennaceur et al. ([2007](#)) observed higher HCB concentrations in the breast milk of older mothers compared to younger mothers. Similarly, Lackmann et al. ([1999](#)) showed that HCB concentration in neonatal serum taken prior to the first breast milk feeding was significantly correlated with maternal age ( $p < 0.01$ ), with 2.7-fold higher serum levels in offspring of 40-year-old women as compared with 20-year-old women. Birth order of the neonates was not reported in the study. One study showed a positive relationship between HCB levels in breast milk and decreased body mass index ([Geyer et al., 1986](#)), but another study did not confirm this finding ([Lovelady et al., 1999](#)).

Attachment A contains data on breast milk concentrations measured globally from 1970 to 1991, [[Table 3-13](#) as reported by IPCS ([1997](#)).] and from 1967 to 2001 [[Table 3-14](#), as reported by ATSDR ([2002b](#))].

#### **Workshop Notes: Data Gaps**

Although there are some data available for HCB ([LaKind et al., 2009](#)), studies reporting levels of PBT chemicals in maternal blood and milk collected simultaneously are relatively rare (e.g., at the workshop, this was identified as a data gap for mirex). These data are useful for developing partition coefficients for use in PBPK modeling.

It is also exceedingly uncommon to find data comparing chemical concentrations in breast milk to measures of maternal exposure from the environment (e.g., from food, dust, air).

### **3.2.3. How Much HCB Passes from Mother to Infant via Breast Milk?**

Abraham et al. ([1994](#)) measured HCB in the feces of one breastfed baby and one formula-fed baby (date unspecified, assumed location Germany). The infants' stool was collected at the beginning and end of 2.5- and 3-day sampling periods at ages 1 and 5 months, respectively. A corresponding sample of at least 100 mL mother's milk was obtained at the one-month period, and one breast milk sample was obtained for the 5-month period. Based on study results, the authors concluded that absorption of HCB in the breastfed infant was "almost complete."

Ribas-Fito et al. ([2005](#)) recruited a birth cohort of 92 mother-infant pairs in Spain (infants born 1997-1999) to measure organochlorine compounds (HCB, dichlorodiphenyl dichloroethane, and polychlorinated biphenyls) in cord serum, colostrum taken 3 days after delivery, breast milk taken 3

weeks after delivery, and infant serum taken at 13 months of age. At 13 months of age, infants that were breastfed had higher concentrations of HCB in serum than those that were formula-fed (4.26 ng/mL among breastfed infants vs. 2.13 ng/mL among formula-fed infants). Additionally, comparison of 13-month serum within the group of breastfed infants showed that those who were breastfed for longer durations had higher concentrations of HCB. The authors did not find a correlation between serum concentrations of HCB in the children and concentrations of HCB in colostrum or breast milk. Statistical analysis considered confounders of infant weight, parity, maternal age, and maternal body mass index.

#### **Workshop Notes: Data Gaps**

Although the primary route of exposure to HCB is oral, inhalation is an exposure route of greater concern for some PBT chemicals, such as chlordane ([Appendix B.1](#)). Thus, breastfed infants may be exposed to PBT chemicals not only through ingestion of breast milk, but also by inhalation. Also, infants, including those who are breastfed, ingest dust, which may contain PBT chemicals. Therefore, dust ingestion could be another additional source of infant exposure to these chemicals.

Workshop participants noted that it is important to understand how all routes of exposure may contribute to infant body burden of a PBT chemical and suggested that these additional exposure routes should be included in modeling approaches whenever appropriate data are available.

### **3.3. Maternal Dose vs. Nursing Offspring Dose in Animals**

#### **3.3.1. Maternal Exposure and Elimination in Milk**

In the Environmental Health Criteria for HCB, IPCS ([1997](#)) concluded that HCB is transferred from mothers to offspring in a number of species and that lactation seems to be the most important route of transfer. Studies cited by IPCS and others identified for the purposes of this briefing packet are summarized below.

##### **3.3.1.1. Mice**

Courtney and Andrews ([1985](#)) compared HCB levels in CD-1 dams and their nursing pups in full litters and litters reduced to only 2 pups. While lactational transfer from dams to pups resulted in approximately 95% depletion of HCB burden in dams by postnatal day (PND) 20, litter size did not have a significant effect on HCB levels in dams or pups. Dams were dosed with 0, 10, or 50 mg/kg-day HCB on gestation days (GD) 11 through 17 (the last week of gestation). Some of the dams (n=23) nursed their full litters while some dams (n=14) had litters reduced to 2 pups. All dams nursed their pups until PND 20. HCB concentrations in liver, brain, muscle, skin, fat, and whole blood of dams were depleted almost

entirely on PND 20 in all exposure groups regardless of full or reduced litter size; depletion in non-pregnant female mice over the same time period was significantly less. For example, HCB concentrations were reduced in the liver and in fat by about 99% in the highest dose group of lactating mice. In non-pregnant female mice, HCB concentrations were only reduced by about 72% in the liver, and HCB concentrations actually increased about 1% in fat in those mice. Similar HCB levels were observed in pups regardless of whether they were weaned as part of a full or reduced litter; pup body burdens corresponded in a dose-dependent manner with maternal exposure levels.

Andrews and Courtney ([1986](#)) measured HCB levels in groups of CD-1 mouse pups to ascertain the gestational and lactational transfer of HCB from dams who received 50 mg/kg-day from GD 6 through 16. Pups born to unexposed dams and cross-fostered and nursed by exposed dams had higher body burdens than pups born to exposed dams and cross-fostered to control dams. Pups born to and nursed by exposed dams had the highest burdens after 4 days of nursing ([Andrews and Courtney, 1986](#)).

### **3.3.1.2. Rats**

Cripps ([1990](#)) showed that both gestational and lactational transfer of HCB occurs in rats. Female rats were orally dosed with 400 or 2,000 µg/g HCB during gestation; offspring were sacrificed at the time of birth or after 2, 4 or 8 weeks. At birth, HCB levels in neonatal liver in the offspring of dams dosed with 2,000 µg/g were 27.3 µg/g; at 5 weeks, these levels had increased to 836 µg/g. Sacrifice and sampling times were not consistent in this study design, and limited additional information was available. Additionally, HCB levels in maternal breast fat of dams dosed with 2,000 µg/g decreased from 2,947 µg/g at the time of delivery to 1,091 µg/g after 3 weeks of nursing ([Cripps, 1990](#)).

Goldey et al. ([1990](#)) reported on the uptake, distribution, and elimination of HCB in dams, fetuses, and nursing pups when dams were exposed to 11 mg/kg HCB over three days at breeding. During pregnancy, fetal blood HCB levels were about 0.9 times maternal blood HCB levels. However, maternal HCB levels decreased as a result of nursing, and HCB levels in pups' liver, kidney, and blood at PND 7 were about 5 times the level in respective maternal tissues.

Mendoza et al. ([1978](#); [1977](#)) performed two cross-foster studies investigating gestational and lactational exposures to HCB in rats; the toxicological results of these studies are discussed in [Section 3.4.2](#). The authors reported that pups born to unexposed dams and cross-fostered to HCB-exposed dams had significantly higher HCB body burden than pups nursed by unexposed dams. Pups born to HCB-exposed dams and nursed by unexposed dams had similar HCB body burdens compared to pups born to and nursed by unexposed mothers. These results suggest that lactation was an important route for HCB transfer to pups ([Mendoza et al., 1977](#)).

Lilienthal et al. ([1996](#)) exposed female rats to diets containing 4 to 16 mg/kg HCB starting 90 days prior to mating and continuing throughout mating, gestation and lactation. Dams, unmated rats with the

same exposure, and offspring all showed dose-related tissue levels of HCB. However, HCB levels were significantly decreased in dams after nursing compared to unmated rats.

Nakashima et al. (1997) fed Sprague-Dawley rats 35.1 nmol HCB per 100 g diet *ad libitum* during gestation and lactation. HCB levels were elevated in the blood, subcutaneous fat, and perirenal fat of 2 pregnant dams one day before parturition compared to 3 nursing dams on PND 16 (Table 3-2). Additionally, HCB levels in blood, subcutaneous fat, and perirenal fat were approximately 6, 29, and 15 times higher in the respective tissues of 43 suckling pups compared to their 3 dams. The authors concluded that HCB is transferred from dams to pups during lactation. In a second experiment reported within the same study, Nakashima et al. (1997) compared placental and lactational transfer of HCB using a cross-foster study design. Lactational transfer during the early days after birth resulted in a significant contribution to HCB burden in pups and reduction of burden in dams, but was not quantified by the authors. The authors estimated that 0.39% of maternal HCB intake was transferred to the fetuses prenatally.

**Table 3-2. Concentration of HCB in Blood and Fat of Exposed Dams and Their Pups**

Tissue type	Pregnant dams <sup>a,b</sup> (n=2)	Fetuses <sup>a</sup> (n=26)	Nursing dams <sup>a,c</sup> (n=3)	Nursing pups <sup>a,c</sup> (n=43)
Blood (nmol/L)	12.01, 15.48	ND	3.84 ± 0.24	27.90 ± 2.10*
Subcutaneous fat (pmol/g)	546.2, 909.5	ND	49.10 ± 3.81	1,495 ± 240*
Perirenal fat (pmol/g)	445.6, 358.2	ND	145.9 ± 26.52	2,351 ± 238*
Whole body (pmol/g)	ND	22.72 ± 4.34	ND	ND

<sup>a</sup> Values represent means ± SEM. When n<3, individual values are listed.

<sup>b</sup> Measured one day prior to presumed parturition; dams fed 31.5 nmol HCB/100g diet during gestation and lactation

<sup>c</sup> Measured 16 days postpartum; dams fed 31.5 nmol HCB/100g diet during gestation and lactation

ND = Not determined

\*Statistically significant compared to corresponding dam at P < 0.05

Source: Nakashima et al. (1997).

Taylor et al. (1991) also illustrated that HCB is transferred from dams to pups via lactation, quickly depleting maternal body burden. Dams were dosed with either 10 or 100 mg/kg HCB two weeks prior to mating; HCB tissue concentrations remained 10-fold different between the two groups during the 20-day gestation period. However, during lactation (measured on PND 4, 5, 10, and 14), tissue concentrations in both dams and pups were only 2- to 3-fold different between the two dose groups. The authors concluded that pups receive a similar dose of HCB via milk regardless of the maternal dose because HCB stored in the dams' tissues equilibrates with lipoprotein-rich blood during lactation.

### 3.3.1.3. Monkeys

Bailey et al. (1980) exposed nursing rhesus monkey dams with infants between 1 and 3 months of age to HCB; dams received 60 mg/kg-day HCB for 60 days. HCB concentrations in milk and serum were measured 14 and 7 days prior to exposure and on exposure days 0, 1, 2, 4, 8, and then weekly through exposure day 60 (or until infant death as two infants became ill before the end of the study). As shown in Table 3-3, serum concentrations were consistently higher in infants than in mothers from day 8 onward (Bailey et al., 1980).

**Table 3-3. Mean HCB Concentrations (µg/g wet weight) in Serum and Milk of Dam and Infant Rhesus Monkeys, Dams Dosed with 60 mg/kg-day while Nursing**

Day of dosing	Number of dams	Number of infants	Concentration in dam's serum	Concentration in milk	Concentration in infant serum
-14*	3	3	<0.01	<0.01	<0.01
-7*	3	3	<0.01	<0.01	<0.01
1	3	0	0.41 ± 0.23	7.51 ± 7.15	--
2	3	3	0.78 ± 0.14	17.31 ± 15.49	0.42 ± 0.18
4	3	0	1.35 ± 0.31	12.90 ± 7.30	--
8	3	3	1.86 ± 0.46	29.37 ± 11.02	2.62 ± 1.26
15	3	3	3.37 ± 0.34	36.47 ± 6.81	5.82 ± 2.35
22	3	3	3.43 ± 0.21	107.13 ± 37.54	14.32 ± 1.87
29	2	2	1.86 ± 0.46	61.20 ± 20.80	2.62 ± 1.26
36	2	0	5.73 ± 2.18	24.75 ± 10.25	--
43	2	1	8.15 ± 0.15	186.00 ± 119.00	28.31
50	1	1	14.40	110.00	28.50
57	1	1	16.16	--	49.44

\*Days listed as lactation days. Negative values refer to measurements taken prior to dosing.

Source: Bailey et al. (1980).

## 3.4. Developmental Effects of HCB

### 3.4.1. Human Studies

In Anatolia, Turkey in the 1950s, a widespread HCB exposure occurred when a large population ate bread made from grain that was treated with HCB. Studies focused on developmental effects resulting from the exposures include Cripps et al. (1984), Gocmen et al. (1989), Peters et al. (1982a; 1982b), and Peters et al. [(1966) as cited in IPCS (1997)]. In addition, the epidemic is described by ATSDR (2002b) in

the Toxicological Profile for Hexachlorobenzene. Doses of HCB from the treated grain were estimated at 0.7 to 2.9 mg/kg-day for a person of “average” size.

Breastfed infants of mothers who ingested the contaminated bread presented with a condition called *pembe yara* (pink sore), which was associated with skin lesions and infant deaths from cardiorespiratory failure ([ATSDR, 2002b](#)). The skin lesions were further diagnosed as *porphyria cutanea tarda*. Porphyria indicates a dysfunction in the normal production of hemoglobin, and HCB exposure has been demonstrated to cause porphyria ([ATSDR, 2002b](#)). *Porphyria cutanea tarda* is a specific form of porphyria that results in tissue damage to the skin. The effects of HCB exposure to infants were irreversible, and there was some evidence that adult women who had been exposed as children had elevated incidences of miscarriages and stillbirths ([ATSDR, 2002b](#)).

In an early report of the grain contamination incident and cases of *pembe yara* [Peters et al. [([1966](#)) as cited in IPCS ([1997](#))], HCB was said to be present in breast milk but was not quantified. Elevated levels of HCB were measured in breast milk 20–30 years after the incident, with a mean level of 510 ng/g-fat in milk from 56 mothers whose children experienced porphyria. The mean HCB level was 70 ng/g fat in milk from 77 women from families without porphyria or from areas outside of the endemic area ([IPCS, 1997](#); [Gocmen et al., 1989](#); [Peters et al., 1982b](#)).

#### **Workshop Notes: Data Gaps**

Workshop participants noted that there are a number of developmental processes that occur in humans during the neonatal period. For many PBT chemicals, there are few or no published epidemiologic studies assessing toxicological endpoints that might result from disruption of these postnatal developmental processes. These data are needed to ensure that any potential hazards of neonatal PBT chemical exposure in humans are identified. Furthermore, dose-response relationships based on human data for postnatal developmental endpoints would be highly beneficial for risk assessment. This is especially true for the evaluation of postnatal developmental effects that are difficult to evaluate in animal models.

See [Section 5](#) for a detailed discussion of this topic.

### **3.4.2. Animal Studies**

The animal toxicology literature on controlled exposures to HCB was reviewed for studies presenting data specifically related to exposure occurring during gestation and/or lactation. To provide a comprehensive understanding of the hazard resulting from these specific types of exposures, all relevant studies that could be retrieved were reviewed and are summarized in [Table 3-4](#). The endpoints examined and effects observed are noted, as are potential points of departure (PODs) for each study. For endpoints in the low-dose range, benchmark dose modeling using U.S. EPA’s Benchmark Dose Software (BMDS) was considered. However, appropriate litter-specific data were not available for



critical effects in the low-dose range, making BMD modeling inappropriate for this application. PODs are arrayed in [Figure 3-1](#) and [Figure 3-2](#) to facilitate comparisons across studies. [Table 3-4](#) serves as a reference key for [Figure 3-1](#) and [Figure 3-2](#).

Studies were classified according to the timing of exposure for the pups. Gestational exposure studies were those in which maternal animals were exposed during gestation and (1) pups were sacrificed prenatally or at parturition, or (2) pups were nursed by unexposed dams. Combined lactational and gestational exposure studies were those in which maternal animals were exposed during gestation or during gestation and lactation and pups were nursed prior to sacrifice. Finally, lactational exposure studies were those in which dams were not dosed until lactation began or pups unexposed during gestation were cross-fostered to exposed mothers.

#### **Workshop Notes: Approaches**

The relative importance of gestational exposure vs. lactational exposure was a major issue raised across chemicals. See [Section 4.2](#) for a detailed discussion of this topic.

Specifically with regard to HCB, it may have been more appropriate to select Barnett et al. ([1987](#)) as the POD study and decreased delayed-type hypersensitivity response as the critical effect. Then, HEDs for both gestational and lactational exposure could be calculated to determine which would be more health-protective.

For some PBT chemicals, including mirex ([Appendix B.4](#)), it was suggested that none of the existing animal studies were adequate to identify a potential hazard from postnatal exposure. The lack of adequate studies with the potential to identify hazards of postnatal exposure was a major issue raised across chemicals. See [Section 4.2](#) for a detailed discussion of this topic and [Section 5](#) for a description of a study design tailored to this purpose.

HCB induced a number of developmental effects in monkeys, mink, rats, and mice; including fetal abnormalities, neurotoxicity, reproductive effects, immunotoxicity, hepatotoxicity, and mortality. Three studies specifically investigated effects following lactational exposure to the offspring via controlled maternal exposure ([Iatropoulos et al., 1978](#); [Mendoza et al., 1978](#); [Mendoza et al., 1977](#)). Iatropoulos et al. ([1978](#)) exposed three rhesus monkey dams to HCB at 64 mg/kg-day for 22, 38, and 60 days (starting on postnatal days 118, 61, and 21, respectively). This dose resulted in mortality in two of the three exposed offspring, and HCB levels measured in milk samples and in infant serum support the importance of lactation as a route of exposure. Results of a cross-fostering study in Wistar rats further demonstrate the importance of lactational exposure relative to gestational exposure ([Mendoza et al., 1978](#); [Mendoza et al., 1977](#)). Changes in liver weights and enzyme activities were more significant in pups exposed solely through lactation compared to those exposed solely during gestation. Furthermore, liver weight and enzyme changes were not significantly different in pups exposed solely through lactation compared to those exposed during both gestation and lactation.

### **Workshop Notes: Study Design**

Workshop participants noted that rodent gestation is quite short. Many developmental processes that occur during the postnatal period in rodents occur during the prenatal period in humans. The “Translating time” website provides a guide to matching neurodevelopment in humans with other species (<http://bioinformatics.ualr.edu/ttime/index.php>).

Similar tools are not yet available to compare the developmental timing of other systems. The identification of critical windows of exposure (in humans and animals) was a major issue raised across chemicals. See [Section 4](#) for a detailed discussion of this topic and [Section 5](#) for a description of a study design tailored to this purpose.

Mendoza et al. ([1977](#)) was selected to serve as the example POD study for this briefing packet. It is important to note that this selection is intended only as an example to stimulate discussion for the workshop participants. This particular study was chosen as the example because the authors observed toxicological responses resulting from lactational HCB exposure. Potential PODs could also be derived from other studies to thoroughly evaluate the relative importance of gestational and lactational exposures for assessing the risk of developmental effects from HCB.

Mendoza et al. ([1977](#)) observed hepatotoxicity in pups following exposure of Wistar rat dams to 6.7 mg/kg-day HCB via the diet during lactation. Because this study included only one HCB dose group, BMD modeling was precluded. Therefore, the LOAEL of 6.7 mg/kg-day is used as an example POD to assess the impact that exposure to a mother may have on lactational exposure of her offspring. This POD is used in [Section 3.6](#), along with results from PBPK models, to estimate an HED.

### **Workshop Notes: Approaches**

Workshop participants disagreed with the choice of Mendoza et al. ([1977](#)) as the basis for the POD.

In many animal studies of lactational exposure to PBT chemicals, dose response has not been well-defined because only one dose level was tested. As discussed in [Section 1.1](#), a POD derived from benchmark dose modeling of data collected at several exposure levels is preferred over a POD derived from a NOAEL or LOAEL. This is true for dose-response assessment of any chemical exposure, including exposure to PBT chemicals.

**Table 3-4. Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational Exposure</b>								
Mouse, CD-1, gavage, treated GD 7–11, fetal examination on PND -2	0, 100	<p><b>Measured:</b> Maternal body weight and relative liver weight, average fetal weight, live fetuses per litter, fetal mortality, fetal physical abnormalities including incidence of cleft palate and clubfoot</p> <p><b>Observed:</b> No differences between treatment or control in terms of maternal effects; <b>increased average number and percent of abnormal fetuses per litter</b></p>	<p>Maternal: DU</p> <p>Develop: NA</p>	<p>Maternal: DU</p> <p>Develop: 100</p>	DU	DU	<p>Courtney et al. (<a href="#">1976</a>)</p> <p>(The number of dams per dose not reported but 10 litters evaluated; inconsistencies in reporting results in tables and text make the significance of maternal findings impossible to report)</p>	Develop: [A1]

Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<p>Rat, Wistar, ~7-16 dams per group, gavage, GD 6-9, 10-13, 6-16, or 6-21, sacrificed GD 22</p> <p>Authors report that dams were dosed in four groups, as follows:  10–80 mg/kg-d GD6-9;  10-80 mg/kg-d GD10-13;  10-60 mg/kg-d GD6-16;  40-120 mg/kg-d GD6-21;  number of dams per group not reported but number of pregnant rats at term ranged from 7-16;  Data presented is pup data, with no information on incidence per litter.</p>	0, 10, 20, 40, 60, 80, 120	<p><b>Measured:</b> Maternal (GD1, 6-15, and PND0) and fetal weight; pathological alterations in dams; number of resorptions, deciduomas, and fetuses dying late in development; pup viability and external and skeletal malformations; microscopic evaluation of pups' mid-sagittal section</p> <p><b>Maternal Observed:</b> Decreased maternal body weight and fetal body weight (associated with maternal toxicity) and increased incidence of hyperaesthesia, tremors, and convulsions in dams at 80 or 120 mg/kg (dams dosed on GD 6-21)</p> <p><b>Developmental Observed:</b> Increased incidence of uni- and bilateral 14th rib in offspring at <math>\geq 10</math> mg/kg-d (dams dosed GD 6-16), at <math>\geq 10</math> mg/kg-d (dams dosed GD10-13), and at <math>\geq 40</math> mg/kg-d (dams dosed GD 6-21) (related to dose and duration of exposure); increased sterna defects (i.e., asymmetrical apposition of sternebrae, hemisternebrae, and delayed ossification) at <math>\geq 40</math> mg/kg-d (dams dosed GD 6-21)</p>	Maternal: 60  Develop: NA	Maternal: 80  Develop: 10	6.12 (GD 6-16)	10.9 (GD 6-16)	Khera ( <a href="#">1974</a> )	Develop (GD6-16): [B1] Develop (GD10-13): [B2]

**Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per treated litter cross-fostered to control dams immediately after parturition, pups examined at PND17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Body weights and organ weights of pups on PND 17 or 21  <b>Observed: Decreased absolute brain weight at PND 17 and 21</b>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. ( <a href="#">1978</a> )	Develop: [C1]
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per treated litter cross-fostered to control dams immediately after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Mean HCB residue concentration in liver, brain, heart, kidney, and spleen; absolute and relative liver weight; liver enzyme activity towards thiophenyl, p-nitrophenyl, and indophenyl acetate (TPA, PNPA, IPA) hydrolysis  <b>Observed:</b> Significant difference in mean HCB levels in liver, brain, heart, kidney, and spleen in pups nursed by exposed dams compared to control dams. Liver esterase activity in control pups significantly lower than in pups nursed to control dams.	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. ( <a href="#">1977</a> )  (Increased HCB residue concentrations found in all examined tissues of gestationally exposed pups [liver, brain, heart, kidney, spleen] compared to control.)	Develop: [D1]

**Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Sprague-Dawley, 20 dams per dose, dosed prior to mating and through three matings (F1a, b and c); F1a, F1b, F1c offspring examined PND1	0, 0.5, 2.5, 12.5	<p><b>Measured:</b> Litters examined on PND 1 for number of pups, number of live and dead pups, number of male and female pups, average pup weight; anogenital distance; gross reproductive tract structure; surface righting (beginning PND1) external malformations; stomach milk and cleft palate; visceral and skeletal evaluation, blood and serum parameters; thyroid hormone determination</p> <p><b>F1 a, b, and F1c on PND1 Observed:</b> Mean anogenital distance increased by 8% in F1a males and by 4% in F1b females at 12.5 mg/kg-d; increased mortality in F1c pups at 12.5 mg/kg-d; average individual pup weight increased by 10% on PND 1 at 2.5 mg/kg-d; anogenital distance increased by 6% in females at 12.5 mg/kg-d; <b>increased incidence of skeletal malformations at 12.5 mg/kg-d for both F1a and F1b litters</b> (31.9% of pups showing variation compared to 8.3% of control for F1a and 25.6% compared to 6.3% for control for F1b); increased average pup weight in F1c males.</p>	Maternal: 5.0  Develop: 0.5	Maternal: 12.5  Develop: 2.5	DU	DU	<p>Wolfe and Pepperl (2005)</p> <p>(Biological significance of decreased number of females with normal cycles at 2.5 and 12.5 mg/kg-d questioned due to comparable female reproductive data between treatment and controls; absolute liver weight increased in dams at 2.5 mg/kg/d reported to be significant, but 9% is not considered to be biologically significant; statistical significance of skeletal malformations not reported)</p>	Develop: [E1]

Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational and Lactational Exposure</b>								
Rat, Sprague-Dawley, 20 dams per dose, dosed prior to mating and through three matings (F1a, b and c); F1c litters examined at various time points from PND 1 through PND 23.	0, 0.5, 2.5, 12.5	<p><b>Measured:</b> Dam body weight, food consumption clinical observations; full evaluation at necropsy. F1c litter examined on PND 1, 4, 7, 14, and 21 for number of pups, number of live and dead pups, number of male and female pups, total body weight, individual body weight; anogenital distance; gross reproductive tract structure; surface righting (beginning PND1); nipple retention and hypospadias (PND 12 and 13); vaginal thread and cleft clitoris (PND 22 or 23); startle response and functional observation battery (PND 22); immunological evaluation (PND 16); 6 control and 18 low-dose pups sacrificed PND 22-23 for full necropsy.</p> <p><b>F1c Observed:</b> Increased mortality in F1c pups until PND 9 (100% by PND9) at 12.5 mg/kg-d; pup weights decreased by 20% on PND 4 and decreased by ~40% on PND 7 at 12.5 mg/kg-d; <b>increased surface righting reflex and increased hindlimb grip strength (males only) at 2.5 mg/k-d; delayed day of prepuce separation in males at 2.5 mg/kg-d.</b></p>	Maternal: 5.0  Develop: 0.5	Maternal: 12.5  Develop: 2.5			Wolfe and Pepperl (2005)  (Results from the immunological evaluation were not reported.)	Develop: [E2]

Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<p>Rat, Sprague-Dawley, unreported number of dams, dams treated 2 weeks prior to breeding by gavage for 4 consecutive days, pups evaluated for neurotoxicity (PND9–120)</p> <p>Blood, fat, liver, brain, and kidneys collected on GD 9, 15, 20 and on PND 4, 7, 10, 14. Milk samples collected during lactation; Brain, blood, and liver tissues collected from 20-day old fetuses</p>	0, 2.5, 25	<p><b>Measured:</b> Overt signs of toxicity in dams and pups; behavioral assays in young pups (~9-16 days): negative geotaxis reflex response, olfactory discrimination/homing test, exploratory and locomotor activity. Behavioral assays in mature offspring: locomotor activity (~60 days), exploratory behavior (100 days), learning behavior (40 days), acoustic startle response (23 days and 120/150 days)</p> <p><b>Observed:</b> No overt toxicity in dams, signs of overt toxicity in pups (severe body weight loss and organ malformations) at 25 mg/kg-d; <b>faster responses in negative geotaxis and olfactory homing assays in offspring at 2.5 and 25 mg/kg-d</b>; increased exploratory behavior and hyperactivity in offspring at 25 mg/kg-day; decrease in amplitude of acoustic startle response of pups (25 mg/kg-day at 23 days; 2.5 and 25 mg/kg-d at 120 days)</p>	Maternal: 25  Develop: NA	Maternal: NA  Develop: 2.5	DU	DU	Taylor and Goldey ( <a href="#">1992</a> )	Develop: [F1]



Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Sprague-Dawley, 30 dams per group, treated 2 weeks prior to breeding by gavage for 4 consecutive days, litters culled to 8 pups/litter and evaluated at various intervals (PND1–120)	0, 2.5, 25	<p><b>Measured:</b> Length of gestation, number of live and dead pups at PND1; number of days to eye opening; maternal and pup body weight at PND 1, 3, 6, 9, 12, 14, and 28; absolute and relative brain weight in pups at PND 28; negative geotaxis reflex (PND 6, 8, and 10); olfactory discrimination (PND 9–11); exploratory behavior (PND15–20); acoustic startle reflex; reflex modification of ASR (PND 90); visual discrimination learning (PND 40); motor activity in mature offspring (PND 60)</p> <p><b>Observed:</b> No overt toxicity in dams, <b>faster responses in negative geotaxis and olfactory homing assays in offspring, at 2.5 and 25 mg/kg-d; increased exploratory activity on PND 19 and 20 at 2.5 and 25 mg/kg-d;</b> decrease in amplitude of acoustic startle response of pups (PND 23) at 25 mg/kg-day; <b>elevated mean startle amplitude (PND90) in males at 2.5 and 25 mg/kg-d</b> and in females only at the highest dose</p>	Maternal: 25  Develop: NA	Maternal: NA  Develop: 2.5	DU	DU	Goldey and Taylor ( <a href="#">1992</a> )	Develop: [G1]

Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Mouse, BALB/C, 2 dams per dose group, diet, GD 1-18, offspring weaned at PND 21, immunological assays began on PND 45	0, 0.5, 5.0	<p><b>Measured:</b> Overt signs of toxicity; delayed-type hypersensitivity (DTH) response; hemolytic plaque assay; mitogen-induced blastogenesis; mixed-lymphocyte response</p> <p><b>Observed:</b> No overt toxicity in dams, <b>DTH response to oxazolone severely depressed at 0.5 and 5.0 mg/kg-d in offspring</b>; decreased mixed lymphocyte response level at 5.0 mg/kg-day (ex vivo); increased distribution of splenic T cells at 5.0 mg/kg-day (low-dose group not analyzed); decreased distribution of splenic B cells at 5.0 mg/kg-day (low-dose group not analyzed)</p>	Maternal: 5.0  Develop: NA	Maternal: NA  Develop: 0.5	DU	DU	<p>Barnett et al. (<a href="#">1987</a>)</p> <p>(Authors conclude that HCB is capable of impacting development of immune response in mice at the T cell level.)</p>	Develop: [H1]
Mouse, CD-1, 3-7 dams per dose group, gavage, GD 6-17, dams sacrificed PND 1 or 21, pups sacrificed PND 1, 8, 10, 15, or 21.	0, 1, 10, 50	<p><b>Measured:</b> Lactic dehydrogenase (LDH) and creatine kinase (CK) activities and isozymes profiles in dams and pups; mortality</p> <p><b>Observed:</b> Decreased LDH-5 and increased LDH-3 in dams at 50 mg/kg-day on PND 1; <b>increased pup mortality at 50 mg/kg-day (71.7%) and 10 mg/kg-d (27.6%)</b>; Increased LDH-5 enzyme in pups at 50 mg/kg-day on PND 21</p>	Maternal: 10  Develop: 1	Maternal: 50  Develop: 10 (FEL)	DU	DU	<p>Courtney et al. (<a href="#">1984</a>)</p> <p>(Study was limited to an evaluation of cardiac enzymes.)</p>	Develop: [I1]

**Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Mink, <i>Mustela vison</i> , unreported number of dams, diet, approximately 1 month prior to breeding through gestation and lactation; 6 female kits/dose examined at 16-17 weeks of age	0, 0.2, 1.0 <sup>b</sup>	<b>Measured:</b> Kit weight; HCB analysis of blood, fat, muscle, kidney, liver, and brain; hepatic mixed-function oxidases; renal function (in vitro; p-aminohippurate and tetraethylammonium [TEA]); histological examination of liver and kidney  <b>Observed: Increased mortality in low- (44.10%) and high dose (77.4%) offspring (0.2 and 1.0 mg/kg-d);</b> elevated hepatic P-450 concentrations and ethoxyresorufin-O-deethylase at 1.0 mg/kg-d, and decreased TEA accumulation in kidneys at 1.0 mg/kg-d; liver cell cytoplasm occupied by ellipsoidal vesicular profiles	Maternal: NE  Develop: NA	Maternal: NE  Develop: 0.2 <sup>b</sup> (FEL)	DU	DU	Rush et al. ( <a href="#">1983</a> )  (No information on timing of mortality or cause precluding conclusions as to contributing exposure period. Study includes data on tissue distribution of HCB in offspring.)	Not included
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per treated litter remaining with treated mother after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Body weights and organ weights of pups on PND 17 or 21  <b>Observed: Decreased absolute brain weight at PND 17 and 21;</b> decreased absolute kidney weight at PND 17; decreased absolute spleen weight at PND 17; increased relative liver weight at PND 21	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. ( <a href="#">1978</a> )  (No significant difference in liver effects as compared to lactation-only or gestation-only exposure)	Develop: [C2]

**Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating PND 23, litters culled to 10 pups with 5 pups per treated litter remaining with treated mother after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<p><b>Measured:</b> Mean HCB residue concentration in liver, brain, heart, kidney, and spleen; absolute and relative liver weight, liver enzyme activity towards thiophenyl p-nitrophenyl, and indophenyl acetate (TPA, PNPA, IPA) hydrolysis</p> <p><b>Observed: Increased liver enzyme activity toward TPA, PNPA, and IPA hydrolysis, and increased relative liver weight on PND 17 and 21</b></p>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	<p>Mendoza et al. (<a href="#">1977</a>)</p> <p>(No significant difference in liver effects compared to lactationally exposed pups, but increased as compared to gestationally exposed pups. Increased HCB residue concentrations found in all examined tissues of the pups (liver, brain, heart, kidney, spleen) compared to control.)</p>	Develop: [D2]
Rat, Sprague-Dawley, 10 dams per dose, diet, 96 days prior to mating through gestation and lactation (2 successive litters raised); offspring (litters not culled) observed through weaning	0, 5.9, 7.8, 9.8, 11.8, 13.7 <sup>a</sup>	<p><b>Measured:</b> Pup mortality, maternal lung histology after second litter, clinical observations, maternal and pup body weight, litter size, pup brain and liver HCB residues</p> <p><b>Observed: Dose-dependent increase in mortality through weaning (11, 19, 30, 45, 94, and 92% for F1a and 11, 19, 22, 20, 45, 100, and 94% for F1b in the 0, 5.9, 7.8, 9.8, 11.8, 13.7 mg/k-d dose groups, respectively);</b> increased incidence of intra-alveolar foamy histiocytes and hypertrophy and proliferation of the lining endothelial cells in pulmonary venules in treated dams (dose not reported)</p>	Maternal: ND  Develop: NA	Maternal: ND <sup>c</sup>  Develop: 5.9 <sup>a</sup> (FEL)	DU	DU	<p>Kitchin et al. (<a href="#">1982</a>)</p> <p>(Authors report LD50 for day 21 cumulative mortality from birth = 100 ppm for F1a generation and 104 for F1b generation. Mortality incidence was reported slightly differently in abstract.)</p>	Develop: [K1]

Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Lactational Exposure</b>								
Monkey, Rhesus, 3 dams per dose, gavage; offspring 21, 61, or 118 days old at onset of exposure and exposed for 60, 38, or 22 days, respectively; 5 controls per age group	0, 64	<p><b>Measured:</b> <i>in situ</i> examination of CNS, salivary glands, mammary tissue, liver, stomach, duodenum, jejunum, ileum, colon, pancreas, adrenals, kidneys, urinary bladder, mesenteric lymph nodes, esophagus, trachea, lungs, heart, thyroid, parathyroid, uterus, eyes, ovaries, and tongue</p> <p><b>Observed: Mortality seen in offspring (66%) with onset of exposure at PND 61 or 118</b> (lung edema associated with mortality in both instances with bronchopneumonia reported in one animal, one infant was listless for 24 hours prior to death with engorgement of all central nervous system vessels and decreased body weight, the second infant showed lethargy, depression, and ataxia prior to death); microscopic changes included: mild centrilobular hepatocellular hypertrophy (n=1), diffuse fatty metamorphosis of liver (n=1), slight degree of proximal renal tubular vacuolation (n=2), mild gliosis in the cerebrum (n=1)</p>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 64 (FEL)	DU	DU	Latropoulos et al. ( <a href="#">1978</a> )  (Authors note that HCB serum concentrations of mothers and infants as well as HCB concentrations in the milk samples are reported in other studies and results indicate: (1) the amount of HCB infants received through milk was equal or greater than the amount given to mothers (2) HCB in the milk samples was very high (3) mean HCB in infant serum at sacrifice was higher than mothers' HCB serum.)	Develop: [L1]

**Table 3-4 (Continued): Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per control litter cross-fostered to treated dams immediately after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Body weights and organ weights of pups on PND 17 or 21 <b>Observed: Increased absolute and relative liver weight at PND 17 and 21;</b> decreased absolute kidney weight at PND 17	Maternal: NE Develop: NA	Maternal: NE Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. (1978) (Significantly increased absolute and relative liver weights compared to pups in gestation-only exposure group at PND 17 and 21.)	Develop: [C3]
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per control litter cross-fostered to treated dams immediately after parturition, pups examined at PND 17 and 21.	0, 6.7 <sup>a</sup>	<b>Measured:</b> Mean HCB residue concentration in liver, brain, heart, kidney, and spleen; absolute and relative liver weight, liver enzyme activity towards TPA, PNPA, and IPA hydrolysis <b>Observed: Increased liver enzyme activity toward TPA, PNPA, and IPA hydrolysis, and increased relative liver weight on PND 17 and 21</b>	Maternal: NE Develop: NA	Maternal: NE Develop: 6.7 <sup>a</sup>			Mendoza et al. (1977) (Significantly increased absolute and relative liver weights, and liver enzyme activity compared to gestationally exposed pups at PND 17 and 21. More than 10 fold increase in organ HCB residue compared to gestationally exposed pups.)	Develop: [D3]

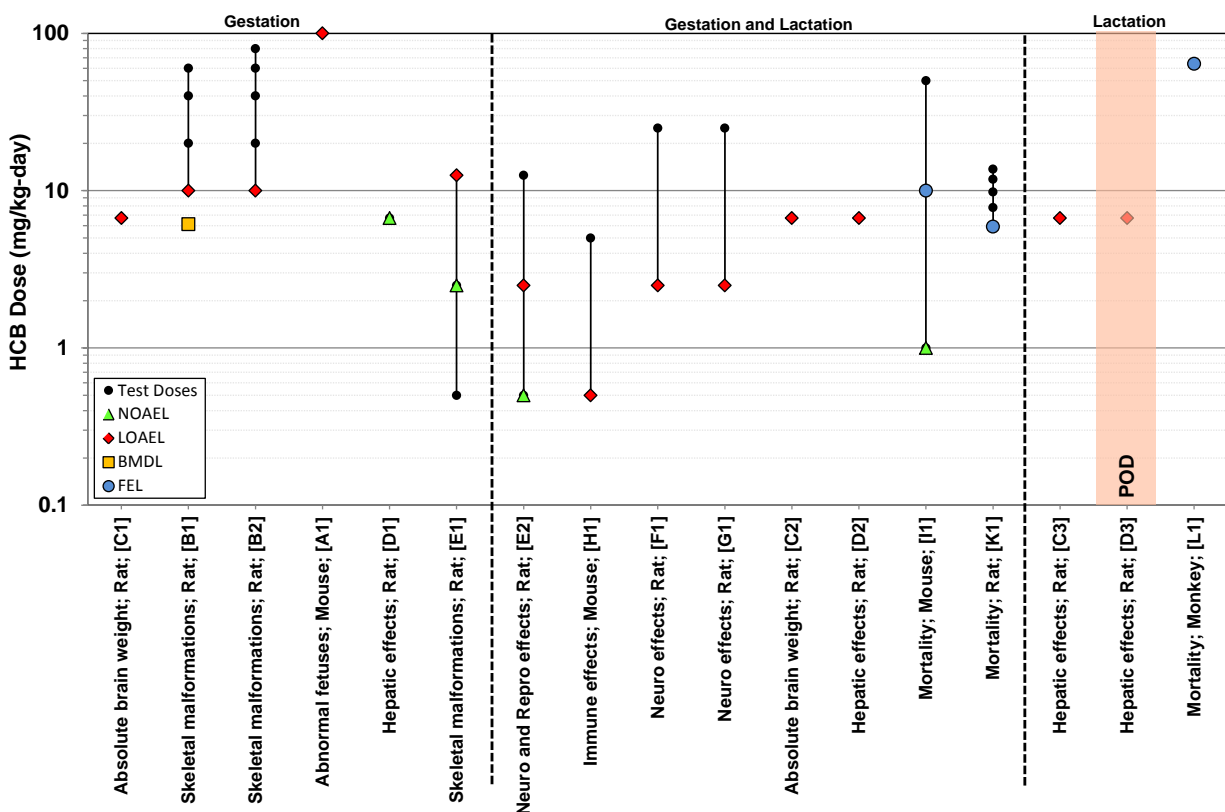
<sup>a</sup> Doses estimated using recommended body weight and food intake values (U.S. EPA, 1988).

<sup>b</sup> Doses estimated using mink body weight and food intake values reported in Aroclor 1016 IRIS, due to lack of information on mink dosimetry (U.S. EPA, 1988): <http://www.epa.gov/iris/subst/0462.htm>.

<sup>c</sup> Authors report maternal effects, however the significance of these findings (and the lowest dose at which findings are significant) are not reported.

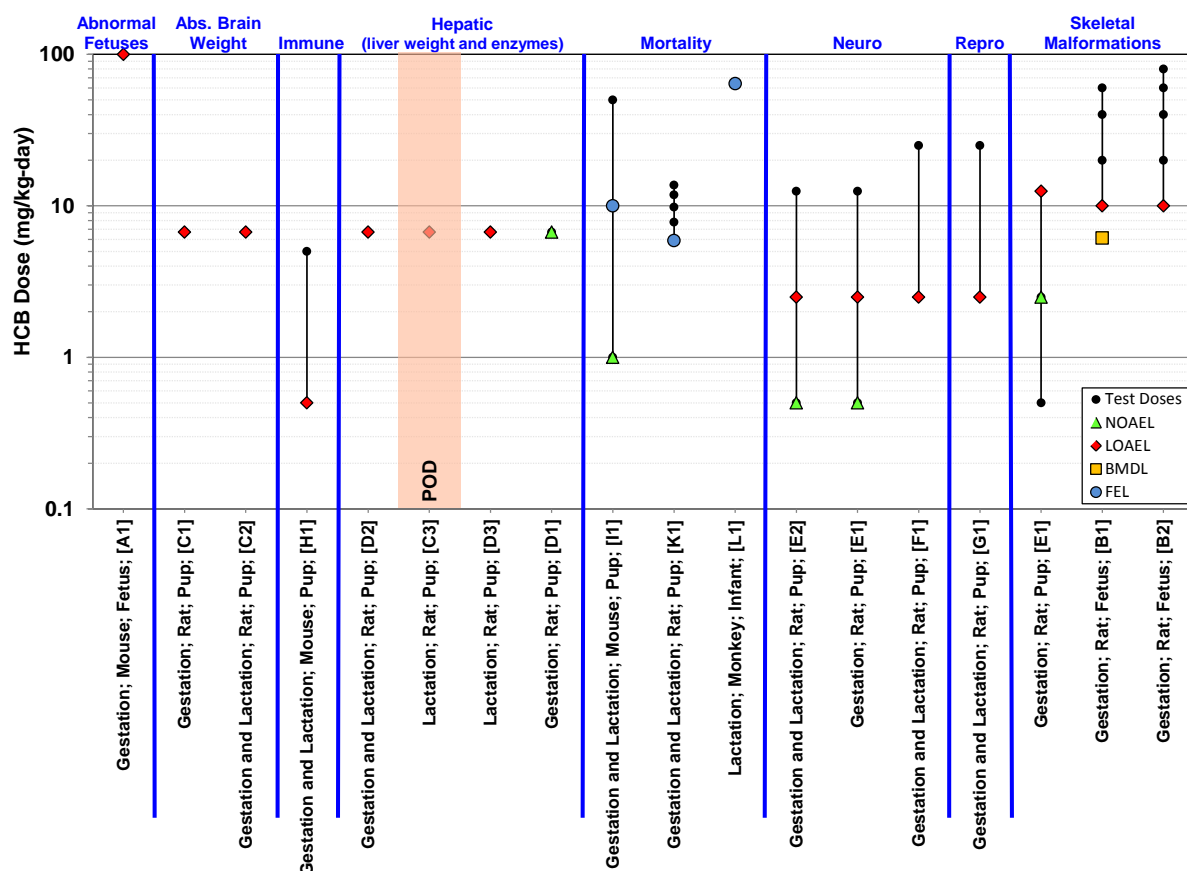
DU = data unsuitable; NA = not applicable; ND = no data; NE = not examined; BMD = benchmark dose; BMDL = the 95% lower bound confidence limit on the BMD; LOAEL = lowest observed adverse effect level; NOAEL = no observed adverse effect level; FEL = frank effect level

### 3.4.3. Exposure-Response Arrays for Developmental Effects



Note: Each array element represents the dose-response data for a unique study design. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. All endpoints pertaining to relative liver weight and liver enzymes are labeled as “Hepatic effects”. “Repro effects” refers to delayed day of prepuce. All endpoints pertaining to negative geotaxis, olfactory homing, surface righting reflex, and hindlimb grip strength are labeled as “Neuro effects”. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table 3-4](#). The study by Rush et al. (1983) is not included in the array due to poor reporting. NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; BMDL = the 95% lower bound confidence limit on the BMD; FEL = frank effect level

**Figure 3-1. Exposure-Response Array Showing All Developmental Effects, Sorted by Exposure Route, in Animals after Exposure to HCB.**



Note: Each array element represents the dose-response data for a unique study design or endpoint. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. “Immune” refers to DTH response to oxazolone. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. All endpoints pertaining to negative geotaxis, olfactory homing, surface righting reflex, and hindlimb grip strength are labeled as “Neuro”. “Repro effects” refers to delayed day of prepuce. “Skeletal Malformations” refers to general category (including incomplete ossification, shortened supernumerary rib, or other), or uni- and bilateral 14th rib. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table 3-4](#). The study by Rush et al. ([1983](#)) is not included in the array due to poor reporting. NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; BMDL = the 95% lower bound confidence limit on the BMD; FEL = frank effect level

**Figure 3-2. Exposure-Response Array Showing All Developmental Effects, Sorted by Effect Type, in Animals after Exposure to HCB.**

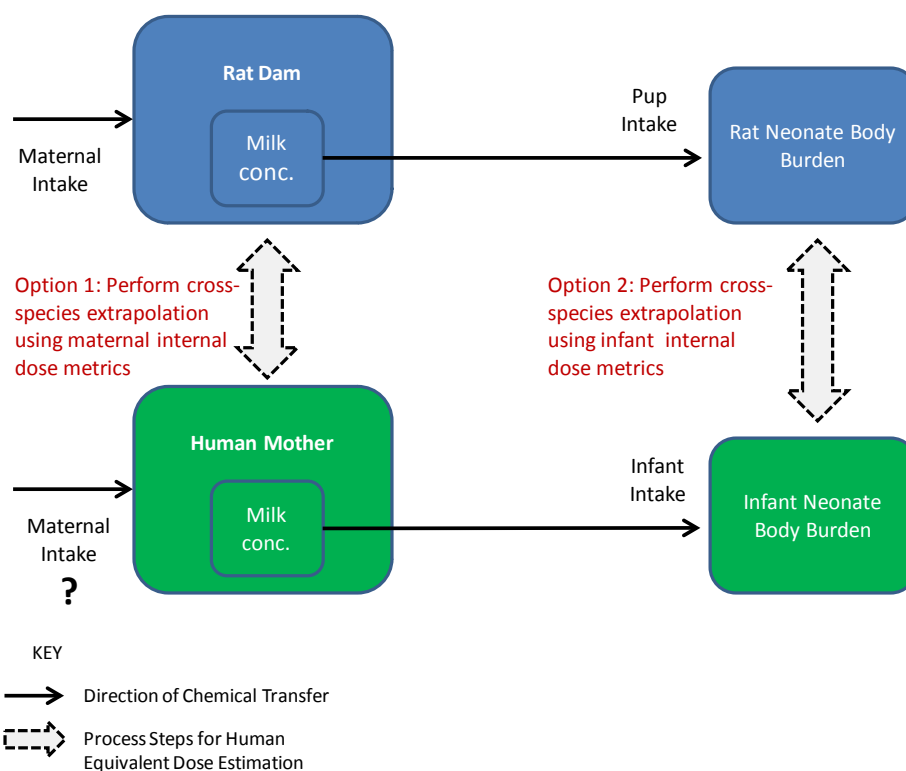


### 3.5. Human Equivalent Dose (HED) Estimation

Once the POD has been selected, the next step is estimation of the HED. This HED is intended to quantify the maternal continuous daily exposure in humans that may result in an adverse developmental effect similar to that observed in animal offspring as described in [Section 3.4.2](#).

The information provided in this section is designed to promote discussion amongst the workshop attendees as to how best to perform the HED estimation. To provide an example to facilitate discussion, one method is chosen and carried through in this document for demonstration purposes only. The HED can be estimated in a number of different ways. Generally speaking, an internal dose metric is selected for the human and the animal, and then these two metrics are assumed to result in the same effect. The decision must be made as to which internal dose metric is appropriate to serve as an indicator of the associated adverse effect.

For developmental effects resulting from lactational exposure, [Figure 3-3](#) provides an example of the pathways that may be chosen to perform cross-species extrapolation to estimate the HED. The assumption is made that the maternal or infant animal intake is known, and the HED is the unknown human continuous daily exposure (depicted with a “?” in the figure). Because exposure occurs through the mother and then passes to the offspring by nursing, cross-species extrapolation may be performed by selecting either an internal maternal dose metric or an internal infant dose metric. Performing the extrapolation on internal maternal dose metrics will not account for species differences regarding infant feeding habits and infant kinetics, while performing the extrapolation on the infants requires more input data and more judgment about how to appropriately link very young members of both species. The overall choice will depend on judgment as to the reliability of the models available and the input data driving the models, balanced against the desire for increased biological accuracy.



**Figure 3-3. Process Diagram for Performing Cross-Species Extrapolation**

The main questions considered in derivation of an HED from an animal-derived POD are shown in the text box below (“Discussion Points for HCB HED Estimation”). This section discusses some of the chemical-specific properties that affect (1) the selection of the modeling method, (2) the kinetic information available for HCB, and (3) the types of models available for assessing HCB exposure in animals and humans. Summary statements for each of the discussion points are provided in the appropriate sections.

#### Discussion Points for HCB HED Estimation

- ? Is HCB metabolized? How should metabolism be addressed in the HED estimation?
- ? What pharmacokinetic data or relevant chemical-specific information are available for HCB in animals and humans to facilitate model implementation?
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ? What PBPK or other simpler biokinetic models are available for HCB?
- ? What is the best method for estimating the HED using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

### 3.5.1. Chemical-Specific Properties Affecting HED Estimation

#### HCB Metabolism

- ? Is HCB metabolized? How should metabolism be addressed in the HED estimation?
- ✓ HCB is metabolized at a very slow rate.

HCB is slowly metabolized in animals and humans. The main metabolite in both animals and humans is pentachlorothiophenol (PCP) ([Renner, 1988](#); [Ingebrigtsen et al., 1986](#)), with other metabolites including chlorinated benzenes, chlorophenols, and benzenes ([Koss et al., 1986](#)). In a study that dosed rhesus monkeys with radiolabeled HCB in the diet for 15 months, the recovered radioactivity in the feces indicated 99% of the excreted HCB was eliminated as parent compound with only 1% consisting of the different metabolites. It is expected that HCB toxicity is at least in part due to the parent compound. For these reasons, accounting for metabolism in the estimation of the HED may not be of high importance. However, if the human equivalent dose is estimated assuming long-term exposure, excluding metabolism will tend to overestimate the human HCB tissue concentrations associated with a given dose.

### **Workshop Notes: Approaches**

Metabolism is not a key issue for HCB. However, it is important for some PBT chemicals, such as DDT ([Appendix B.2](#)). Workshop participants identified issues that may be complicated by PBT chemical metabolism.

If metabolism of the chemical differs between animals and humans, selection of a POD from an animal study may be complicated because animal offspring may be exposed to different metabolites or to different proportions of parent compound and metabolite(s) compared to human offspring.

In order to use modeling approaches to estimate offspring exposure in animals and humans, half-life estimates are needed for the parent compound and for metabolite(s) in all relevant species.

Essentially, maternal metabolism may result in offspring exposure to a mixture of PBT chemicals (i.e., parent compound together with any persistent metabolite(s)). Some PBT chemicals, such as chlordane ([Appendix B.1](#)), enter the environment as mixtures. Human mothers are exposed to multiple components of the mixture, and the mixture components may be subject to maternal metabolism, increasing the complexity of the mixture to which offspring are exposed. Thus, risk assessment of PBT chemical mixtures can be complicated by many of the same issues encountered with PBT chemical metabolism.

These issues are discussed in depth in [Section 4](#).

### **3.5.2. Available Pharmacokinetic Data for HCB**

Before discussing the potential modeling techniques for HCB, the available database of pharmacokinetic data is summarized. This database should help to determine which modeling techniques may be most appropriate and subject to the least uncertainty. Potential parameters that may be useful in estimating HED by a variety of methods are presented in [Table 3-5](#). A discussion of the parameters and their sources follows the table. The various applications and uses of these parameters are discussed in the context of different modeling techniques in [Section 3.5.4](#).

### **Pharmacokinetic Data**

- ? What pharmacokinetic data or relevant chemical-specific information are available for HCB for both animals and humans to facilitate model implementation?
- ✓  $K_{ow}$
- ✓ Human and rat half-lives
- ✓ Absorption fractions and fraction of chemical stored in fat in humans (assumptions can be made based on other PBT chemicals)
- × No fraction of chemical stored in fat for rats
- ✓ Partition coefficients from PBPK models for rats and humans

### **Workshop Notes: Approaches**

If Barnett et al. ([1987](#)) were used as the POD study, as suggested by the workshop participants, then pharmacokinetic parameters for mice, as opposed to rats, would be needed.

**Table 3-5. Available Pharmacokinetic Parameters for HCB in Humans and Rats**

Parameter (units)	Variable	Value	Note/source
<b>Chemical-Specific Only</b>			
Octanol-water partition coefficient	$\log(K_{ow})$	5.73	Hansch et al. ( <a href="#">1995a</a> )
<b>Human Chemical Parameters</b>			
Fraction of ingested chemical that is absorbed by the infant (dimensionless)	$f_{ai}$	1.00	Assumed
Fraction of ingested chemical that is absorbed by the mother (dimensionless)	$f_{am}$	Variable; 85.4% at zero blood concentration and decreasing for increasing blood concentration	Schlummer et al. ( <a href="#">1998</a> )
Fraction of chemical that is stored in maternal fat (dimensionless)	$f_f$	0.9	Assumed ( <a href="#">U.S. EPA, 1998</a> )
Biological elimination constant for HCB ( $\text{day}^{-1}$ )	$k_{elim}$	0.00322	Estimated as $\ln(2) / t_{1/2}$
Half-life in humans HCB (days)	$t_{1/2}$	215	Yesair et al. ( <a href="#">1986</a> )
Partition coefficients- in different human body compartments for HCB	Rate constants found in Yesair et al. ( <a href="#">1986</a> ); estimated for rats and applied to humans; can also be estimated using $K_{ow}$		
Metabolic rates for HCB	None identified in the literature		
Parameter (units)	Variable	Value	Note/source
<b>Animal Chemical Parameters</b>			
Fraction of ingested chemical that is absorbed by the rat pup (dimensionless)	$f_{ai}$	0.8	Assumed
Fraction of ingested chemical that is absorbed by the rat dam (dimensionless)	$f_{am}$	0.8	Koss and Koransky ( <a href="#">1975</a> )
Fraction of chemical that is stored in rat dam/maternal fat (dimensionless)	$f_f$	0.9	Assumed
Biological elimination constant for chemical ( $\text{day}^{-1}$ )	$k_{elim}$	0.087	Estimated as $\ln(2) / t_{1/2}$
Half-life of chemical in rats (days)	$t_{1/2}$	8*	Koss et al. ( <a href="#">1983</a> )
Partition coefficients in different rat compartments for the HCB	Partition coefficients found in Yesair et al. ( <a href="#">1986</a> ) and Lu et al. ( <a href="#">2006</a> )		
Metabolic rates for HCB	None identified in the literature		

\*The reasoning and process behind the half-life selection for HCB is discussed in detail toward the end of [Section 3.5.2](#).

Hansch et al. ([1995a](#)) estimated a log octanol-water partition coefficient (**log  $K_{ow}$** ) for HCB of 5.73.

In the absence of specific information on HCB in humans, the input values presented here were patterned after those in the U.S. EPA's *Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions* ([U.S. EPA, 1998](#)). Chapter 9 of this methodology (hereafter "Combustor Assessment") presents a framework for evaluating exposure to persistent lipophilic chemicals in breast milk. Thus, the assumptions made in that document are applied to HCB when values specific to HCB could not be located.

#### **Workshop Notes: Data and Data Gaps – Human Data**

Workshop participants supported the use of a log(Kow) of 5.73 for HCB. However, log(Kow) is a critical parameter affecting the output of the simpler modeling approaches. And, for other chemicals, there was some uncertainty surrounding this parameter. For example, workshop participants identified several log(Kow) values for mirex in the literature: NTP ([2011a](#)) gives a value of 5.28, McKim et al. ([1985](#)) report a value of 7.5, and Veith et al. ([1979](#)) provide a value of 6.89.

Workshop participants identified the fraction of HCB stored in fat in both humans and rats as a data gap.

- 0.9 was used because it represents a health protective assumption.
- It was suggested that it might be possible to get a rough estimate of the value for rats from Nakashima et al. ([1997](#)).

Workshop participants strongly disagreed with the selected half-life value for HCB. Half-life is a critical parameter affecting the output of the simpler modeling approaches.

- Human epidemiology studies have observed that HCB levels in children at 7 years of age continue to be influenced by the length of time a child was breastfed ([Karmaus et al., 2001](#)). This suggests that the half-life is longer than 215 days.
- The half-life estimated by Yesair et al. ([1986](#)) may be based on the first phase of HCB elimination, as opposed to the slower second phase of elimination from non-blood (i.e., fat) compartments. This two phase process has been described for other PBT chemicals.
- Workshop participants suggested that the HCB half-life of 6 years estimated by To-Figueras et al. ([2000](#)) be used instead.

Human partition coefficients are not always available for PBT chemicals, and this was identified as a data gap for HCB and mirex. For HCB, the coefficients were found for rats and applied to humans. In the absence of experimental animal data, human partition coefficients may be estimated using log K<sub>ow</sub>.

Uncertainty in the half-life estimates and parameters used for modeling were major issues raised across chemicals. See [Section 4.3](#) for a detailed discussion of these topics.

**Workshop Notes: Data and Data Gaps – Human Data**

Workshop participants observed that data are often unavailable to support infant-specific parameters for modeling purposes, including absorption fraction. In those instances, participants suggested using conservative assumptions, such as 1 in the case of the absorption fraction, to bridge these knowledge gaps until data become available.

Uncertainty in the parameters used for modeling was a major issue raised across chemicals. See [Section 4](#) for a detailed discussion of this topic.

Schlummer et al. ([1998](#)) estimated the **gastrointestinal absorption fraction** for HCB in seven humans ranging in age from 24 to 81 years old. In the younger subjects, absorption was 70-82%; however, absorption decreased in the older subjects fed the same meal, with 1% in a 53 year old and net excretion (rather than absorption) in 76 and 81 year old subjects. In addition, the blood concentrations also varied by age, with increasing blood levels with age. Thus, the authors determined a regression equation that relates absorption to the blood concentration. The relationship predicts 85.4% absorption when the blood concentration is zero and decreases 0.2% for each ng/g lipid of HCB in the blood. For the purposes of this briefing packet, women of child bearing age are expected to be age 21 to 50, although most mothers are expected to be in the lower age range and expected to have the higher absorption reported by Schlummer et al. ([1998](#)). Infants were assumed to absorb 100% of the dose in the absence of specific information.

Quantitative information about the **fraction of HCB in the body that is stored in fat in humans** could not be found in the literature. The reviewed literature generally described HCB as highly lipophilic. The Combustor Assessment states that for highly lipophilic compounds, >90% of the compound may be stored in the fat ([U.S. EPA, 1998](#)). In the absence of more specific information, this parameter was set to 0.9 in the example calculations.

No direct measures of **HCB half-life in humans** were available in the literature. However, Yang et al. ([1978](#)) estimated half-lives of 91 and 114 days in two monkeys. PBPK modeling by Yesair et al. ([1986](#)) estimated human female half-lives of 215 days when exposed to HCB for 15 weeks at 15 years of age. For this briefing packet, the PBPK estimate of 215 days is used.

For **partition coefficients in humans**, no partition coefficients estimated from human empirical data are available. Yesair et al. ([1986](#)) estimated coefficients in rats using empirical data in the development of a PBPK model and then applied the same coefficients to humans. Coefficients were estimated for the brain, systemic circulation, kidney, liver, intestinal lumen, richly perfused tissue, poorly perfused tissue, breast tissue, and the offspring compartments. Partition coefficients can also be estimated based on  $K_{ow}$  ([Poulin and Krishnan, 1996](#)); and, for highly lipophilic compounds, partition coefficients can be assumed to be equal to the ratio of lipids between compartments ([Haddad et al., 2000b](#)).



### **Workshop Notes: Data and Data Gaps – Animal Data**

Partition coefficients for the laboratory species of interest are not always available for PBT chemicals, and this was identified as a data gap for DDT.

Uncertainty in the parameters used for modeling was a major issue raised across chemicals. See [Section 4](#) for a detailed discussion of this topic.

Animal parameters were determined from values found in the literature where available. The search focused on rats, since rats are the species studied in the candidate POD study ([Mendoza et al., 1977](#)). Studies on **HCB absorption in rats** revealed that absorption fractions depend on whether the HCB was administered in oil or in aqueous solution. Koss and Koransky ([1975](#)) found absorption fractions of 0.8 when HCB was administered to rats in oil while absorption from aqueous solutions ranged from 2-5%. In this briefing packet, an absorption rate of 80% is assumed for the dam. In the absence of specific information, an absorption rate equal to the maternal rate (0.8) is assumed for the pups.

No estimate could be found in the literature for the fraction of absorbed HCB deposited in the body lipids of rats (**fraction deposited in fat for rats**). Human studies found a concentration 60 times higher of radioactive HCB in fat compared to blood and 30 times higher in fat than in the liver or the brain, indicating preferential transport to and storage in the fat ([Ingebrigtsen and Nafstad, 1983](#)). In the absence of specific information, the same fraction assumed for humans (90%) was assumed for rats.

**HCB half-life values in rats** tend to increase in time. When rats were dosed every other day for six weeks, the half-life was estimated to be 8 days right after exposure ended, 10 weeks after three months, and 12 months after 1.5 years ([Koss et al., 1983](#)). In the POD study selected in this briefing packet ([Mendoza et al., 1977](#)), the dams were dosed for a total of eight weeks (i.e., two weeks prior to mating, three weeks during gestation, and three weeks during lactation). Dosing did not proceed after weaning. Thus, the 8 day half-life was selected for the half-life in this briefing packet since it represents a half-life following exposure.

**Partition coefficients for rats** were estimated in the study by Lu et al. ([2006](#)) as discussed in [Section 3.5.4.1](#). Coefficients are also calculated from empirical data in Yesair et al. ([1986](#)).

### **3.5.3. Appropriate Dose Metric for Selected Point of Departure (POD)**

#### **Dose Metric**

- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ✓ Peak or average concentrations from maternal or infant dose metrics
- ✗ The critical window could not be defined

Adverse developmental effects may be associated with HCB exposure during a critical period of development. Metrics may be selected to look at either peak or average maternal or infant exposure, depending on the effect in question and the dosing protocol in the animal study. For developmental effects, if a critical window can be determined, then the model solutions during the critical window may be used for the cross-species extrapolation; otherwise, peak or average concentrations from different exposure metrics (see below) may be used based on the judgment of the assessor as to what is most appropriate. For the proposed POD presented here, no critical window could be determined. Possible dose metrics for specified lactation durations include:

- The average maternal body burden,
- The peak maternal body burden,
- The average maternal fat concentration,
- The peak maternal fat concentration,
- The average milk concentration,
- The peak milk concentration,
- The average infant body burden, and
- The peak infant body burden.

#### 3.5.4. Available Models for Estimating HCB Internal Dose

##### Available Models

- ? What PBPK or other simpler biokinetic models are available for HCB?
- × Data-validated human and rat PBPK models are available that include lactation
- ✓ Simple first order models are available
- ✓ Simple biotransfer methods are available

##### 3.5.4.1. Multi-Compartment PBPK Models

The following section discusses potential models that may be used to estimate internal dose metrics based on maternal intake. The section starts with the most biologically-robust class of models, physiologically-based pharmacokinetic (PBPK) models. The section then discusses simpler first-order kinetic modeling techniques and a biotransfer method for estimating maternal exposure. Thus, the section generally progresses from the most biologically robust to the more simple models. The final choice in technique depends on weighing both the biological sophistication of the method and the uncertainty in the model parameters, as discussed in [Section 3.6](#).

Generally speaking, the most biologically complete kinetic model for a given chemical is a multi-compartment, data-validated, PBPK model. Validated models exist for HCB in rats (the species in the

candidate POD study) and humans. The models are discussed below, with special attention given to their ability to address lactational exposure.

#### **Workshop Notes: Approaches**

As discussed in [Section 6](#), workshop participants agreed that validated PBPK modeling approaches should be used whenever they are available.

- Starting with the POD from the animal study, use a validated PBPK model for the chemical in that species to predict the average or peak blood concentrations in animal offspring
- If the POD study includes data on offspring body burden, then these data can be used directly rather than applying a model.
- Use this dose metric for cross-species extrapolation to a human infant.
- Estimate the maternal dose using a validated PBPK model for the chemical in humans.

#### **CONSIDERATIONS:**

- PBPK models can account for fat mobilization in the mother after parturition.
- PBPK models allow for the consideration of the whole "perinatal" period rather than separating prenatal and postnatal exposures.
- PBPK models are more robust than simpler models, which often rely heavily on measurements of log(Kow) or half-life.
- Unlike first-order models, PBPK models can account for variations in metabolism or in half-life, such as those connected to increases in chemical dose or body burden.

However, when validated models are not available for a given chemical in the relevant species, then there are alternative approaches that can be used in the interim until the appropriate models are developed and validated. See [Section 6](#) for a detailed discussion of the available approaches.

### 3.5.4.2. Rat Models

#### **Workshop Notes: Data Gaps**

Validated PBPK models are available to describe the kinetic profile of HCB in rats. One of these models even contains descriptions for lactation and the nursing pup. However, such models are often unavailable in relevant laboratory species for other PBT chemicals, including mirex.

Workshop participants suggested that these models should be developed and used for all PBT chemicals. Even if some of the parameters required for model development are unknown, the process of working to develop the model would help to identify data gaps.

The following are examples of the types of data needed to develop models describing kinetics during lactation and in the nursing offspring:

- growth data for animal offspring
- data on offspring lipid composition as a function of growth
- data on offspring feeding patterns
- partitioning of chemical from blood into fat, milk, muscle, and liver compartments
- metabolism constants

Two PBPK models for HCB are discussed in this section. The first model by Yesair et al. ([1986](#)) simulates the distribution and excretion of HCB following oral exposure. The model describes gestation and lactation, as well as the nursing pup. Other compartments include intestinal lumen, blood, feces, liver, metabolites, kidney, urine, brain, richly perfused tissues, and poorly perfused tissues. Metabolism was included in the liver compartment by simulating first-order transfer from the liver compartment to the metabolite compartment. The rate constants for metabolism were estimated from the literature, although the paper does not specify the sources. Metabolites were then eliminated via the feces and urine.

The model was validated using rat data from several studies, including Koss and Koransky ([1975](#)), Koss et al. ([1978](#)), and Courtney et al. ([1979](#)). The model was judged by the authors to agree well with the experimental data in multiple compartments. In addition, data from Kitchin et al. ([1982](#)) were used to compare the lactational transfer in the model to experimental data, and the model results were found to compare well. The model code is not provided and was not requested from the authors. In addition, the model equations are not provided in the study. Thus, implementation of the model would include both coding and testing the model against results in the paper.

The second model is by Lu et al. ([2006](#)). This study incorporated two additional pharmacokinetic enhancements for HCB in rats. Based on findings from Yang et al. ([1975](#)), HCB binding with erythrocytes and the subsequent effects on sequestration and elimination were included in the model. In addition, passive diffusion from the blood compartment to the gastrointestinal lumen was modeled. These enhancements were judged by the authors to improve the performance of the model in estimating

concentrations shortly (i.e., hours) after exposure. However, this model does not include descriptions for lactation and the nursing pup, so these descriptions would have to be added if this model were implemented here. The model code is provided as supplemental material to the paper.

### 3.5.4.3. Human Models

After developing and validating the Yesair et al. (1986) rat model discussed in Section 3.5.4, the model was applied by the study authors to humans by changing the physiological parameters to reflect human values. However, the model was not validated against available human data.

#### **Workshop Notes: Approaches**

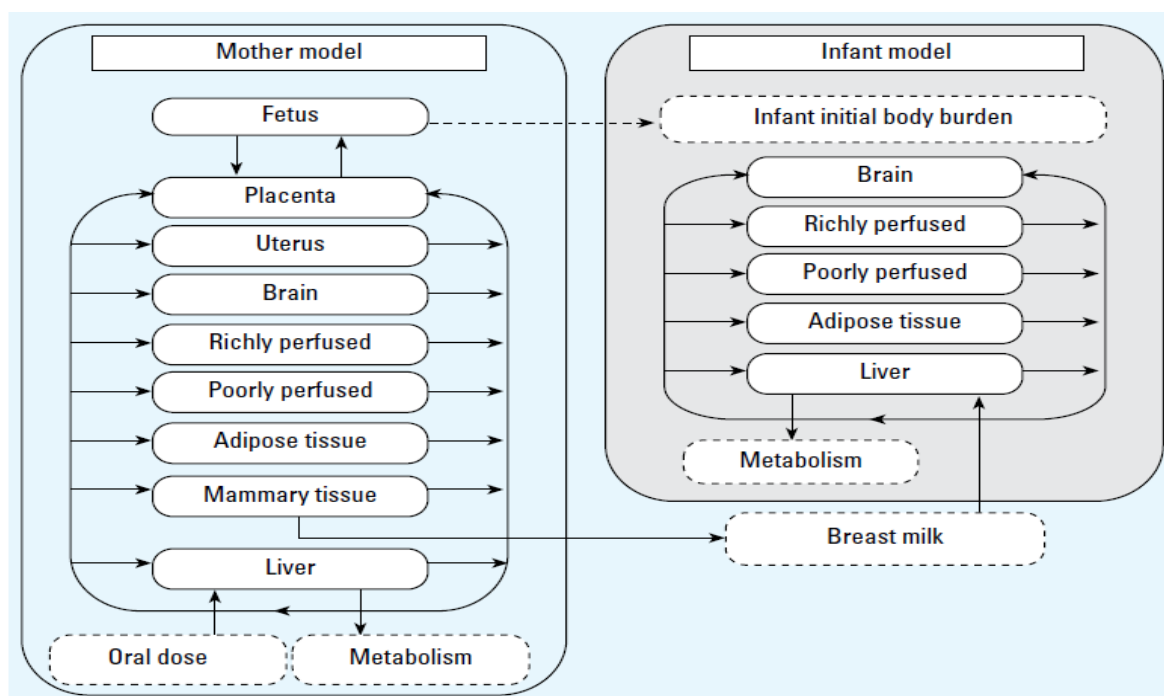
Workshop participants suggested the use of the model by Verner et al. (2009) when a validated chemical-specific PBPK model is not available for humans. Also, a new version of the model was recently released (Verner et al., 2013).

The model has been validated for HCB. Concentrations of HCB in the infant were simulated after a year of exposure. Serum, blood and breast milk HCB concentrations were compared, and the model could predict 74% variability.

The model has also been validated for p,p'-DDT and p,p'-DDE based on blood measurements in children enrolled in two longitudinal birth cohorts. When maternal blood levels were used to estimate daily intake in mothers and subsequently simulate children's blood levels in the two cohorts, the model could explain 47% and 57% of p,p'-DDT, and 48% and 83% of p,p'-DDE levels measured in children of ~6 months of age.

A generic maternal-infant multi-compartment model for persistent organic pollutants (POPs) has been developed (Verner et al., 2009; Verner et al., 2008). The primary utility of this model for HCB is that the chemical-specific parameters can be estimated using only the half-life and octanol-water partition coefficient ( $K_{ow}$ ) of the compound.

The full maternal and infant model (Verner et al., 2009) is intended to simulate the entire life-cycle of a woman up to age 55 years and includes nine maternal tissue compartments and five infant tissue compartments, as shown in Figure 3-4. The oral dose is modeled as being directly absorbed into the liver and assumed to be fully bioavailable. First-order hepatic metabolism is included and is intended to represent all excretion in the absence of breastfeeding or childbirth. The elimination rate constant is determined for the chemical from the adult whole-body half-life. The concentrations in each compartment are determined using partition coefficients, and the chemical-specific partition coefficients are estimated using an equation based on  $K_{ow}$  (Poulin and Krishnan, 1996) and using the fraction of blood and tissue that are lipid and water (Price et al., 2003). Physiological parameters such as body weight and fat volume fraction are assumed to vary in time to capture changes over the life of the woman.



**Figure 3-4. Maternal and Infant POP Model (Verner et al., 2009)**

The model published by Verner et al. (2009) has been validated, specifically for infants, against human cord blood, breast milk, and infant blood concentrations for seven POPs ( $p,p'$ -DDE,  $p,p'$ -DDT, HCB,  $\beta$ -HCH, PCB-138, PCB-153, and PCB-180). The computer code was not provided for this model.

The more recent toxicokinetic model published by Verner et al. (2013) was evaluated more extensively than the 2009 model and included the model code in Supplemental Material to the manuscript. Whereas model accuracy was only evaluated for predictions of 6-month-old children's blood POP levels based on maternal blood levels, the two-compartment model predictions were evaluated against blood levels at 6, 16 and 45 months in children from two different cohorts (Inuits and Slovaks) based on either maternal blood, cord blood or breast milk levels. Where the same data were used to evaluate both models, they yielded similar results.

**Workshop Notes in Focus: Summary of Verner Publications**

Verner et al. ([2009](#))

- The infant model must consider growth of infant
- If the daily intake of mother is not known, it can be estimated based on mother's blood concentration assuming steady-state conditions.
- The concentration in milk can be estimated based on the milk:blood partition coefficient (Pmb).
- The Pmb should equal the ratio of neutral lipid equivalent between both matrices ([Haddad et al., 2000a](#)).
- Infant daily ingested milk volumes are known and therefore the daily infant dose can be estimated.
- The tissue dosimetry is determined with the infant PBPK model.

Verner et al. ([2013](#))

- The approach is essentially the same but the PBPK model is reduced to model of two compartments representing the maternal and fetal/child total body lipids in function of age.
- The infant dose is estimated based on the milk lipid concentration and daily volume transferred to infant per BW.

**ASSUMPTIONS:**

- It is assumed that all of the chemical is stored in neutral lipid. For many PBT chemicals, this is a sound assumption. However, if there are data to suggest that some of the chemical partitions into aqueous compartments, then relevant toxicokinetic processes should be included.
- For both approaches, infant body burden at birth is estimated based on maternal blood concentrations on the day of delivery. The blood concentration in infant and mother are assumed to be equal (on a neutral lipid basis). In this way, gestational exposure to the infant is captured. If data for a given chemical indicate that infant or cord blood concentrations at birth are more or less than maternal blood concentrations at that time, then relevant toxicokinetic processes should be included.

### **Workshop Notes: Approaches**

Workshop participants stated that the two-compartment model may be sufficient in the absence of a validated PBPK model for a given chemical in humans or the relevant laboratory species.

- Starting from the POD, use this model to estimate body burden of the offspring in the animal study.
- If the POD study includes data on offspring body burden, then these data can be used directly rather than applying a model.
- Attribute the resulting body weight-adjusted body burden to a human infant after 12 months of breastfeeding.
- Using human exposure factors and the same model, back-calculate human maternal average daily intake.

#### **CONSIDERATIONS:**

- Appropriate for very lipophilic chemicals
- Most appropriate when there is no metabolism or metabolism is relatively slow (i.e., when metabolites are known to be of no concern)
- The model is easy to use and is reasonably accurate with a slight bias toward conservatism, which favors protectiveness of human health.
- Model predictions are very sensitive to the half-life. If the half-life value is uncertain, there will be a great deal of uncertainty in the model output (e.g., milk concentrations and estimated infant dose).

### **3.5.5. First-Order Single-Compartment Model ([U.S. EPA, 1998](#))**

A first-order single-compartment model is an intermediate step between a full, multi-compartment PBPK model and simpler dosimetric conversions or biotransfer models. The model presented here is patterned after the first-order models presented in the Combustor Assessment ([U.S. EPA, 1998](#)) and the dioxin reassessment ([U.S. EPA, 2012b](#)) and is adapted to fully incorporate time-dependence during lactation. The general model equations are presented first, and then the model is applied to both humans and rats in the next section. Rats are the focus here since that is the species in the candidate POD study. Because the equations are readily available, they are presented in greater detail than the equations in the PBPK models discussed in [Section 3.5.4.1](#). However, this is not meant to imply that the first-order models are superior for HCB.

The single body compartment represented by the model is generally defined as “body burden,” or the total average concentration of chemical in the body. In a first-order model, PBT chemical elimination from the body is represented as a rate constant multiplied by the total body burden. This rate constant is estimated from the whole-body elimination half-life of the PBT chemical, which is often readily



available in the literature. The body burden is based on the chemical dose normalized by the total body weight. Thus, the simple model can be represented by the following differential equation<sup>2</sup>:

$$\frac{\partial BB(t)}{\partial t} = f_{am} DI_{mat}(t) - k_{elim} \times BB(t)$$

Equation 1

Where:

- BB(t) = the time-dependent total body burden (ng/kg),
- $f_{am}$  = the fraction of ingested chemical absorbed by the mother (dimensionless),
- $DI_{mat}(t)$  = the time-dependent maternal dose (ng/kg-day), and
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ .

This equation can be converted to a difference equation and iterated in time:

$$BB_{t+\Delta t} = BB_t + \Delta t (f_{am} DI_{mat,t} - k_{elim} \times BB_t)$$

Equation 2

Where:

- $BB_{t+\Delta t}$  = the total body burden at the next time step (ng/kg),
- $BB_t$  = the total body burden at the current time step (ng/kg),
- $\Delta t$  = the time step (days),
- $f_{am}$  = the fraction of ingested chemical absorbed by the mother (dimensionless),
- $DI_{mat,t}$  = the maternal dose at the current time step (ng/kg-day), and
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ .

This equation will approximate the solution to the differential equation. To help eliminate errors introduced by converting the differential equation to an algebraic equation, the time step ( $\Delta t$ ) should be much smaller than the elimination time scale.

This equation allows approximation of the maternal body burden; however, additional assumptions can be made to estimate the time dependent infant intake from breast milk. First, the concentration in the

<sup>2</sup> Strictly speaking, this equation is only correct if the maternal body weight is not changing in time. However, this approximation is sufficient given the overall uncertainty in using a first-order model.

maternal milk fat is assumed to be equal to the concentration in the maternal fat and can be estimated as:

$$C_{\text{milk fat},t} = \frac{BB_t \times f_f}{f_{fm}}$$

Equation 3

Where:

- $C_{\text{milk fat},t}$  = the concentration of chemical in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_f$  = the fraction of chemical that is stored in maternal fat (dimensionless), and
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW).

Although nonpolar compounds partition to some degree into the aqueous compartment, the most commonly analyzed organic chemicals, including HCB, are highly concentrated in the lipid phase; the concentration of HCB in breast milk is not expected to be significantly underestimated by ignoring the aqueous phase.

To account for the fact that lactation acts as an additional removal mechanism from the mother, the elimination rate during lactation is increased to equal:

$$k_{\text{elac}} = k_{\text{elim}} + \frac{CR_{\text{milk},t} \times f_f \times f_{\text{mbm}}}{f_{fm} \times BW_{\text{Mat}}}$$

Equation 4

Where:

- $k_{\text{elac}}$  = the maternal elimination rate during lactation (days<sup>-1</sup>),
- $k_{\text{elim}}$  = the first order elimination rate (days<sup>-1</sup>) = ln(2)/half-life (days),
- $CR_{\text{milk},t}$  = the time-dependent infant ingestion rate of milk (kg/day),
- $f_f$  = the fraction of chemical that is stored in maternal fat (dimensionless)
- $f_{\text{mbm}}$  = the fraction of fat in milk (dimensionless),
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW), and
- $BW_{\text{Mat}}$  = the maternal body weight (kg).

Then, the time-dependent infant intake can be estimated as:

$$DI_{INF,t} = \frac{(C_{milk\ fat,t} \times f_{mbm}) \times CR_{milk,t}}{BW_{INF,t}}$$

Equation 5

Where:

- $DI_{INF,t}$  = the time-dependent infant daily ingestion (ng/kg-day),
- $C_{milk\ fat,t}$  = the time-dependent concentration of chemical in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_{mbm}$  = the fraction of fat in milk (dimensionless),
- $CR_{milk,t}$  = the time-dependent infant ingestion rate of milk (kg/day), and
- $BW_{INF,t}$  = the time-dependent infant's body weight (kg).

The time-dependent body weights and ingestion rates are incorporated in the models by using different point estimates over the course of the simulation (see [Sections 3.5.5.1](#) and [3.5.5.2](#)). Finally, if the infant is modeled using a first-order model as well, the infant body burden can be estimated as:

$$BB_{INF,t+\Delta t} = BB_{INF,t} + \Delta t (f_{ai} DI_{INF,t} - k_{elim,INF} \times BB_{INF,t})$$

Equation 6

Where:

- $BB_{INF,t+\Delta t}$  = the infant total body burden at the next time step (ng/kg),
- $BB_{INF,t}$  = the infant total body burden at the current time step (ng/kg),
- $\Delta t$  = the time step (days),
- $f_{ai}$  = the fraction of ingested chemical absorbed by the infant (dimensionless),
- $DI_{INF,t}$  = the infant dose at the current time step (ng/kg-day), and
- $k_{elim,INF}$  = the first order elimination rate in the infant ( $\text{days}^{-1} = \ln(2)/\text{half-life (days)}$ ).

The above equations approximate the maternal body burden and exposure to a nursing infant. The model does not, however, include any fetal exposure *in utero*.

This model is generic and not species specific. For purposes of illustration, the model was implemented in Excel and time integrated through the life of the mother ([ICF, 2013a, b](#)). Then, the time-dependent solution can be used to estimate the internal dose metrics in [Section 3.6](#).

The above model was parameterized so that the maternal dose, the infant body weight, and the infant ingestion rate all vary with time. In the case of the maternal dose, this allows the model to follow dosing protocols from animal studies. In the case of the body weight and ingestion rate, this allows the model to more accurately simulate effects on infants during very early exposure when body weight is changing rapidly and when critical windows for developmental effects may occur.

#### **Workshop Notes: Approaches**

Here, the adult whole-body half-life is used to calculate the first order elimination rate in the infant ( $k_{elim,INF}$ ). For many PBT chemicals, the half-life in adults differs from the half-life in juveniles. If offspring body burden is used as the dose metric for interspecies extrapolation, it would be preferable to use the half-life of the chemical in offspring to calculate the first order elimination rate in offspring. However, this information is often unavailable in the literature.

Workshop participants stated that steady-state conditions likely apply in almost all human exposure scenarios if the human half-life for HCB is less than or equal to 215 days. However, as stated above, some workshop participants suggested that there is evidence for a much longer half-life for HCB in humans (i.e., maybe as long as 20 years).

If the half-life for HCB in humans is much longer than 215 days, it might not be appropriate to assume steady state conditions. The same can be said for other PBT chemicals with very long half-lives (e.g., mirex). Workshop participants also expressed concern regarding the assumption of steady-state conditions in laboratory animals, where experimental exposures may be short relative to PBT chemical half-life.

Workshop participants suggested that data from epidemiological studies (e.g., serum or breast milk chemical concentrations) could be used with this model to evaluate the half-life selected for a particular PBT chemical in humans. If this model is run with the correct half-life, it should be able to predict the epidemiological data.

See [Section 4](#) for a detailed discussion of uncertainty in half-life estimates and [Section 6](#), for further discussion regarding the choice of dose metric for the interspecies extrapolation.

#### **3.5.5.1. Input Parameters and Application to HCB in Humans**

For humans, the model was implemented using a time step of 1 week. This represents a balance between a time step short enough to capture changes in the infant during lactation and long enough so that data are not applied beyond their measurement range. Since the Exposure Factors Handbook ([U.S. EPA, 2011a](#)) provides infant estimates on a monthly basis (see below), input values across weeks within a single month were kept equal.

The input parameters used for HCB for humans are shown in [Table 3-6](#). In addition to using mid-range (i.e., mean) values in the model, low and high end estimates were also used for some parameters,

where available, to determine the approximate range of human variability. Values for the parameters that are not chemical-specific were retained from the Combustor Assessment ([U.S. EPA, 1998](#)) or taken from the Exposure Factors Handbook ([U.S. EPA, 2011a](#)). HCB-specific values are the same as those presented and discussed in [Section 3.5.2](#).

As discussed in [Section 3.5.2](#), the absorption rate of HCB depends on the relative concentration of the chemical in the blood at the time of intake. Thus, the model was implemented assuming low daily exposure and constant absorption at 80% throughout the maternal lifetime. This may tend to overestimate the absorption, particularly in women who get pregnant at an older age.

#### **Workshop Notes: Approaches**

Workshop participants suggested changes to some of the values presented in [Table 3-6](#). Also, some clarifications were suggested to better explain how the data were used in the exposure models.

In [Table 3-6](#), 3 different values are considered for maternal age at pregnancy, the fraction of mother's weight that is fat ( $f_{fm}$ ), and the fraction of fat in breast milk ( $f_{mbm}$ ). The values in the column labeled "Low" were used to estimate the low end of a potential range of human variability in infant exposure. Similarly, the columns labeled "Mid" and "High" were used to estimate mid-range and high-end infant exposures, respectively.

In the original version of this briefing packet, the values for the fraction of mother's weight that is fat were listed as 0.2, 0.3 and 0.4 for "Low", "Mid" and "High" calculations, respectively. This was an error. In this version, the order in which these values are listed has been corrected.

Workshop participants suggested that the values presented in [Table 3-6](#) for fraction of mother's weight that is fat are too low.

Workshop participants also suggested that the values presented in [Table 3-6](#) for fraction of fat in breast milk are too low. A more current reference, U.S. EPA ([2011a](#)), reports the mean fraction of fat in breast milk to be 0.036. The mean minus 2 SD (0.021) can be used to calculate low-end infant exposures, the mean (0.036) can be used for mid-range exposures, and the mean plus 2 SD (0.051) can be used to determine the high-end of the range.

Accounting for human variability and uncertainty in the parameters used for modeling were major issues raised across chemicals. A detailed discussion of these topics is located in [Section 4](#).

**Table 3-6. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model ([ICF, 2013a](#))**

Parameter (units)	Variable	Low	Mid	High	Note/source
Human Parameters (not chemical-specific)					
Maternal age at pregnancy (years)	Age	18	25	40	Assumptions
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 1 (kg)	BW <sub>INF,t</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 2-3 (kg)		5.9			
Infant body weight, Month 4-6 (kg)		7.4			
Infant body weight, Month 7-12 (kg)		9.2			
Infant ingestion rate, Month 1 (kg milk/day)	CR <sub>milk,t</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, Month 2-3 (kg milk/day)		0.69			
Infant ingestion rate, Month 4-6 (kg milk/day)		0.77			
Infant ingestion rate, Month 7-12 (kg milk/day)		0.62			
Fraction of mother’s weight that is fat (kg maternal fat/kg BW) <sup>b</sup>	f <sub>fm</sub>	0.4	0.3	0.2	Timson and Coffman ( <a href="#">1984</a> ); U.S. EPA ( <a href="#">1998</a> )
Fraction of fat in breast milk (dimensionless) <sup>c</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Parameter (units)	Variable	Value			Note/source
Human Chemical-Specific Parameters					
Fraction of ingested chemical that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1.00			Assumed
Fraction of ingested chemical that is absorbed by the mother (dimensionless)	f <sub>am</sub>	0.80			Schlummer et al. ( <a href="#">1998</a> )
Fraction of chemical that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9			U.S. EPA ( <a href="#">1998</a> )
Biological elimination constant for chemical (day <sup>-1</sup> )	k <sub>elim</sub>	0.00322			Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans (days)	t <sub>1/2</sub>	215			Yesair et al. ( <a href="#">1986</a> )

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost postpartum.

<sup>b</sup> Represents mean ± standard deviation. In this table, "Low" and "High" refer to the low and high ends of the infant dose range that will result from using the given parameters in the model. Because the fraction of the mother's weight that is fat is inversely related to infant dose in the model, higher values for this parameter produce lower estimates of infant dose. Thus, the high end value for maternal fat corresponds to the "low" end for infant dose, and the low end value for maternal fat corresponds to the "high" end for infant dose.

<sup>c</sup> Represents the typical range and midpoint of the range.

**Workshop Notes: Model Parameters in the First-Order,  
Single Compartment Human Model**

**Fraction of Fat in Breast Milk:**

Workshop participants noted that lipid content in milk is a parameter that may affect the infant dose of PBT chemicals and may be important to consider. More information about this factor would be useful.

- At least in humans, this parameter changes across the period of breastfeeding. Thus, breast milk lipid measurements based on samples taken at a single time point may not represent average lipid concentrations over the duration of breastfeeding.
- In fact, this parameter changes even during the course of a single feeding. Foremilk, which is expressed in the beginning of a feeding, contains mostly water. It is not until closer to the end middle/end of a feeding when the hindmilk, which is rich in lipids, is expressed. So, some uncertainty in the value used for this parameter might result if it is based on breast milk lipid measurements from samples expressed only at the beginning of a feeding.
- This parameter can be modeled. EPA's Multimedia Ingestion Risk Calculator (MIRC) [described in U.S. EPA (2009)], based on the model found in U.S. EPA (1998), factors in this changing concentration over an assumed breastfeeding duration of 12 months.

**Half-Life in Humans:**

Workshop participants agreed that the first-order model can be useful in the absence of a validated PBPK model for a given chemical in the relevant species. However, the reliability of the first-order model is very sensitive to the accuracy of the half-life value. If the half-life value is uncertain, there will be a great deal of uncertainty in the model output.

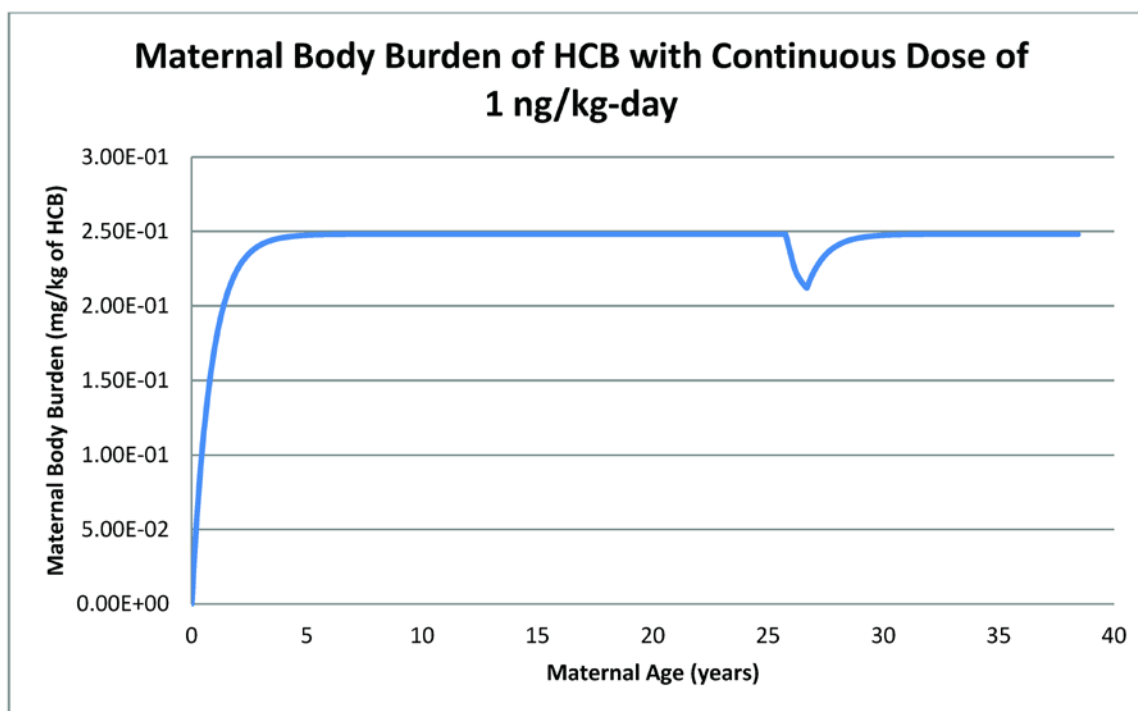
As mentioned above, workshop participants disagreed with the half-life value used for HCB in this briefing packet. Uncertainty in half-life estimates was a major issue raised across chemicals. See [Section 4.3.3](#) for a detailed discussion of this topic.

**Maternal Body Weight:**

Workshop participants observed that maternal weight and percent body fat may change dramatically over the course of lactation. These are also parameters for which there is considerable variability across the population.

Accounting for human variability and uncertainty in the parameters used for modeling were major issues raised across chemicals. See [Section 4.3](#) for detailed discussions of these topics.

An example showing the maternal body burden assuming pregnancy at age 25 years and a daily intake of 1 ng HCB/kg-day is shown in [Figure 3-5](#). Because the models are linear with respect to dose, this intake level is chosen for illustrative purposes and the body burden would scale linearly with any change in intake. The dip at age 25 years and 9 months is due to the additional elimination during lactation.



**Figure 3-5. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day HCB**

One useful metric from the model is the estimate of the ratio between infant lactational dose and average daily maternal dose. For the model parameterized as discussed above, assuming the “mid” parameters ([Table 3-6](#)) and pregnancy at age 25 years, the average infant lactational dose to average daily maternal dose ratio was estimated to be 2.3 (1 month lactation duration) and 1.8 (12 month lactation duration). The overall range of the ratio, including those calculated using peak infant lactational dose estimates and accounting for human variability in maternal age at pregnancy (18-40 years), maternal body fat content (20-40%), and milk lipid content (2-4%), was from 0.9 to 5.0 (see [Table 3-7](#)).



**Table 3-7. Estimated Ratio of Infant Lactational Dose (ng/kg-day) to Average Daily Maternal Dose (ng/kg-day) for HCB Based on First-Order Model ([ICF, 2013a](#))**

Infant Nursing Duration	Pregnancy at 18 years; Low parameters	Pregnancy at 25 years; Mid parameters	Pregnancy at 40 years; High parameters
1 month, Peak Infant Intake	1.2	2.4	4.7
1 month, Average Infant Intake	1.2	2.3	4.6
12 months, Peak Infant Intake	1.3	2.6	5.0
12 months, Average Infant Intake	0.9	1.8	3.3

### 3.5.5.2. Input Parameters and Application to HCB in Rats

For rats, the model was implemented using a time step of one half day (one week used for humans). This was deemed sufficient to resolve changes during the typical three-week lactation period. The input parameters used for HCB are shown in [Table 3-8](#). A gestation time of 3 weeks and lactation duration of 3 weeks were assumed for the analysis as representative typical values in rats ([U.S. EPA, 2002](#)). A litter size parameter was added so that the nursing rat fed 10 pups at a time, rather than the single infant assumed in the human model. This litter size, which matches the number of pups per litter in the candidate POD study, increased the maternal lactational elimination rate.

The physiological parameters needed for the model were not presented in the candidate POD study. Milk ingestion rates in rats were calculated from experimentally determined milk production values of 29.7, 54.3, 59.1, and 43.7 g/rat/day measured on lactation days 2, 7, 14, and 21, respectively ([Knight et al., 1984](#)). The values for days 2, 7, and 14 were used for weeks 1, 2, and 3, respectively, dividing by 10 (the number of pups in each litter) to get per pup ingestion rates of 3.0, 5.4, and 5.9 g milk/day. Knight et al. (1984) also presented litter weights at different time points in a figure. This figure was digitized using the GetData Graph Digitizer ([Fedorov, 2008](#)), and the values were divided by 10 pups per litter to get pup body weights of 6.6, 13.9 and 28.0 g for weeks 1, 2 and 3, respectively.

#### **Workshop Notes: Uncertainty**

Workshop participants expressed some concern regarding the estimation of milk ingestion rate for rat pups.

- Milk production is a physiological response to suckling.
- Specific litter sizes and pup sizes make a big difference on the dynamics of dose to the offspring.
- These parameters should be addressed in models estimating offspring dose.

Uncertainty in the parameters used for modeling was a major issue raised across chemicals. See [Section 4](#) for a detailed discussion of this topic. See [Section 5](#) for a description of a study design optimized to reduce uncertainty in the milk ingestion rate for rodents.

Knight et al. (1984) reported the weight of lactating Wistar dams at day 2, 7, 14 and 21. The average across these days for the control group (273 g) was used in the model. Fisher et al. (1990) reported fat as a percentage of body weight to be 6.0 to 12.0% in lactating F344 dams. A midpoint of 9.0% was used for this application. The fraction of fat in milk was reported to be 15.0% in Fisher et al. (1990), compared to a value of 4% in humans (Welch and Findlay, 1981). The same chemical-specific values as presented in Section 3.5.2.) were used: maternal absorption fraction, the fraction of chemical stored in the fat relative to the total body burden, and the half-life.

#### **Workshop Notes: Approaches**

As discussed in the text box on page 21, workshop participants suggested Barnett et al. (1987) as an alternate candidate POD study. If Barnett et al. (1987) were used instead of Mendoza et al. (1978; 1977), then physiological and chemical-specific parameters for mice, as opposed to rats, would be needed. The litter size and dam weight are not reported by the study but could be estimated for mice as a litter size of 6 (The Jackson Laboratory, 2012) and a dam weight of 0.025 kg (Brown et al., 1997).

#### **Workshop Notes: Model Parameters in the First-Order, Single Compartment Rat Model**

##### **Fraction of Fat in Milk:**

- In humans, this parameter changes across the period of breastfeeding. Workshop participants wondered if this also happens in rats.

##### **Weight of Lactating Dam:**

- Workshop participants noted that the dam weight may decrease dramatically during lactation.
- The value provided in Table 3-8 is an average of the dam weight on lactation days 2, 7, 14, and 21. To better capture the change in weight with time, this value could be varied in time instead of using an average value.

Uncertainty in the parameters used for modeling was a major issue raised across chemicals. See Section 4 for a detailed discussion of this topic.

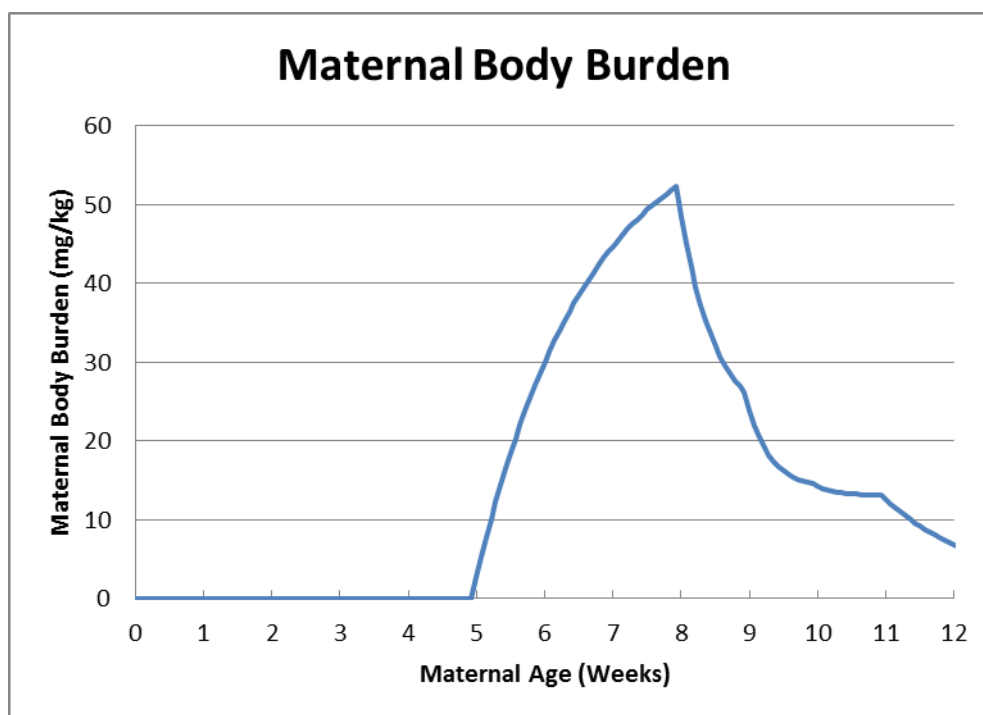
**Table 3-8. Model Parameters and Their Values Used in First-Order Single-Compartment Model in Rat (ICF, 2013b)**

Parameter (units)	Variable	Estimated Value	Note/source
<b>Rat Parameters (not chemical-specific)</b>			
Dam age at pregnancy (weeks)	Age	5	Assumption
Dam weight during lactation (kg)	BW <sub>Mat</sub>	0.273	Knight et al. (1984)
Litter size (number of pups)	Litter	10	Mendoza et al. (1978; 1977)
Pup body weight, Week 1 (kg)	BW <sub>INF,t</sub>	0.0066	Knight et al. (1984)
Pup body weight, Week 2 (kg)		0.014	
Pup body weight, Week 3 (kg)		0.028	
Pup ingestion rate, Week 1 (kg milk/day)	CR <sub>milk,t</sub>	0.0030	Knight et al. (1984)
Pup ingestion rate, Week 2 (kg milk/day)		0.0054	
Pup ingestion rate, Week 3 (kg milk/day)		0.0059	
Fraction of dam's weight that is fat (kg maternal fat/kg BW)	f <sub>fm</sub>	0.09	Fisher et al. (1990)
Fraction of fat in milk (dimensionless)	f <sub>mbm</sub>	0.15	Welch and Findlay (1981)
<b>Rat Chemical-Specific Parameters</b>			
Fraction of ingested chemical that is absorbed by the pup (dimensionless)	f <sub>ai</sub>	0.8	Assumed
Fraction of ingested chemical that is absorbed by the dam (dimensionless)	f <sub>am</sub>	0.8	Koss and Koransky (1975)
Fraction of chemical that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	Assumed
Biological elimination constant for chemical (day <sup>-1</sup> )	k <sub>elim</sub>	0.087	Calculated as ln(2) / t <sub>1/2</sub>
Half-life in rats (days)	t <sub>1/2</sub>	8 days*	Koss et al. (1983)

\*The reasoning and process behind the half-life selection for HCB is discussed in detail toward the end of [Section 3.5.2](#).

The dosing protocol in the POD study ([Mendoza et al., 1977](#)) was applied to this rat model. In the study, the dams were dosed daily from two weeks before mating until PND 21. The model is able to capture transfer to the nursing pup during lactation, but it does not model any offspring body burden during gestation.

An example figure showing the maternal body burden using the candidate POD dose (6.7 mg/kg-day) is shown in [Figure 3-6](#). One useful metric from the model is the estimate of the ratio between offspring lactational dose and average daily maternal dose. For the model parameterized as discussed above ([Table 3-8](#)), the average offspring lactational dose to average daily maternal dose ratio was estimated to be 5.4 (1 week lactation duration) and 3.3 (3 week lactation duration, see [Table 3-9](#)).



**Figure 3-6. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study ([Mendoza et al., 1977](#)) and an Administered Dose of 6.7 mg/kg-day HCB (POD)**

#### **Workshop Notes: Approaches**

If Barnett et al. ([1987](#)) were used as the POD study, as suggested by the workshop participants, then the dosing protocol from that study would be used to generate [Figure 3-6](#), above.

**Table 3-9. Estimated Ratio of Offspring Lactational Dose (mg/kg-day) to Average Daily Maternal Dose (mg/kg-day) for HCB in Rats Based on First-Order Model ([ICF, 2013b](#))**

Pup Nursing Duration	Ratio
1 week, Peak Pup Intake	4.9
1 week, Average Pup Intake	3.5
3 weeks, Peak Pup Intake	4.9
3 weeks, Average Pup Intake	1.9

### 3.5.6. Biotransfer Method

The simplest technique for linking infant lactational dose with average daily maternal dose involves estimating the elimination of HCB in breast milk using a biotransfer factor. This method is discussed in the Combustor Assessment ([U.S. EPA, 1998](#)). The technique is based on a study by Travis et al. ([1988](#)) and assumes that the milk fat chemical concentration is proportional to maternal chemical intake, with the proportionality constant represented by a biotransfer factor:

$$C_{\text{milk fat}} = DI_{\text{MAT}} \times BTF_m \times BW_{\text{Mat}}$$

Equation 7

Where:

- $C_{\text{milkfat}}$  = the concentration of chemical in the maternal milk fat (ng/kg milk fat),
- $DI_{\text{MAT}}$  = daily maternal intake of chemical (ng/kg BW-day),
- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $BW_{\text{Mat}}$  = the maternal body weight (kg).

The biotransfer factor is then estimated using a regression equation based on the octanol-water partition coefficient ( $K_{ow}$ ) using the expression:

$$BTF_m = 0.00062 \times K_{ow}$$

Equation 8

Where:

BTF<sub>m</sub> = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and  
 K<sub>ow</sub> = the octanol-water partition coefficient (unitless).

The regression equation was estimated using six highly lipophilic compounds with log(K<sub>ow</sub>) in the range of 5.16 to 6.5. According to the Combustor Assessment ([U.S. EPA, 1998](#)), the model tends to over-predict concentrations. Thus, the Combustor Assessment suggests the equation should only be used if parameters for more sophisticated kinetic models cannot be found in the literature and if the K<sub>ow</sub> of the chemical in question is within the range of the K<sub>ow</sub> used to fit the model. This method is included for consideration.

The daily infant chemical dose can then be estimated for the biotransfer method using the equation:

$$DI_{INF} = \frac{(C_{milk\ fat} \times f_{mbm}) \times CR_{milk} \times ED_{INF}}{BW_{INF} \times AT}$$

Equation 9

Where:

DI<sub>INF</sub> = the daily infant chemical dose (ng/kg-day),  
 C<sub>milkfat</sub> = the concentration of chemical in the maternal milk fat (ng/kg milk fat),  
 f<sub>mbm</sub> = the fraction of fat in milk (dimensionless),  
 CR<sub>milk</sub> = the infant ingestion rate of milk (kg/day),  
 ED<sub>INF</sub> = the infant exposure duration (year),  
 BW<sub>INF</sub> = the infant's body weight (kg), and  
 AT = the averaging time (year).

Using the above equations, the ratio between the infant lactational dose and average daily maternal dose can be estimated as:

$$\frac{DI_{INF}}{DI_{MAT}} = \frac{0.00062 \times K_{OW} \times f_{mbm} \times CR_{milk} \times ED_{INF} \times BW_{Mat}}{BW_{INF} \times AT}$$

Equation 10

### 3.5.6.1. Input Parameters and Application to HCB in Humans

The input parameters used for HCB in the biotransfer model are shown in [Table 3-10](#). In addition to using mid-range (e.g., mean) values in the model, low and high end estimates were also used for some

parameters where available to determine the approximate range of human variability. The  $ED_{INF}$  and AT values are equal to each other (either 1 month or 12 months) and cancel from the equation.

**Table 3-10. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age**

Parameter (units)	Variable	Low	Mid	High	Note/source
Maternal weight during lactation (kg) <sup>a</sup>	$BW_{Mat}$	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, 1 month (kg)	$BW_{INF, 1}$	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, average over 12 months (kg)	$BW_{INF, 12}$	7.8			Weighted mean, U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, 1 month (kg milk/day)	$CR_{milk,1}$	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, average over 12 months (kg milk/day)	$CR_{milk,12}$	0.66			Weighted mean, U.S. EPA ( <a href="#">2011a</a> )
Fraction of fat in breast milk (dimensionless) <sup>b</sup>	$f_{mbm}$	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Octanol-water partition coefficient	$\log(K_{ow})$	5.73			Hansch et al. ( <a href="#">1995a</a> )

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost postpartum.

<sup>b</sup> Represents the typical range and midpoint of the range.

Based on the  $K_{ow}$  value and using [Equation 8](#), the biotransfer factor for HCB is 333. Using this  $BTF_m$ , the average infant lactational dose to average daily maternal dose ratio was estimated for infants at 1 and 12 months of age and is presented in [Table 3-11](#). For the model parameterization presented here, these ratios tend to be one order of magnitude higher than the estimates from the simple one-compartment kinetic model.

**Table 3-11. Estimated Ratio of Infant Lactational Dose (ng/kg-day) to Average Daily Maternal Dose (ng/kg-day) for HCB Based on Biotransfer Method**

Age	Low	Mid	High
1 month, Average Infant Intake	53.1	79.6	106.1
12 months, Average Infant Intake	42.3	63.4	84.5

### **Workshop Notes: Approaches**

A simpler approach than the biotransfer method, based on a method presented in Rogan and Ragan ([1994](#)), was proposed by workshop participants as a conservative method to estimate maternal continuous daily exposure when insufficient data are available for a given chemical.

- Once the average pup body burden (mg/kg bw) at the POD from the POD study is established, this value is set as the benchmark for the average human infant body burden during lactation (assumed to be a 12 month duration).
- The dose to the infant over this time period (mg/kg-day) is calculated: average human infant body burden (mg/kg bw)/infant age (365 days).
- Chemical concentration in milk (mg/kg fat) is calculated: ((dose to the infant (mg/kg-day)\* infant body weight (kg) (weighted average over 12 months))/(infant milk intake (kg milk/day) (weighted average over 12 months)\*fraction of fat in breast milk (kg fat/kg milk))).
- Total maternal body burden (mg/kg bw) is calculated: chemical concentration in milk (mg/kg fat)\*fraction of mother's weight that is fat (kg fat/kg bw).
- Continuous daily dose to mother is calculated (assumed that mother is 25 years of age at the time of first parturition): total maternal body burden (mg/kg bw)/mother's age (9,490 days).

#### **ASSUMPTIONS:**

- There is no chemical elimination: every molecule of a chemical that enters the body stays in the body. This is an extremely conservative assumption. Some workshop participants objected to this assumption because, even for PBT chemicals with very long half-lives, there is some fecal elimination. There are simple mathematical models that can be used with only log(Kow) or half-life information; and, these do account for some elimination. However, for chemicals for which there are no solid half-life data or if there's uncertainty in the reported log(Kow)s, this method may provide a first approximation or worst-case scenario that could be used until better data are obtained.
- It is assumed that all of the chemical is stored in fat. For many PBT chemicals, this is a sound assumption. However, if there are data to suggest that some of the chemical partitions into aqueous compartments, then it would be preferable to use a more complex model that can accommodate those data.



### 3.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation



The previous sections discuss the tools and input data available to estimate internal dose metrics for HCB in rats and humans. The next step is to select a method for performing cross-species extrapolation by weighing biological sophistication against methodology and data uncertainty. Cross-species extrapolation is used to estimate a human equivalent dose, or the daily dose likely to lead to the same developmental effect in humans as that observed in animals.

[Table 3-12](#) presents potential dose metrics and methods for performing the cross-species extrapolation. This list may not be exhaustive but is intended to foster discussion at the workshop. For illustrative purposes in this briefing packet, one method for HED calculation was selected (shown in bold type), and sample calculations were performed.

In selecting the dose metric to use for cross-species extrapolation and the technique to use to estimate the dose metrics, the discussion points listed in the text boxes throughout this section should be considered. This information is synthesized in the following text box.

As an example, the points were considered, and an example was provided to foster discussion. The chemical-specific information available should be considered to help guide the decision on whether a maternal or offspring dose metric should be utilized. Both human ([Verner et al., 2009](#)) and rat ([Yesair et al., 1986](#)) PBPK models for HCB exist in the literature and have the necessary inputs. The chemical is metabolized in the body, but metabolism is slow. For these reasons, the database was considered sufficient to support selection of infant/pup dose metrics to perform the cross-species extrapolation.

**Table 3-12. Potential Dose Metrics and Methods for HED Estimation**

Should the Cross-Species Extrapolation Be Performed on Maternal or Offspring Dose Metric?	What Technique Is Used to Estimate the Dose Metrics?	What Dose Metric Should Be Used for Cross-Species Extrapolation?
<p>Maternal Concentration</p> 	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Maternal average or peak body burden during lactation</li> <li>• Maternal average or peak fat concentration during lactation</li> <li>• Maternal average or peak milk concentration during lactation</li> </ul>
<p>Offspring Concentration</p> 	<ul style="list-style-type: none"> <li>• <b>PBPK model in rats and humans</b></li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Infant average or peak body burden during lactation</b></li> </ul>
<p>Other?</p>	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Other?</li> </ul>

### **Workshop Notes: Approaches**

Choice of dose metric for the interspecies extrapolation was a major issue raised at the workshop.

#### **Lactational Dose vs. Target Tissue Dose & Offspring Body Burden**

- Some workshop participants preferred to use offspring lactational dose as calculated from chemical concentrations in milk and estimates of milk consumption by offspring.
- It was suggested that the ideal metric would be a measure of chemical concentration in the target tissue affected in offspring. These data were not available for any of the chemicals discussed at this workshop. However, for highly lipophilic compounds, lipid-adjusted blood concentrations might be assumed to be similar to lipid-adjusted chemical concentrations in target tissues. Therefore, lipid-adjusted blood concentrations might be used as a measure of target tissue dose or offspring body burden.

#### **Peak vs. Average Offspring Exposure**

- Workshop participants suggested that the average target tissue concentration during the critical window of exposure for the relevant developmental effect be used for the offspring exposure metric.
- If the precise critical window of exposure for the relevant developmental effect is unclear, workshop participants suggested that the average target tissue concentration obtained in the pup over the duration of exposure be used for the offspring exposure metric.
- Use of the average rather than the peak provides a more health-protective value. And, unless the critical window of exposure is known to have occurred at the same time as the peak exposure, it isn't clear that dosing at the maximal value was required to elicit the effect.
- Use of the average may be preferred especially if the effect involves an endocrine pathway. In this case, there may be a non-monotonic response in which a lower dose may have an effect equal to or greater than a higher dose.

See [Section 6](#) for further discussion of this topic.

Next, a model was selected to characterize the dose metrics for HCB. Both human and rat HCB PBPK models are available. For the first-order model, some of the necessary parameters are unavailable for rats and humans, including the fraction of chemical stored in fat. For this reason, the PBPK models are selected in this example as the method to estimate the dose metrics in rats and humans.

Finally, the infant/pup dose metric was selected. Increased liver weight and enzyme activity were more pronounced in the cross-fostered animals exposed during lactation only. For this reason, lactation is likely an important exposure route for the effect. However, increased liver weight and enzyme activity are not effects on development, per se. Therefore, it is unlikely that their occurrence is determined by exposure during a specific window of susceptibility. Thus, the average infant body burden may be the most appropriate dose metric for cross-species extrapolation.

The selections described above were not applied in this briefing packet due to the significant effort necessary to implement and run the PBPK models.

#### **Discussion Points for HCB HED Estimation**

- ? Is HCB metabolized? How should metabolism be addressed in the HED estimation?
  - ✓ HCB is metabolized at a very slow rate.
- ? What pharmacokinetic data or relevant chemical-specific information are available for HCB for both animals and humans to facilitate model implementation?
  - ✓  $K_{ow}$
  - ✓ Human and rat half lives
  - ✓ Absorption fractions and fraction of chemical stored in fat in humans (assumptions can be made based on other PBT chemicals)
  - ✗ No fraction of chemical stored in fat for rats
  - ✓ Partition coefficients from PBPK models for rats and humans
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
  - ✓ Peak or average concentrations from maternal or infant dose metrics
  - ✗ The critical window could not be defined
- ? What PBPK or other simpler biokinetic models are available for HCB?
- ✗ Data-validated rat PBPK and human PBPK model are available that include lactation
  - ✓ Simple first order models are available
  - ✓ Simple biotransfer methods are available
- ? What is the best method for estimating the human equivalent dose using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

### 3.7. Attachment A: Concentrations of HCB in Human Milk

**Table 3-13: Mean Levels of HCB in Breast Milk as Reported in IPCS ([1997](#)).**

Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk	Reference
Australia	1970	39	0.042 (rural)	Newton and Greene ( <a href="#">1972</a> )
		28	0.063 (urban)	
Australia	1979-1980	137	0.007 (0.002-0.019) (rural)	Stacey et al. ( <a href="#">1985</a> )
		130	0.008 (0.002-0.017) (urban)	
Australia	-	60	0.017 (0.0007-0.32)	Quinsey et al. ( <a href="#">1995</a> )
Australia	1990-1991	128	0.0036 <sup>a</sup> (<0.01-0.216)	Stevens et al. ( <a href="#">1993</a> )
Brazil	1987-1988	30	0.00048 (0.00024-0.0036) <sup>b</sup>	Beretta & Dick ( <a href="#">1994</a> )
Canada	1986	412	0.0008 (max = 0.014)	Mes et al. ( <a href="#">1993</a> )
Canada	1982	210	0.002 (max = 0.009)	Mes et al. ( <a href="#">1986</a> )
Canada	1975	100	0.002 (max = 0.021)	Mes and Davies ( <a href="#">1979</a> )
Canada	1989-1990	536	0.0013 <sup>b</sup>	Dewailly et al. ( <a href="#">1991</a> ) as cited in IPCS ( <a href="#">1997</a> )
Canada	1978	127	0.00051	Frank et al. ( <a href="#">1988</a> )
	1979	15	0.0004	
	1980-1981	12	0.00028	
	1983-1984	13	0.00052	
	1985	18	0.00026	
Finland	1984-1985	143 <sup>b</sup>	0.002 <sup>b</sup>	Mussalo-Rauhamaa et al. ( <a href="#">1988</a> )

**Table 3-13 (Continued): Mean Levels of HCB in Breast Milk as Reported in IPCS (1997)**

Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk	Reference
Finland	1982	50	0.0023 (0.0007-0.006)	Wickström et al. ( <a href="#">1983</a> )
France	1990-1991	20	0.002 (0.00004-0.008) <sup>b</sup>	Bordet et al. ( <a href="#">1993</a> )
Federal Republic of Germany	1984	144	0.021 <sup>b</sup>	Fürst et al. ( <a href="#">1994</a> )
	1985	220	0.019 <sup>b</sup>	
	1986	157	0.015 <sup>b</sup>	
	1987	144	0.015 <sup>b</sup>	
	1988	196	0.013 <sup>b</sup>	
	1989	145	0.01 <sup>b</sup>	
	1990	286	0.0095 <sup>b</sup>	
	1991	113	0.0074 <sup>b</sup>	
Federal Republic of Germany	1985-1987	167	0.0126 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Federal Republic of Germany	1979-1981	2709	0.048 <sup>b</sup>	BUA ( <a href="#">1993</a> )
	1986	3778	0.013 <sup>b</sup>	
	1987	1897	0.014 <sup>b</sup>	
	1988	2994	0.011 <sup>b</sup>	
	1989	3256	0.01 <sup>b</sup>	
	1990	5340	0.009 <sup>b</sup>	
Former German Democratic Republic	1990-1991	483	0.007 <sup>b</sup>	BUA ( <a href="#">1993</a> )
India	1987	16	0.042 (0-0.25) <sup>d</sup>	Nair and Pillai ( <a href="#">1989</a> )

**Table 3-13 (Continued): Mean Levels of HCB in Breast Milk as Reported in IPCS (1997)**

Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk	Reference
Israel	-	100	0.00256	Weisenberg et al. ( <a href="#">1985</a> )
Italy	-	56	0.058 <sup>c</sup>	Franchi & Focardi ( <a href="#">1991</a> )
Italy	1987	64	0.006 (0.004-0.009)	Larsen et al. ( <a href="#">1994</a> )
Netherlands	1972-1973	202	0.036 <sup>a,b</sup>	Greve and Van Zoonen ( <a href="#">1990</a> )
	1983	278	0.008 <sup>a,b</sup>	
New Zealand	1988	38	0.0011	Bates et al. ( <a href="#">1994</a> )
Norway	1991	28	0.0007	Johansen et al. ( <a href="#">1994</a> )
Spain	1984-1987	240	0.089(0.039-0.21) <sup>b</sup>	Conde et al. ( <a href="#">1993</a> )
	1990-1991	358	0.048(0.037-0.073) <sup>b</sup>	
Sweden	1978	20	0.0042 (0.002-0.009) <sup>b</sup>	Norén ( <a href="#">1983</a> )
Sweden	-	2	0.0007-0.004 <sup>b</sup>	Norén ( <a href="#">1983</a> )
Sweden	1972	227	0.003 (0.002-0.004) <sup>b</sup>	Norén ( <a href="#">1988</a> )
	1976	245	0.003 (0.003-0.004) <sup>b</sup>	
	1980	340	0.003 (0.003-0.004) <sup>b</sup>	
	1984-1985	102	0.001 (0.0008-0.001) <sup>b</sup>	
Sweden	1989	140	0.0012 <sup>b</sup>	Norén ( <a href="#">1993</a> )
Sweden	1986-1987	40	0.0017 <sup>b</sup>	Vaz et al. ( <a href="#">1993</a> )
Thailand	1985-1987	3	0.0003 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Turkey	1988	51	0.0035 <sup>b</sup>	Basri Üstünbas et al. ( <a href="#">1994</a> )
Turkey	20-30 years post-exposure during 1955-1959	56	0.021 <sup>b</sup>	Gocmen et al. ( <a href="#">1989</a> )

**Table 3-13 (Continued): Mean Levels of HCB in Breast Milk as Reported in IPCS (1997)**

Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk	Reference
United Kingdom	1989-1991	193	0.001 (<0.001-0.005)	WPPR ( <a href="#">1992</a> )
USA	1979	40	0.00052	Bush et al. ( <a href="#">1985</a> )
USA	1985-1987	8	0.0007-0.0008 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Vietnam	1985-1987	12	<0.00017 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Yugoslavia	1978	10	0.006 (0.002-0.017)	Kodric -Smit et al. ( <a href="#">1980</a> )

<sup>a</sup> Median value.

<sup>b</sup> Expressed in cited reference as mg/kg milk fat and subsequently converted to a wet weight (whole milk) basis using either the % fat reported, or if not given, a value of 4.2% fat was assumed ([DNHW, 1987](#)).

<sup>c</sup> Number of positive samples.

<sup>d</sup> Expressed in cited reference on a dry weight basis and subsequently converted to wet weight (whole milk) using an assumed value of 88% moisture ([DNHW, 1987](#)).



**Table 3-14. Mean Levels of HCB in Breast Milk as Reported by ATSDR ([2002b](#))**

Location of study	N/n	Concentration <sup>a</sup>	Reference
New Zealand			
Auckland, urban	11/n/a	0.020	Bates et al. ( <a href="#">1994</a> )
Northland, rural	10/n/a	0.021	
Christchurch, urban	9/n/a	0.030	
Canterbury, rural	8/n/a	0.063	
Australia			
Victoria, Australia	60/59	0.41	Quinsey et al. ( <a href="#">1995</a> )
Victoria, Australia (1985/1986)	158/153	0.005 <sup>b</sup>	Monheit and Luke ( <a href="#">1990</a> )
Italy			
Total	27/NR	--	Larsen et al. ( <a href="#">1994</a> )
Rome	NR	0.25	
Pavia	NR	0.12	
Milan	NR	0.20	
Florence	NR	0.14	
Czech Republic			
Prague	17/NR	0.639	Schoula et al. ( <a href="#">1996</a> )
Kladno	12/NR	0.570	
Uherske Hradiste	7/NR	0.482	
Other European locations			
France (multiple locations)	20/19	0.147	Bordet et al. ( <a href="#">1993</a> )
Madrid, Spain	63/52	1.0	Conde et al. ( <a href="#">1993</a> )
Madrid, Spain: Industrialized area	NR	1.74	
Sweden (1972–1980)	227–1,500/NR	0.110–220	Norén and Meironyté ( <a href="#">2000</a> )
Bratislava, Slovak Republic	26/26	0.339	Prachar et al. ( <a href="#">1993</a> )
Canada			
Canada (1967–1992)	NR	0.002–0.00044 <sup>b</sup>	Craan and Haines ( <a href="#">1998</a> )
Quebec	NR	0.002–0.00040 <sup>b</sup>	
Ontario	NR	0.002–0.00048 <sup>b</sup>	
Arctic Quebec Inuit women	107/107	0.136	Dewailly et al. ( <a href="#">1993</a> )

**Table 3-14 (Continued): Mean Levels of HCB in Breast Milk as Reported by ATSDR (2002b)**

Location of study	N/n	Concentration <sup>a</sup>	Reference
Arctic Quebec Caucasian women	50/48	0.028	
Canada	412/no data	0.026	Mes et al. ( <a href="#">1993</a> )
Canada (multiple locations, in 1992)	497/497	0.0044 <sup>b</sup> 0.015 <sup>c</sup>	Newsome et al. ( <a href="#">1995</a> )
<b>Other North America</b>			
Arkansas, USA	942/57	0.03	Mattison et al. ( <a href="#">1992</a> )
Hawaii, USA (1979–1980)	54/54	0.046 ± 0.049	Takei et al. ( <a href="#">1983</a> )
Veracruz, Mexico	43/43	0.047	Waliszewski et al. ( <a href="#">1996</a> )
<b>Other</b>			
Akumadan, Ghana	20/19	0.04	Ntow ( <a href="#">2001</a> )
Northern Thailand	25/9	0.0051 <sup>b</sup>	Stuetz et al. ( <a href="#">2001</a> )
Porto Alegre, Brazil	30/19	0.02	Beretta and Dick ( <a href="#">1994</a> )

<sup>a</sup> µg/g on lipid basis unless otherwise noted<sup>b</sup> whole milk sample<sup>c</sup> milk fat sample

N = number of samples; n = number of samples with measurable levels; NR = not reported

Source: Reproduced from Pohl and Tylenda ([2000](#)) by ATSDR ([2002b](#)).

## 4. GLOBAL ISSUES, DATA GAPS AND UNCERTAINTIES

Before and during the workshop, participants were asked to identify the presence and nature of gaps in data and parameter measures, potential issues, problems, complications and uncertainties related to conducting risk assessments of PBT chemicals in breast milk. The factors identified can be grouped into five general categories: risk communication; critical windows of exposure; variability and uncertainty in modeling data and parameters; PBT chemical metabolism; and exposure to chemical mixtures. Workshop discussions of the issues identified for each category are summarized in this section.

### 4.1. Risk Communication

Evidence to date suggests that breastfeeding is the healthiest option for infants despite the presence of environmental chemicals in breast milk ([Mead, 2008](#)). The American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend exclusive breastfeeding until 6 months of age and continued breastfeeding until 12 months ([AAP, 2012](#); [WHO, 2003](#)). Estimates of PBT chemical levels in breast milk could cause concern among breastfeeding women and their families, even if these levels are thought to be below those associated with health effects. To help prevent unwarranted anxiety in breastfeeding women and in those deciding whether or not to breastfeed, workshop participants suggested making risk communication a high priority as a factor to be addressed when discussing and assessing potential risks of early life exposure to PBT chemicals in breast milk ([Lakind et al., 2004](#)).

While it is important to assess early life exposures as part of an overall risk assessment, workshop participants emphasized that messaging strategies should be developed early in the process that are mindful of the potential for women to be discouraged inadvertently from breastfeeding. One example of successful risk communication comes from the state of Oregon, where risk assessment guidance has been adopted to protect infants from the consumption of PBT chemicals in breast milk ([Oregon DEQ, 2010](#)). At clean-up sites where PBT chemicals are present, the Oregon Department of Environmental Quality (Oregon DEQ) guidance directs state risk assessors to apply chemical-specific infant risk adjustment factors (IRAF) to adult risk values to calculate risk to breastfeeding infants. The IRAF is designed to account for the dose magnification from average daily maternal dose to infant lactational dose. For details about the derivation of IRAFs, see Oregon DEQ ([2010](#)). Another state agency in Oregon, the Oregon Health Authority (OHA), has also adopted use of IRAFs in its calculation of fish consumption advisories in water bodies where PBT chemicals are the risk driving chemicals. Oregon DEQ staff and OHA staff in the Office of Environmental Public Health reached out to fellow Public Health staff in breastfeeding promotion programs early in the process of developing the IRAFs and the Oregon DEQ ([2010](#)) guidance. The focus of these inter- and intra-agency conversations was to ensure that language encouraging breastfeeding is incorporated into risk communication strategies and language in risk assessments where IRAFs are to be used. Breastfeeding promotion officials also helped Oregon DEQ and other OHA staff refine risk communication language to clarify in the guidance that the goal is not to reduce breastfeeding, but rather to reduce maternal exposure to PBT chemicals so that infants can

enjoy the optimal health benefits of breastfeeding. Early and continuous communication with breastfeeding promotion officials also avoided potential conflict between agencies with differing mandates and gained the support of public health professionals in the effort. Overall, the central message to convey is that the goal of addressing risk to nursing infants is not to discourage breastfeeding, but rather to accurately account for all possible risk and potentially reduce maternal exposure so that chemical levels in breast milk are reduced.

## **4.2. Critical Windows of Exposure**

### **4.2.1. Toxicological Consequences of Prenatal and Postnatal PBT Chemical Exposures**

The developmental period is critical because it sets the stage for lifelong health. Adverse effects on a developing organism may be triggered by exposure to a chemical toxicant during the prenatal period, infancy, adolescence and even later (e.g., in humans, the nervous system continues to develop throughout childhood and into early adulthood) ([Makris et al., 2008](#); [Adams et al., 2000](#)). Throughout development, critical and/or sensitive windows of exposure (i.e., developmental periods of vulnerability during which adverse effects may be triggered by exposures to environmental agents or other stressors) vary for different endpoints, depending on the duration or timing of the programmed developmental process, and may also differ in their relative sensitivity. Further, for many endpoints, these critical and/or sensitive windows of exposure have not been delineated.

For developmental endpoints with very wide or undefined sensitive windows of exposure, it is important to consider the potential impacts of exposure at all lifestages when the relevant developmental process might occur ([Cohen Hubal et al., 2008](#)). For example, human neurodevelopment continues throughout childhood and adolescence, but the timing of specific neurodevelopmental events is often unknown ([Adams et al., 2000](#)). If a particular neurodevelopmental endpoint observed in young adults is suspected to have been influenced by exposure to an environmental toxicant, the relevant exposure could have occurred during the prenatal period, during infancy, and/or later in childhood or adolescence. Therefore, exposures at all of these developmental stages may inform hazard identification and dose-response assessment.

Since elevated exposures to PBT chemicals may occur during the postnatal period, comprehensive hazard identification for these chemicals requires the evaluation of toxicological endpoints sensitive during infancy. Although most human development occurs during the prenatal period, certain systems do continue to develop in humans after birth, as described below.

The prenatal period of development is critical, and prenatal exposures to xenobiotics may cause devastating developmental disruptions. For example, in humans, embryonic exposure to thalidomide is well-known to cause spontaneous abortion and/or severe limb defects ([Vargesson, 2009](#)). Also, as

discussed in [Section 4.2.3](#), developmental processes that occur prenatally are often more sensitive to chemical disruption than those occurring later (i.e., in the postnatal period, which can include infancy, childhood, and even later lifestages).

However, certain systems do continue to develop in humans postnatally. For example, neurodevelopment occurs over a very prolonged period in humans, and there are different components of neurological function that develop prenatally and postnatally ([Adams et al., 2000](#); [Rice and Barone, 2000](#)). For example, Verner et al. ([2010](#)) used a PBPK model for PCB 153 in humans to identify critical windows of exposure during which PCBs may impair human infant attention and activity level (assessed at 11 months of age by video coding of infants' behavior during the administration of the Bayley Scales of Infant Development). The model was used to simulate infant blood PCB 153 levels at delivery and on a month-by-month basis during the first year of life. The association between inattention and estimated infant blood PCB 153 level was greatest (larger standardized beta in multivariable regression models) and only statistically significant at delivery, suggesting that prenatal exposure has the strongest effect on this neurobehavioral domain. On the other hand, non-elicited activity duration, another neurobehavioral domain, was only significantly associated with postnatal exposure estimates: the strongest association was with the estimated level of PCB 153 in infant blood at approximately 4 months of age. Whether these associations are attributable exclusively to either prenatal or postnatal exposures remains unclear as regression models were not adjusted for exposures during other time windows (i.e., regression models of prenatal exposure were not controlled for postnatal exposure and vice versa).

Pre- and postnatal exposure to organophosphate pesticides has been associated with impaired neurobehavioral development in infants, toddlers and preschoolers. Neurodevelopmental effects associated with prenatal exposure include abnormal reflexes, impaired performance on both the psychomotor and mental development components of the Bayley Scales of Infant Development, poorer cognitive abilities, maternally reported pervasive developmental disorder, and inattention ([Bouchard et al., 2011](#); [Marks et al., 2010](#); [Engel et al., 2007](#); [Eskenazi et al., 2007](#); [Rauh et al., 2006](#); [Young et al., 2005](#)). Postnatal organophosphate exposure has been associated with the following neurodevelopmental effects: diagnosis of attention-deficit/hyperactivity disorder (ADHD); maternally reported pervasive developmental disorder; and longer reaction time ([Bouchard et al., 2010](#); [Eskenazi et al., 2007](#); [Grandjean et al., 2006](#)). Gascon et al. ([2011](#)) reported an association between postnatal exposure to PBDE 47 and certain symptoms of ADHD and poor social competence in children at 4 years of age.

Immune and pulmonary endpoints are also susceptible to postnatal disruption ([Dietert et al., 2000](#)). The immune system continues to develop after birth, and neonatal dioxin exposure has been associated with decreased allergy at 8 years of age ([ten Tusscher et al., 2003](#)). Although decreased allergy may not seem like an adverse effect, it may serve as an indication that some aspect of immune function is impaired. For example, allergic disease has been shown to be reciprocally related to incidence of infection ([Cookson and Moffatt, 1997](#)). And, ten Tusscher et al. ([2003](#)) also observed persistently decreased platelet count, increased thrombopoietin, and increased CD4+ T-helper and CD45RA+ cell

counts in children postnatally exposed to dioxin. Also, a large portion of bronchial and lung development occurs postnatally in both humans and rodents ([Miller and Marty, 2010](#); [Dietert et al., 2000](#)). For example, in humans, alveolarization continues through at least age two, and growth of the lung continues through late adolescence. Evidence is accumulating that developmental exposure to some chemicals may disrupt lung development. For example, maternal exposure to air pollution during pregnancy has been shown to increase asthma risk in offspring ([Ho, 2010](#)). Exposures to environmental factors during the first year of life have also been shown to impact asthma risk ([Peden, 2000](#)). Prenatal or postnatal exposures may alter early development of the airways and immune system, resulting in permanent alterations that create a lasting vulnerability to asthma later in life ([Ho, 2010](#); [Dietert and Zelikoff, 2008](#)).

Some aspects of reproductive development can be altered by early life exposure to PBT chemicals in both humans and laboratory rodents ([Lemasters et al., 2000](#)). For example, human male infants have a “mini-puberty” at 2-5 months of age when Sertoli cell number increases followed by a quiescent phase until puberty; rodent species pass on to puberty without a quiescent phase ([Zivkovic and Hadziselimovic, 2009](#)). Reduction in Sertoli cell numbers, which may result from chemical exposure during this sensitive period of “mini-puberty”, leads to reduced sperm counts in adulthood ([Aafjes et al., 1980](#)). Furthermore, pubertal timing in males and females may be affected by pre- and postnatal chemical exposures ([Schoeters et al., 2008](#); [Blanck et al., 2000](#)).

Proper endocrine function is critical for both fetal and postnatal development. For example, even transient changes in thyroid hormones during critical periods of growth may lead to adverse neurological outcomes ([Williams, 2008](#); [Glinioer, 2001](#)). The timing of thyroid deficiency (early pregnancy, late pregnancy, postnatal) and its degree are critical to the type and severity of neurological deficit exhibited in infants and children ([Zoeller and Rovet, 2004](#)). Visual processing, memory and gross motor skills are predominantly affected by hormone insufficiencies in early pregnancy, visuospatial skills and fine motor deficits characterize insufficiencies in later stages of pregnancy, and hormone deficiencies in the late-stage fetus and neonate result in deficits in language skills and verbal memory. Therefore, thyroid hormone is essential across all stages of fetal and neonatal brain development ([de Escobar et al., 2008](#); [Zoeller and Rovet, 2004](#)). Thyroid hormone is also important for normal lung development ([van Tuyl et al., 2004](#)). The ratio of surfactant protein mRNA expression to that of corresponding proteins was influenced by both prenatal and postnatal thyroid hormone deficiency. Developmental exposure to PBT chemicals has been observed to effect thyroid hormone homeostasis. For example, perinatal dioxin exposure has been associated with increased levels of thyroid stimulating hormone (TSH) ([Koopman-Esseboom et al., 1994](#); [Pluim et al., 1993](#)) and decreased levels of both triiodothyronine (T3) and thyroxine (T4) in the blood of infants ([Nagayama et al., 1998](#)). Notably, the blood thyroid hormone levels observed in infants in these studies were all within the normal range ([LaKind et al., 2008](#)). However, because proper endocrine function is essential to support developmental processes, it may be important to consider the potential for known endocrine-disrupting chemicals to impact both prenatal and postnatal development.

Epigenetic changes, such as DNA methylation, might serve as biomarkers of developmental effects, enabling the early detection of effects programmed during development that do not manifest until much later in life. Because DNA methylation is easy to detect in small quantities of commonly-sampled biological tissues (e.g., blood), development of validated biomarkers based on DNA methylation may facilitate the detection of health effects and their associations with perinatal chemical exposures in human populations.

Currently, a great deal of work has been published to support the use of DNA methylation as a biomarker for cancer ([Li et al., 2012](#)). In recent years, it has been recognized that exposure to environmental carcinogens specifically during development may lead to increased cancer risk ([Anderson et al., 2000](#)); this may be true even for chemicals with non-mutagenic modes of action, such as endocrine disruptors ([Birnbaum and Fenton, 2003](#)). Thus, there may be a developmental aspect to the carcinogenic mode of action for some chemicals, and developmental exposure to carcinogens may impact cancer risk to a greater extent than non-developmental exposure. It is thought that altered DNA methylation plays a role in carcinogenesis and promotes cancer progression by activating oncogenes, inhibiting tumor suppressor genes and inducing chromosome instability ([Li et al., 2012](#)). Many studies have used DNA isolated from blood samples, or buccal cells, to investigate DNA methylation as a diagnostic biomarker of cancer. Associations between specific DNA methylation patterns and risk have been identified for breast cancer, bladder cancer, colon cancer, colorectal adenoma, gastric cancer, head and neck squamous cell carcinoma, lung cancer, ovarian cancer, nasopharyngeal carcinoma, pancreatic cancer, and prostate cancer. Once validated, some of these methylation patterns might be useful as biomarkers in epidemiological studies to detect associations between developmental exposures and increased cancer risk.

Epigenetic markers may be similarly useful for the detection of non-cancer endpoints. For example, asthma risk, stated above to be increased as a consequence of prenatal and postnatal environmental exposures, is also programmed during development based on epigenetic changes ([Ho, 2010](#)). Increased hypermethylation of the promoter region of the gene for acyl-CoA synthetase long-chain family member 3 in umbilical cord blood DNA was associated with both increased maternal exposure to polycyclic aromatic hydrocarbons (PAHs) during pregnancy and increased asthma risk of offspring in childhood ([Perera et al., 2009](#)). Once validated, methylation at this site may be used as a biomarker of both increased asthma risk and prenatal exposure to PAHs.

Also, altered metabolic programming during the prenatal and postnatal periods may result in obesity and insulin resistance, which are major risk factors associated with type 2 diabetes and cardiovascular disease ([Plagemann et al., 2012](#); [Dyer and Rosenfeld, 2011](#)). Prenatal exposures to PCBs, DDE and DDT have been linked to increased body mass index in children at age 6.5 years ([Valvi et al., 2012](#)), and studies of children exposed to secondhand tobacco smoke provide support for the idea that postnatal exposure to environmental chemicals may have lasting impacts on metabolic health. For example, exposure to secondhand tobacco smoke during childhood has been associated with being overweight at age 6 years ([Raum et al., 2011](#)) and insulin resistance at age 10 years ([Thiering et al., 2011](#)). But, these

outcomes have not been adequately studied with regard to postnatal exposures to specific PBT chemicals. Metabolic programming is driven by epigenetic changes ([Liguori et al., 2010](#)). Some of these changes might be exploited as biomarkers in epidemiological studies to detect associations between chemical exposures during development and increased risk of metabolic disease in later life. For example, the promoter region of the gene for peroxisome proliferator activated receptor  $\gamma$  coactivator-1  $\alpha$  is hypermethylated in DNA isolated from the pancreatic islets of patients with type 2 diabetes ([Ling et al., 2008](#)). Although this is an interesting finding supporting the idea that epigenetic biomarkers of diabetes may have future diagnostic, and perhaps predictive, utility, more research is needed to identify epigenetic biomarkers for metabolic disease in more accessible biological tissues.

#### **4.2.2. Identifying Critical Windows of Exposure**

To better understand the relative importance of PBT chemical exposures at different lifestages, workshop participants emphasized the need to delineate the critical and/or sensitive windows of exposure during which infants are more vulnerable to chemical insults. This can be achieved in both experimental and epidemiologic settings where dose-response relationships are characterized for different periods of exposure. As mentioned previously, human data are preferred for use in risk assessment. But, in studies of human exposure to PBT chemicals, it can be difficult to determine whether a developmental effect resulted from prenatal exposure, postnatal exposure or both. Some analytical techniques have been applied to help establish the critical window of exposure related to a given effect in humans. For example, the impact of PBT chemical exposure on neurodevelopmental effects has been compared between breastfed (pre- and postnatal exposure) and formula-fed (prenatal exposure only) infants. Patandin et al. ([1999](#)) found that increased prenatal PCB exposure was strongly associated with decreased scores on the Kaufman Assessment Battery for Children in formula-fed infants at 42 months of age. In contrast, none of the associations between PCB exposure and performance on these tests approached statistical significance in breastfed infants. Similarly, after dividing the study population into children who were breastfed for more than 6 weeks and those who were not, Jacobson and Jacobson ([2002](#)) reanalyzed data from earlier studies linking prenatal PCB exposure to cognitive deficits assessed using the McCarthy Scales of Children's Abilities at 4 years of age and the Wechsler Intelligence Scales for Children, the Woodcock Reading Mastery tests, and the Wide Range Achievement tests at 11 years of age. They found the same pattern that was reported by Patandin et al. ([1999](#)): PCB-related decrements in cognitive functioning were observed only in children who had not been breastfed. These results support an association between neurodevelopmental effects and prenatal exposure to PCBs. Furthermore, they support the idea that breastfeeding itself is beneficial to cognitive development and may, to some extent, compensate for the detrimental effects of prenatal exposure ([Pan et al., 2009](#); [Boersma and Lanting, 2000](#); [Koopman-Esseboom et al., 1996](#)). However, at least some of the infants in the "formula-fed" group of the study by Jacobson and Jacobson ([2002](#)) were breastfed during the first six weeks of life, and it is possible that postnatal exposure during this early period of infancy contributed to observed cognitive deficits.



Another method for distinguishing between pre- and postnatal exposures to PBT chemicals in epidemiological studies has been to use a measure of breast milk levels multiplied by total breastfeeding duration as a postnatal exposure metric. Using PCB 153 data from Inuit mothers and children in the Nunavik region of Canada, Ayotte et al. (2003) found that a model based on maternal body burden and breastfeeding duration explained only 36% of infant PCB 153 plasma concentration variance at 6 months of age. Furthermore, even if it were completely accurate, a postnatal exposure metric calculated by multiplying maternal body burden by breastfeeding duration is only reliable if accumulated postnatal exposure is the important determinant of developmental toxicity. Following the same general assumption, another popular measure of postnatal exposure is the child's plasma or serum chemical concentration at the time of testing. But, it is important to consider that development occurs in stages, and the timing of toxicant exposure may be as important as the accumulated dose in determining the damage that results (Rice and Barone, 2000). Therefore, these measures of accumulated exposure may not be the most relevant metrics for developmental endpoints that are most vulnerable during a distinct time period. For example, if the development of a particular neurological function is most susceptible to disruption during the first month of life, then the most relevant exposure metric would be a measure of infant exposure during the first month of life rather than a measure of accumulated exposure at a later time point. Measures of accumulated exposure may be useful for identifying effects resulting from exposure during the postnatal period, but it is important to acknowledge that these measures may also lead to misclassification error if the critical window of exposure ends relatively soon after birth.

Toxicokinetic models can be used to estimate internal dosimetry of the breastfed infant using information on (1) lipid-adjusted concentration of a chemical in maternal blood, cord blood or breast milk samples, and (2) maternal and infant physiology (i.e., infant sex, maternal age at pregnancy, duration of breastfeeding, weight and height of both infant and mother). This information, used with an adequate lifestage PBPK model, can allow the estimation of internal concentrations vs. time profiles from birth all the way up to 45 months. Depending on the hypothesized mechanism of toxicity, relevant estimates of exposure can be abstracted from these profiles. For compounds that can exert their toxicity at any period, estimations of peak body burden or accumulated exposure (e.g., area under the concentration vs. time curve) can be used. Conversely, measures of body burden during a specific period can be used if the developmental process can only be altered over a certain period. This kind of exposure estimation approach can be used in epidemiological studies to identify the critical windows of exposure in humans by assessing the associations that may exist between effects and exposures at different time periods (e.g., every month after birth). This was the method used by Verner et al. (2010) to demonstrate that non-elicited activity and inattention in children at 11 months of age are best associated with PCB 153 exposures at 4 months of age and during pregnancy, respectively.

Information on critical windows of exposure may also come from studies in laboratory animals. A cross-fostering study design can be used to isolate prenatal and postnatal exposures (Holladay and Smialowicz, 2000). In a cross-fostering study, some offspring are removed from their dams at birth and raised by surrogates. The unexposed control group consists of offspring born to and nursed by unexposed dams. Effects of prenatal exposure are assessed using offspring born to exposed dams but

nursed by unexposed dams. Similarly, effects of postnatal exposure are assessed using offspring born to unexposed dams but nursed by exposed dams. The effects of exposure during both gestation and lactation can be observed in offspring born to and nursed by exposed dams. Examples of cross-fostering studies are cited in the briefing materials for HCB ([Section 3](#) and [Appendix B.3](#)) ([Mendoza et al., 1978](#); [Mendoza et al., 1977](#)), chlordane ([Appendix B.1](#)) ([Ingle, 1952](#)), DDT ([Appendix B.2](#)) ([Craig and Ogilvie, 1974](#)), and mirex ([Appendix B.4](#)) ([Chernoff et al., 1979b](#); [Gaines and Kimbrough, 1970](#)). Alternatively, chemicals may be administered directly to neonatal animals to identify effects of postnatal exposure ([Makris et al., 2008](#)).

However, it is important to keep in mind that critical windows of exposure may be different between humans and laboratory animals ([Makris et al., 2008](#)). Effects triggered by postnatal exposure in some animal species may be induced by prenatal exposure in humans ([Selevan et al., 2000](#)). Also, in an animal such as a rat, a critical window of exposure may be a few days long while the same window in humans may last for weeks or months. Although some information relating critical windows in different species exists, more information is needed to fully understand the similarities and differences in windows of exposure that affect developmental processes in humans and laboratory animal species.

If the mode of action is known for a given chemical, this may provide some information on its potential to disrupt postnatal developmental processes. For example, dendritic architecture determines neuronal connectivity and has been observed to be abnormal in patients with neurodevelopmental disorders ([Webb et al., 2001](#)). Dendritic arborization is an example of a process that occurs postnatally in humans and is important in the establishment of dendritic architecture. Therefore, if a chemical is known to alter neurodevelopment through an effect on dendritic arborization, then it may be plausible for that chemical to exert its effect during the postnatal period in humans. On the other hand, if the chemical is known to cause an effect on neurodevelopment by inhibiting a molecular pathway that is only active in the fetal brain during gestation, then postnatal exposure to this chemical may be unlikely to cause the effect. However, it is important to note that chemicals often exert toxicity through more than one mode of action. And, even if a chemical is known to cause a particular effect through a mechanism relevant only during gestation, that does not preclude that chemical from producing other developmental effects during the postnatal period.

#### **4.2.3. Relative Magnitude of Prenatal and Postnatal PBT Chemical Exposures**

Most often, studies are not available to precisely define critical and/or sensitive windows of development and exposure. When data from animal studies identify health effects that could result from prenatal or postnatal exposure in humans, it is important to be able to predict infant exposure at both of these time periods, using models that can accommodate all the information that is known about a chemical, to estimate a maternal dose that will limit both prenatal and postnatal exposure to levels of minimal risk. Even if it takes a larger chemical exposure to an infant to disrupt a postnatal developmental process than it takes to disrupt prenatal development, for lipophilic chemicals, infants may receive a much larger chemical exposure through consumption of human milk than they do through

placental transfer prior to birth. Because of PBT chemical accumulation in body lipids over time and elimination in breast milk, it may be necessary to lower maternal exposure to a greater extent to limit lactational exposure to an infant than would be necessary to limit prenatal exposure. Therefore, when assessing effects of PBT chemicals on development, it is important to account for both prenatal and postnatal exposures.

In both humans and animals, during pregnancy, fetal blood levels of PBT chemicals are often very similar to maternal blood levels. However, this is not always the case. For some chemicals, there is significant transplacental movement leading to high levels of fetal exposure. For example, Kim et al. (2011) demonstrated that the ratio of perfluorotridecanoic acid (PFTTrDA) between umbilical cord and maternal serum is 1.93, indicating that the fetus is exposed to cord blood concentrations of this perfluorinated chemical that are nearly twice that of the mother. Similarly, Needham et al. (2011) found that concentrations of some lightly chlorinated PCBs were higher in cord serum than maternal serum (e.g., ratio of 2.2 for PCB 28). In other cases, chemical transfer across the placenta may be limited, resulting in a fetal dose that is low compared to maternal dose (You et al., 1999). And, the ability of a chemical to cross the placenta may differ between humans and animals, complicating the extrapolation of dose-response data from animal studies to human exposure scenarios. Despite the exceptions, in the absence of appropriate validated PBPK models, prenatal dose can be estimated based on maternal body burden as this will often be an accurate or conservative assumption.

However, because many PBT chemicals are stored in body lipids and eliminated via lactation, the daily dose received by nursing offspring during the postnatal period may be much higher than that received by the mother. This concept is supported by the observation that human infant blood levels of PBT chemicals are 4-5 times higher than maternal blood levels at the end of a 4-month breastfeeding period. In order for this phenomenon to be observed in an animal study, maternal exposure has to occur for some period of time prior to lactation to build up a store of chemical in maternal body lipids. As discussed in Section 5, the impact of maternal exposure prior to lactation is maximized if, before mating, a PBT chemical is administered until the chemical concentration in the dam's body lipids comes to steady state. In many reproductive and developmental studies in animals, dams are exposed for only a short time; it is relatively rare for the overall length of exposure to allow a dam's body burden to come to steady state prior to gestation.

Because of differences in elimination of PBT chemicals between humans and animals, even if the design of an animal study allows maximal accumulation of a chemical in maternal body lipids prior to lactation, the ratio between offspring lactational exposure and maternal exposure in that study may be far smaller than the ratio between infant lactational exposure and average daily maternal exposure in humans exposed to the same chemical. This difference arises from differences in elimination of PBT chemicals between humans and animals and differences in the number of concurrent offspring. For example, the elimination half-life, a major factor influencing the bioaccumulation of a PBT chemical, may be much longer in humans than it is in animals (Poiger and Schlatter, 1986), contributing to a greater PBT chemical accumulation in maternal body lipids and a greater lactational dose in humans. Additionally,

rodents typically nurse litters of offspring, each of whom gets only a share of the total milk supply and any chemicals present in milk. Thus, the ratio between individual offspring lactational dose and maternal dose may be smaller than it is in humans, who commonly nurse only one infant.

### 4.3. Variability and Uncertainty in Modeling Data and Parameters

As discussed in [Section 6](#), workshop participants developed and evaluated modeling approaches to support dose-response and exposure assessment for lactational exposures to PBT chemicals. Using accurate or representative parameters in these models is critically important for accurate results, especially when using the simpler models discussed in [Section 6](#). For some parameters, particularly the half-life of the chemical, varying model input can change output by an order of magnitude or more. However, it can be challenging to determine the best number to use for a given parameter because of variability and uncertainty. Some of the modeling parameters used in the approaches discussed in [Section 6](#) are species-specific, others are chemical-specific, and the rest are both species- and chemical-specific. Species-specific parameters often vary, as individual members of a species differ in many ways. At times, species-specific parameters may also be uncertain due to limited data availability. And, chemical-specific data are often limited, leading to significant uncertainty in many chemical-specific parameters. Discussed below are methods to address variability and uncertainty in the parameters used in models that were identified by workshop participants.

#### 4.3.1. Variability in Species-Specific Parameters

Variability is an important factor to address for the following species-specific parameters, among others: maternal age at pregnancy; body weight; adiposity; milk lipid content; milk intake; duration of lactation; parity; previous breastfeeding duration; and years since previous breastfeeding. The modeling approaches discussed in [Section 6](#) may require estimates of these parameters for human women, adult female animals, human infants, and animal offspring.

Issues relevant to individual parameters are listed below:

**Body Weight** – As discussed below, in animal studies, offspring body weight may vary as a function of litter size, which is another parameter required by some of the modeling approaches.

**Adiposity** – Some modeling approaches can accommodate increases or decreases in maternal adipose tissue during gestation and lactation, but these parameters are also highly variable. Furthermore, more sophisticated modeling approaches may benefit from estimates of lipid levels in distinct tissues. These values are expected to vary both within and across species, but tissue-specific data are often unavailable.

**Milk Lipid Content** – At least in humans, this parameter changes across the period of breastfeeding. Some modeling approaches can accommodate these changes ([U.S. EPA, 1998](#)). But, in fact, this

parameter varies even over the course of a single feeding. Foremilk, which is expressed in the beginning of a feeding, contains mostly water. It is not until closer to the end of a feeding when the hindmilk, which is rich in lipids, is expressed. This high fat milk is where chemical doses are especially large. It can be challenging to account for this because of inter-individual variability in feeding duration and the rate of milk ingestion.

**Milk Intake** – In humans, there is often a period of time when an infant is still breastfeeding but is also receiving nutrition from supplemental sources, such as formula or solid foods. The length of this time period and the extent of supplementation are both highly variable across the population. In animals, milk intake may vary with litter size, which is another parameter required by some of the modeling approaches. In general, offspring in smaller litters consume more milk, and those in larger litters consume less. As a consequence, offspring body weight may also vary as a function of litter size.

**Parity, Previous Breastfeeding Duration, Years Since Previous Breastfeeding** – These are all parameters known to impact chemical concentrations in breast milk. Because PBT chemicals may be eliminated during lactation, doses to the first breastfed child are expected to be higher than those to subsequent children, although data indicate that this cannot be assumed for every member of this chemical class ([LaKind et al., 2009](#)). Nonetheless, if maternal exposure is limited to a level posing minimal risk to the first child, later children may also be protected. For this reason, the approaches outlined in [Section 6](#) assume a parity of 1.

#### 4.3.2. Methods to Address Variability

For parameters that vary across the human population, average values can be used in the modeling approaches described in [Section 6](#). However, when a parameter varies across a large range, the difference in model output using an average value may differ significantly from that using a 5<sup>th</sup> or 95<sup>th</sup> percentile value. Consequently, the results of risk analysis using the model output may be very different depending on which parameter value is used. In the interest of public health protection, it is often considered desirable to assess risk to the susceptible members of the population. If risk to the susceptible members of the population is minimized, then the health of those less susceptible is also protected. In order to identify populations susceptible to lactational PBT chemical exposure, it is necessary to account for variability in the parameters mentioned above and to assess the impact that this variability may have on model output. To this end, stochastic modeling, discussed further in [Section 6](#), can be incorporated into modeling approaches in order to account for variability in the parameters used.

However, it is important to understand that some forms of human variability (i.e., toxicodynamic variability) cannot be addressed by stochastic modeling. It is possible that the effects and/or dose response of a PBT chemical are different across subgroups of the population (i.e., the potential impact of exposure at a given level may differ across population groups). For example, certain individuals may be more sensitive to *p,p'*-DDT exposure because glutathione S-transferase polymorphisms may modulate

how  $p,p'$ -DDT affects neurodevelopment ([Morales et al., 2008](#)). The most effective way to address interindividual variability in sensitivity or susceptibility is to gather toxicological dose-response data in the sensitive or susceptible population of interest or in an animal model of that population.

### 4.3.3. Uncertainty in Species- and Chemical-Specific Parameters

First and foremost, the determination of any experimentally-derived modeling parameter depends on the use of accurate methods of measurement. Uncertainty in the reliability of a study's measurements will lead to uncertainty in the parameters reported by the study and uncertainty in the output of models run using those parameters. When selecting parameters to use in the modeling approaches discussed in [Section 6](#), it is important to evaluate the methods used in the studies providing the parameters. If there is more than one study reporting values for the same parameter, it is preferable to use the value reported by a study using superior methodology. Parameter uncertainty may result if (1) there is only one study available for a given parameter, and (2) that study used inferior methodology.

Uncertainty was identified by workshop participants to be an important factor to address for certain species-specific parameters (i.e., milk lipid content and milk intake) and chemical-specific parameters (i.e., absorption fractions, log  $K_{ow}$ , half-life of elimination, and partition coefficients). The modeling approaches discussed in [Section 6](#) may require estimates of these parameters for human women, adult female animals, human infants, and animal offspring. Issues relevant to individual parameters are listed below:

**Milk Lipid Content** – In humans, because milk lipid content changes across the lactational period, breast milk lipid measurements based on samples taken at a single time point may not represent average lipid concentrations over the duration of breastfeeding. Also, milk lipid content varies even over the course of a single feeding. So, there may be some uncertainty in the value used for this parameter if it is based on breast milk lipid measurements from samples expressed only at the beginning of a feeding.

**Milk Intake** – It is difficult to estimate the amount of milk ingested by a human infant or a nursing animal. Milk flow is partly dependent on suckling. In some studies, suckling rate is a function of flow rate (i.e., the higher the milk flow rate, the lower the suckling rate) ([Bowen-Jones et al., 1982](#)). More research is needed in this area; estimates of milk intake are generally uncertain because they are based on assumptions.

**Absorption Fractions** – Absorption fractions in adult humans and/or animals are often unknown, but it is even more rare to have data to inform this parameter in human infants and animal offspring. Chemical absorption by the oral route can be influenced by factors such as gut flora, nutrition, and expression of active transporters. All of these factors are known to differ between adults and neonates; and, although the specific impacts of these differences on chemical absorption are not clear, it is reasonable to expect that absorption of a particular chemical may differ between adults and infants. For

example, although the exact mechanisms are not fully understood, lead is absorbed up to 5 times more in children than in adults ([Ginsberg et al., 2004](#)).

Furthermore, although the examples addressed at this workshop focused on the oral exposure route, chemical exposure may also result from inhalation and dermal routes. For exposure assessment purposes, it is important to have absorption fractions for each potential route of exposure. When absorption fraction data are unavailable or uncertain, it may be necessary to make the conservative assumption that 100% of the ingested (or inhaled) chemical is absorbed.

**Log  $K_{ow}$**  – Uncertainty may be an issue even for biochemical properties that are relatively straightforward to measure, such as log  $K_{ow}$ . For mirex ([Appendix B.4](#)), workshop participants identified several log  $K_{ow}$  values in the literature: NTP ([2011a](#)) gives a value of 5.28, McKim et al. ([1985](#)) report a value of 7.5, and Veith et al. ([1979](#)) provide a value of 6.89. Log  $K_{ow}$  is a critical parameter affecting the output of a number of modeling approaches. And, using mirex as an example, uncertainty in this parameter may result in model outputs ranging across two orders of magnitude depending on which log  $K_{ow}$  value is used. When there are multiple log  $K_{ow}$  values reported for a chemical, if one of them was derived using better methodology than the others, then that value should be used for modeling. If there is no obvious difference in validity among reported values, it may be necessary to choose the highest value as a conservative assumption.

**Half-Life (i.e., Elimination Rate)** – To calculate an elimination rate, the experimental value needed is a chemical's half-life, either in the whole body or in fat. Data for chemical half-lives are often unavailable or inconsistent. When available, chemical half-lives of elimination are often calculated based on serum, plasma or whole blood measurements. Chemicals enter breast milk from the blood, so blood distribution and the kinetics of the chemical in serum, plasma and blood are important. But, it is important to acknowledge that chemical concentrations in serum, plasma or blood may not accurately reflect total body burden. Half-life of a PBT chemical measured in serum, plasma or blood may be much shorter than the half-life in fat, where these chemicals are stored. There is some uncertainty regarding the impact that different types of lipid may have on chemical distribution. For example, chemical solubility in serum lipid may differ from that in milk fat or adipose tissue. It is common to assume that distribution to lipid is the same, regardless of the lipid type. But, research is needed to support or refute this assumption. Good correlations have been shown between maternal serum and breast milk concentrations for some chemicals from matched samples taken close in time, as has been illustrated for PBDEs ([Marchitti et al., In Press](#)). However, there are some indications in the literature that some PBT chemicals may distribute differently to different lipid compartments ([LaKind et al., 2009](#)). And, a two phase elimination process has been described for some PBT chemicals, consisting of a faster first phase of elimination from blood followed by a slower second phase of elimination from fat compartments. As discussed in the DDT briefing packet ([Appendix B.2](#)), for some PBT chemicals, modeling approaches have been adapted to accommodate a “deep fat” compartment. The partition coefficient from the fat to the deep fat compartment is assumed to be higher than from the deep fat to the fat compartment, which means that the deep fat compartment essentially sequesters the chemical



where it is not readily available for elimination. Further evidence that serum chemical concentrations may not provide an accurate measure of body burden comes from studies that have tracked serum chemical levels during lactation. Since lactation is an elimination mechanism for PBT chemicals, it is expected that maternal body burden will decrease throughout the lactational period, assuming that maternal exposure during lactation is not significantly greater than it was prior to lactation. However, studies measuring maternal serum chemical concentrations at different times during lactation have observed highly variable changes: serum chemical concentrations decrease in some women, but they stay the same or increase in other women ([LaKind et al., 2009](#)). These results suggest that changes in body burden may not be reliably detected by measuring concentrations of a chemical in serum.

Aside from the difficulties in measuring chemical half-lives in whole body lipids based on serum, plasma or blood samples, there are additional issues that contribute to uncertainty in half-life estimations in humans. For ubiquitous exposures, including exposures to many PBT chemicals, the interpretation of human data may be complicated by ongoing exposure. Also, because lipophilic PBT chemicals are stored in body lipids, changes in body composition influence their apparent half-lives ([Milbrath et al., 2009](#); [Chevrier et al., 2000](#)). An increase or a reduction in the size of the lipid compartment will change chemical concentrations and distort half-life estimations. Studies measuring the half-lives of chemicals in humans do not always control for variables like weight gain or loss, and this might contribute to some inconsistency across studies. Notably, chemical half-lives in offspring appear to be shorter than those in adults, at least partly because of changes in body composition, as discussed above. Other factors that may contribute to differences in chemical half-lives between adults and offspring include (1) differences in the expression of active transporters and various enzyme detoxification systems, and (2) changes in kidney function with age that might facilitate chemical excretion ([Clewett et al., 2004](#); [Gentry et al., 2003](#)).

When half-life data are unavailable or uncertain, it may be necessary to use modeling approaches that do not include this parameter ([Section 6](#)). Workshop participants mentioned that data from the National Health and Nutrition Examination Survey (NHANES) might be used to estimate the half-life of a chemical in humans. NHANES is a major program of the Centers for Disease Control and Prevention (CDC) designed to assess the health and nutritional status of adults and children in the United States (<http://www.cdc.gov/nchs/nhanes.htm>). The survey collects data on environmental exposures based on measures of environmental chemicals or their metabolites in blood or urine. Ritter et al. ([2011](#)) utilized a population modeling approach with cross-sectional biomonitoring data similar to that found in NHANES to estimate half-lives for individual PCB congeners. In their model, they corrected for the effects of changes in body composition and ongoing exposure using the ages of study participants and empirical daily intake data derived from total diet studies.

**Partition Coefficients** – Partition coefficients are required in order to use the more sophisticated PBPK modeling approaches described in [Section 6](#). These values quantify the partitioning of a chemical between blood and organs and tissues such as fat, muscle, mammary tissue and liver. If these



parameters are not available or are highly uncertain, then it may be necessary to use simpler modeling approaches.

#### 4.3.4. Methods to Address Uncertainty

Workshop participants suggested that the impact of missing or uncertain parameters on simulated dosimetry for durations of 1 or 2 years in the human infant be evaluated, possibly through the use of a global sensitivity analysis ([Saltelli et al., 2008](#)). If modeling is very sensitive to uncertain parameters, then conservative assumptions may be needed. But, if modeling is not very sensitive to the uncertain parameters, then estimated values might be used without substantially increasing the uncertainty in the model output. Also, data from epidemiological studies could be used as a check on the most influential parameters, such as half-life estimates used in the simpler modeling approaches. For example, if the first order kinetic model is run with the correct half-life, it should be able to roughly predict observed epidemiologic data. If it does not, then it may be necessary to use modeling approaches that do not include half-life as a parameter. Most importantly, when the results of a dose-response assessment are reported, the uncertainty in the overall assessment and in each of its parameters must be transparently acknowledged.

### 4.4. Metabolism

Metabolism has a profound effect on chemical kinetics. Because of this, workshop participants identified additional considerations important for PBT chemicals that are metabolized by humans and/or laboratory animals. Metabolism rates and pathways often differ substantially among animal species ([Thompson et al., 2008](#)). In order to use data from animal studies in human health risk assessment of metabolized chemicals, it is important to delineate the metabolic pathways of the chemical in all relevant species. For example, biliary excretion may differ significantly between animals and humans. Also, patterns of metabolic enzyme expression change over the course of development such that the metabolic pathway for a given chemical may differ in children and adults ([Hines, 2008](#)). Thus, when assessing risk of developmental effects in young children, it may also be important to delineate metabolic pathways of the chemical at different lifestages. Furthermore, when animal data are used, it is important to note that the timing of metabolic enzyme expression during development may differ between species. Therefore, data comparing metabolic pathways in adult laboratory animals to those in animal offspring may be useful. Metabolic pathways can be delineated *in vitro* using species- and lifestage-specific hepatocytes, microsomal fractions and/or metabolic enzymes. So, data on active metabolic pathways and metabolites produced as a result of chemical exposure in different species may be available or easily attainable. However, it can be more challenging to determine metabolic rates in an intact organism.

If a PBT chemical is readily converted to easily eliminated metabolites, then that chemical will be unlikely to accumulate in maternal fat stores, and infant lactational dose may not be higher than

average daily maternal exposure. However, some PBT chemicals are metabolized slowly or incompletely in humans, and some PBT chemical metabolites persist, accumulate in body lipids, and partition into breast milk. Metabolism may impact not only kinetics but also toxicity: the types of health effects caused by a metabolite may differ from those caused by its parent compound ([Dekant, 2009](#)). For example, the metabolite, p,p'-DDE, is a much more potent androgen receptor antagonist than its parent compound, p,p'-DDT ([Kelce et al., 1995](#)). Thus, for chemicals that are metabolized, metabolism rates and whole-body half-life information are needed for the parent compound and all metabolites. For the extrapolation of dose-response data from animal studies to assess risk of postnatal developmental effects in breastfed human infants, it is especially useful to know metabolism rates and elimination half-lives for a parent compound and its metabolites in both humans and relevant laboratory animals. As discussed in [Section 6](#), when the appropriate data on metabolic pathways and rates are available, PBPK models can be used to address metabolism and metabolic differences between species and lifestages ([Thompson et al., 2008](#)).

Because of the potential for differences in metabolism between humans and animals, when effects observed in an animal study are used to inform hazard identification and dose-response assessment for lactational exposures to metabolized PBT chemicals, it is important to consider which chemical species (i.e., metabolite(s) and/or parent compound) may be responsible for the observed effects and to analyze whether and to what extent human infants might be exposed to those specific chemicals in breast milk. For example, workshop participants noted that it is very important to account for DDT metabolism in humans and laboratory animal species when selecting an appropriate study to use for hazard identification and dose-response assessment. As discussed in the DDT briefing packet ([Appendix B.2](#)), the three most persistent DDT constituents are p,p'-DDT, o,p'-DDT, and DDE. There is a high degree of variability among species in the metabolism of DDT and DDE over time ([WHO, 2011](#)):

**Rats:** DDT is metabolized to DDE and DDD. DDE and DDD are converted to DDA, with conversion from DDD being the relatively faster pathway.

**Mice:** DDT is metabolized to DDE and DDD. DDD is converted to DDA, but DDE is not ([Gingell and Wallcave, 1974](#)).

**Rabbits:** DDT is metabolized to DDE and DDD ([Hart et al., 1972](#)).

**Humans:** DDT is metabolized to DDD, which is further degraded and excreted as DDA ([ATSDR, 2002a](#)). The conversion of DDT directly to DDE is very limited in humans compared to rodents, and is a slow metabolic process. The limited amount of DDE created in humans persists for long periods of time in the fat, and DDE is not metabolized to DDA in humans.

Since exposure to “DDT” includes exposure to three PBT chemicals, it is important to explore the toxicity of all three, individually and in combination, including the potential impacts of these chemicals on postnatal development.

The study selected in the briefing packet ([Appendix B.2](#)) reported increased aggression at 3 months of age and decreased testes weight at 6 months of age in male mice following maternal exposure to 0.02 mg/kg-day o,p'-DDT on GD 11–17 ([Palanza et al., 1999](#)). Because mice metabolize o,p'-DDT to o,p'-DDE, the dose-response data from this study could be used to assess risk from perinatal exposure to these chemicals in humans. However, for this to be successful, modeling approaches would have to be able to account for o,p'-DDT metabolism in mice and humans. Alternatively, studies by Veeramacheni et al. ([2007](#)) or Yamasaki et al. ([2009](#)) might have been selected. Veeramacheni et al. ([2007](#)) reported reproductive effects in the offspring of rabbits exposed to 8.85 mg/kg-day p,p'-DDT from GD 15 to PND 28. Because rabbits metabolize p,p'-DDT to p,p'-DDE, the dose-response data from this study could be used to assess risk from perinatal exposure to these chemicals in humans. However, for this to be successful, modeling approaches would have to be able to account for DDT metabolism in rabbits and humans. Yamasaki et al. ([2009](#)) reported increased mortality and reproductive effects in the offspring of rats exposed to 50 mg/kg-day p,p'-DDE from GD 6 to PND 20. The dose-response data from this study could be used to assess risk from perinatal exposure to DDE in humans, but modeling approaches would have to be able to account for DDE metabolism in rats, which does not occur in humans. In order to fully explore the toxicity of p,p'-DDT, o,p'-DDT, and DDE, it may be necessary to use data from all three of these studies and to use modeling approaches capable of addressing metabolism in humans, mice, rabbits and rats.

## 4.5. Mixtures

While metabolism of some PBT chemicals in humans and animals creates a mixture of parent compound and metabolite(s) to which offspring may be exposed, other PBT chemicals exist as mixtures even in the absence of metabolism (e.g., chlordane, PCBs). And, human mothers may be exposed simultaneously to multiple components of these mixtures as well as many other chemicals (persistent and non-persistent) in the environment ([LaKind et al., 2008](#)). The current NHANES assessment reports human exposure to more than 200 chemicals. Mixture components may also be metabolized, further increasing the complexity of the chemical mixture present in human milk. Workshop participants noted that human health risk assessment based on exposure to individual chemicals may underestimate risk when populations of interest are exposed to chemicals with overlapping, additive or synergistic toxicities, but these chemicals are not included in the assessment.

U.S. EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* ([U.S. EPA, 2000](#)) recommends several approaches to quantitative health risk assessment of a chemical mixture, depending upon the type of available data. In the ideal approach, toxicity data on the mixture of concern are available, and the quantitative risk assessment is done directly from these data. Alternatively, when toxicity data are not available for the mixture of concern, use of toxicity data on a "sufficiently similar" mixture is recommended. If toxicity data are not available for the mixture of concern or a sufficiently similar mixture, U.S. EPA ([2000](#)) recommends that the mixture be evaluated through an analysis of its components (e.g., using dose addition for similarly acting chemicals and

response addition for independently acting chemicals). When data are available on the effects of interactions between components of the mixture, these data should be considered in the risk assessment. Otherwise, it is assumed that interaction effects at low dose levels either do not occur at all or are small enough to be insignificant to the risk estimate.

At this time, very limited toxicity data are currently available for the specific mixture(s) of PBT chemicals found in human milk ([e.g., Desaulniers et al., 2005](#)). However, for most individual PBT chemicals, toxicity data specific to postnatal developmental effects are also unavailable. Thus, as discussed in [Section 5](#), additional toxicological research along these lines is needed to apply the assessment approaches recommended by U.S. EPA ([2000](#)) to postnatal developmental risk. There are some toxicity data available for PBT chemicals, such as chlordane, that exist as mixtures in the environment. Evaluation of approaches to assess postnatal developmental risk from exposure to these relatively simple mixtures illuminates some issues that will be important for future attempts to consider the full ensemble of chemicals present in human milk.

As discussed in the chlordane briefing packet ([Appendix B.1](#)), technical-grade chlordane is a mixture of about 10 major constituents, including trans-chlordane, cis-chlordane, heptachlor, and trans-nonachlor ([ATSDR, 1994](#)). Oxychlordane and heptachlor epoxide are persistent metabolites of chlordane constituents. In humans, following metabolism, the most persistent constituents of the chlordane mixture are trans-nonachlor, oxychlordane, heptachlor epoxide and cis-nonachlor ([Dearth and Hites, 1991a](#)). But, there are at least six additional bioaccumulative components of technical chlordane beyond these four relatively well-studied constituents. There are also “contaminant” compounds in technical chlordane, and their persistence in the environment and in human tissue has not been thoroughly investigated. Metabolism of chlordane in rats is nearly identical to metabolism in humans, but rats metabolize trans-nonachlor to trans-chlordane, and humans do not. It is unclear whether or not mice are able to metabolize trans-nonachlor to trans-chlordane.

Exposure to “chlordane” includes exposure to numerous PBT chemicals, and it is important to explore the toxicity of all of these, including the potential impacts of these chemicals on postnatal development. The study selected in the briefing packet ([Appendix B.1](#)) reported behavioral effects in offspring following exposure of rat dams to 0.10 mg/kg-day technical chlordane via the diet during gestation and lactation ([Cassidy et al., 1994](#)). The dose-response data from this study could be used to assess risk from perinatal exposure to chlordane in humans. However, modeling approaches would be needed to account for chlordane metabolism in rats and humans. And, because rats metabolize trans-nonachlor to trans-chlordane, offspring of rat dams dosed with a mixture containing trans-nonachlor may not be exposed to trans-nonachlor: it may be metabolized by the dam before it reaches the pups via lactation. Humans do not metabolize trans-nonachlor to trans-chlordane, so human infants may be exposed to trans-nonachlor in breast milk. Reliance on this study may fail to address endpoints that could result from offspring exposure to trans-nonachlor. A more comprehensive approach might include data from additional studies in which rat offspring were exposed directly to trans-nonachlor.

Because of inter-species differences in metabolism of chlordane constituents, the proportions of the various constituents and metabolites that reach offspring in milk may be very different in laboratory animal species and humans. Thus, it is important to develop a clear understanding of the relative toxicities of individual chlordane components and metabolites. Otherwise, there will be significant uncertainty in the predictive power of animal studies administering technical chlordane to pregnant or lactating animals. It would be desirable to have comprehensive toxicological data from studies administering individual chlordane constituents and metabolites (e.g., trans-nonachlor, oxychlordane, heptachlor epoxide, cis-nonachlor) to pregnant and/or lactating animals or directly to their offspring to evaluate postnatal developmental endpoints. Toxicological data on various combinations of mixture constituents can be compared to data on individual constituents to detect interaction effects (e.g., synergy or antagonism).

If mixture components share common metabolic pathways, competitive inhibition of metabolism can be a factor for high dose exposures. It is important to understand and consider this when extrapolating the results of high-dose animal studies to environmentally relevant human exposures.

Estimation of half-life is complicated for mixtures because different components of the mixture (and their metabolites) are eliminated at different rates. To extrapolate an estimate of offspring exposure in animals to humans, the half-life used in the animal model should be for the same mixture component (or metabolite) as the half-life used in the human model. Ideally, this mixture component (or metabolite) would also be linked to the toxicity observed in the animal study. Modeling approaches would also have to be used to account for inter-species differences in distribution and metabolism of mixture components in order to be able to predict behavior of the mixture based on the half-life of a single component. It may be more accurate to model each mixture component and metabolite individually, but this would require separate half-life estimates for each mixture component and metabolite in all relevant species.

Workshop participants suggested future steps that researchers could begin to facilitate a more comprehensive assessment of risk from early life exposures that would consider the entire mixture of PBT chemicals found in human milk in place of the assessment of only one chemical at a time. In U.S. EPA's *Framework for Cumulative Risk Assessment* ([2003a](#)), approaches are provided to deal with complications in risk assessment caused by multiple chemicals, exposures and effects. These approaches would likely apply to the assessment of risk from exposure to mixtures of chemicals found in breast milk. Furthermore, the collection of biomonitoring data might allow for the determination of the composition of a "typical" breast milk PBT chemical mixture to which infants may be exposed. Other possible factors that may be necessary or useful to consider include differences in the content of the PBT chemical mixture found in breast milk that is representative of a national population in contrast to that of a specific geographical region (e.g., near pollution sources) or a particular lifestyle choice (e.g., sportfish consumption) ([Lakind et al., 2004](#)). Next, toxicological research in animals might be conducted using PBT chemical mixtures based on the findings of biomonitoring data to identify hazards and to gather mixture-specific dose-response information on postnatal developmental toxicity. Toxicological testing of

the wide array of chemical mixtures found in the environment may yield information important for assessing the risk of health effects in various populations.

Mixtures of chemicals found in human milk might also be tested using *in vitro* high-throughput screening assays to detect disruption of signaling pathways critical for normal development. Pathways used extensively in early prenatal development (i.e., before organogenesis and cytodifferentiation) include Wntless-Int, Transforming Growth Factor  $\beta$ , Hedgehog, Receptor tyrosine kinase, Notch-Delta, and Cytokine receptor ([NRC, 2000](#)). Pathways important later in prenatal development (i.e., during organogenesis and cytodifferentiation) include Interleukin-1-Toll Nuclear Factor-Kappa B, Nuclear hormone receptor, Apoptosis, and Receptor phosphotyrosine phosphatase ([NRC, 2000](#)). As discussed earlier, epigenetic changes established in early life modulate gene expression during development. Some of these changes are permanent, and through epigenetic mechanisms, exposures in early life may increase the risk of developing chronic diseases in later childhood or adulthood (e.g., asthma, type 2 diabetes, cardiovascular disease). With this in mind, it may be important to include methods for detecting epigenetic alterations in any high-throughput screening panel meant to identify potential developmental toxicants.

In the future, epigenetic changes may also be exploited by epidemiological studies as biomarkers of exposure to particular chemical mixtures. For example, DNA methylation profiles may give some indication of the chemical mixture to which an individual or population has been exposed. An epigenome-wide scan of DNA from the cord blood of infants whose mothers did or did not smoke during pregnancy revealed differential methylation at 26 sites across 10 genes ([Joubert et al., 2012](#)). Thus, DNA methylation at these sites may indicate prenatal exposure to cigarette smoke. However, much research will be needed to identify patterns of epigenetic markers specific for the wide array of current environmental exposures.

## 5. STUDY DESIGN CONSIDERATIONS

As mentioned in [Section 4.1](#), estimating the risk of PBT chemical exposure to breastfed infants may cause concern among breastfeeding women and their families. Thus, it is important to have confidence in the results of human and animal studies that form the basis for these estimations. Workshop participants discussed this issue in breakout groups, and this section reports their observations regarding study designs that maximize the utility of resulting data for application to the assessment of risk from early life exposure. Elements of study design specific to human or animal studies are described below. But, whether a study is conducted in humans or animals, for the purposes of assessing potential health effects resulting from early life exposure, it is important to investigate developmental endpoints sensitive during infancy. For example, as described in [Section 4.2.1](#), postnatal exposures can impact neurodevelopment, immune function, asthma risk, reproductive development, and risk of developing metabolic disease later in life. Developmental studies measuring only endpoints sensitive during the prenatal period (e.g., severe congenital abnormalities) are not sufficient to support conclusions regarding the potential for risk from postnatal exposure. Also, as discussed in [Section 4](#), when studies are conducted to support the assessment of risk from exposure to a chemical, it is important that those

studies use state-of-the-art analytical techniques to measure chemical concentrations in environmental media or biological samples.

## 5.1. Human Studies

As discussed in [Section 1](#), information from human studies is preferred for use in risk assessment. And, for some PBT chemicals, such as DDT, there may be enough human data to adequately assess developmental risk without using animal data. However, modeling approaches may still be required to convert the dose metric from an epidemiological study, which, for PBT chemicals, is often a measure of maternal body burden, to a dose metric that can be used in conjunction with chemical concentrations in environmental media. And, even for DDT, a PBT chemical that has been relatively well-studied in humans, few epidemiological studies have assessed the effects of postnatal exposure or measured specific endpoints known to be sensitive during the postnatal period, such as impaired executive function, increased asthma risk, and changes in pubertal timing ([Section 4](#)).

Sometimes, human studies indicate an association between a chemical exposure and a developmental effect, but the sensitive window of development and its place in the life span are unknown, there is a lack of sufficient knowledge of the cellular and molecular mechanisms that produce the effect, and/or it is not possible to quantify the association between the effect and a particular chemical in humans. When the dose response for a particular chemical cannot be quantified using human data, and also in cases where human data are completely unavailable or where available human studies are not adequately powered to detect relevant developmental effects, animal studies may be used to identify hazards of exposure during development and to establish a dose response for resulting health effects. However, some developmental effects that occur in humans are difficult to evaluate in laboratory animal models, especially non-primate animals (e.g., effects on language development or IQ, some types of behavior and learning) ([Adams et al., 2000](#)). In these cases, human data from well-designed epidemiological studies are needed to support hazard identification and dose-response assessment.

Even when human data cannot be used as the basis for a quantitative dose-response assessment, they may be useful to support (1) the identification of sensitive populations, and (2) the human relevance of effects observed in animal studies. Also, as discussed in [Section 4](#), modeling approaches to extrapolate animal data to a human exposure scenario require input parameters based on human data. Building upon previous efforts to optimize the collection and use of biomonitoring data in a similar context ([Berlin et al., 2005b](#); [Berlin et al., 2005a](#); [Fenton et al., 2005](#); [Lakind et al., 2005b](#); [Lakind et al., 2005a](#); [Lakind, 2005](#); [Lakind et al., 2004](#)), workshop participants identified several types of human biomonitoring data that may be particularly useful to reduce uncertainty in assessments of the toxicological risk from early life exposure to PBT chemicals:

- *Data such as those found in NHANES* reveal the extent of exposure to a particular chemical across a population and establish exposure trends. NHANES data may also provide information



on common co-exposures, which can support assessment of risk associated with exposure to chemical mixtures.

- *Maternal exposure data* (e.g., dietary exposure) collected in the same populations in which chemical concentrations in breast milk are measured. These data serve to inform the relationship between average daily maternal exposure and infant lactational dose of the PBT chemical.
- *Lipid-adjusted PBT chemical concentrations in breast milk, serum and adipose tissue samples from the same individuals.* These data would be useful for understanding chemical distribution across various lipid compartments in the body. Ideally, these studies would also measure lipid-adjusted chemical concentrations in serum from breastfeeding infants born to those same women. This information could be used in a model to determine the infant lactational dose of a PBT chemical. Other biological samples that could be analyzed for PBT chemical content to inform and/or evaluate predictions of chemical kinetics in the infant include placenta, amniotic fluid, cord blood, meconium, infant blood and infant feces.
- *Lipid-adjusted PBT chemical concentrations in serum taken from the same women over time* that (1) include estimates of average daily exposure to the chemical from environmental media, and (2) control for changes in body weight. These data could be used to derive estimates of the elimination half-life in humans.

As discussed in [Section 4.2](#), estimating infant internal concentration vs. time profiles can prove to be very useful to unravel associations between chemical exposures and health effects as well as to determine critical windows of exposure ([Verner et al., 2010](#)). In order to develop these profiles, epidemiologists need to collect information on mothers and their infants that can be used to determine all pertinent physiological parameter values essential for PBPK modeling (i.e., infant sex, maternal age at pregnancy, duration of breastfeeding, weight and height of both infant and mother). Furthermore, a lipid-adjusted measure of chemical concentration in maternal blood near the time of parturition is very important. This information can be used together with chemical-specific parameters (i.e., half-life and  $K_{ow}$ ) to estimate infant internal exposure, which can then be used in epidemiological analyses to identify associations that may exist between exposure and health effects.

## 5.2. Animal Studies

In [Section 6](#), modeling approaches are presented that will facilitate the extrapolation of developmental dose-response data from animal studies to a human lactational exposure scenario. However, meaningful use of these models requires that dose-response data are available for developmental effects occurring as a result of exposure to a chemical. Workshop participants observed that some current protocols for standard reproductive and developmental studies in animals do not adequately assess the impact of PBT chemical bioaccumulation in maternal tissues and lactational elimination on postnatal developmental



outcomes in offspring. As discussed below, workshop participants suggested that current standard protocols be expanded to include additional time periods and endpoints for use with chemicals expected to be persistent and bioaccumulative. For emerging chemicals, tendency for persistence and bioaccumulation can be predicted based on  $\log K_{ow}$ .

One example of a modification of study design to more fully address issues associated with lactational exposure to PBT chemicals would be to increase the pre-mating exposure duration recommended for females. In a National Toxicology Program (NTP) modified one generation study ([NTP, 2011b](#)), test material is not administered until gestation day 6. In order to investigate the full impact of PBT chemical bioaccumulation on lactational dose using this study design, the duration of maternal exposure prior to pregnancy could be extended to allow for chemical accumulation in maternal body lipids prior to lactation and to better reflect the typical exposure pattern occurring in humans.

When a chemical with a long half-life is administered every day, the initial elimination rate is very low, but each dose results in both an increased body burden and increased elimination rate until a point is reached where the elimination rate equals the chemical administration rate. After this, assuming that dosing continues at the same rate, the chemical body burden will not increase further. At this point, the body burden is said to have reached “steady state”. In an animal study, to account fully for the bioaccumulative properties of PBT chemicals, it is desirable to expose F0 females to steady state based on chemical concentrations in fat (or blood, if fat sampling is not practical) prior to mating. However, the time taken to reach steady state is about 4 or 5 times the elimination half-life ([Gibaldi and Perrier, 1982](#)). And, because animals reproduce more reliably when they’re young, if the half-life of a chemical is particularly long, it may not be feasible to wait for the dams to come to steady state prior to mating. For example, female rat fertility begins to decline after about 270 days ([Merry and Holehan, 1979](#)). Thus, if a chemical’s half-life in rats is greater than or equal to about 70 days, females will be difficult to breed by the time their chemical body burden approaches steady state. In these cases, two-generation reproductive toxicity studies are useful to provide additional time for bioaccumulation to occur.

As discussed in [Section 4.2](#), it would also be useful to include a cross-fostering component in reproductive and developmental studies in order to isolate prenatal and postnatal exposures. This provides important information on sensitive windows of exposure and effect. To reduce uncertainty when animal studies are used to support hazard identification and dose-response assessment for lactational exposures, it is important to investigate developmental endpoints that may be susceptible to disruption during the postnatal period in humans ([Makris et al., 2008](#)). These toxicological endpoints are not included in some current protocols for standard reproductive and developmental toxicity studies (e.g., the National Toxicology Program (NTP) reproductive assessment by continuous breeding ([NTP, 2011b](#))). Relevant neurodevelopmental endpoints can be tested using assays of schedule-controlled behavior, attention and associative processing, spatial learning, and behavioral inhibition ([Rice and Barone, 2000](#)). Postnatal effects on immune development can be tested using humoral and cell-mediated immunity assays ([IPCS, 1996](#)), host resistance tests, assays of autoimmunity ([WHO, 2006](#)) and animal models of asthma ([Bates et al., 2009](#)). Postnatal developmental effects on reproductive

endpoints can be detected by counting Sertoli cells in male offspring ([Aafjes et al., 1980](#)) and by monitoring pubertal onset endpoints, such as the age of preputial separation in males and the age of vaginal opening and/or the age at first estrus in females ([Buck Louis et al., 2008](#)). And, as mentioned in [Section 4.2.1](#), endocrine disruption of any type may indicate a potential for a chemical to alter postnatal developmental processes.

A study including assessment of all postnatal developmental endpoints would require resources beyond those available to most researchers. In theory, preliminary *in vitro* assays could be used to detect disruption of signaling pathways important for specific developmental outcomes. This would allow researchers to select the endpoints investigated in a full animal study (or even a human study) based on the likelihood that those endpoints will result from chemical exposure. However, at present, the connections between cellular signaling pathways and specific developmental outcomes are not always well-understood. More research is needed to define these connections more precisely to improve the efficiency and confidence with which researchers can select toxicological outcomes to test.

At present, the Organisation for Economic Co-operation and Development's extended one-generation reproductive toxicity study may be the most comprehensive protocol available for the detection of reproductive and developmental abnormalities resulting from chemical exposure ([OECD, 2011](#)). This protocol includes (1) the assessment of potential impacts of chemical exposure on the developing nervous and immune systems, and (2) administration of test material for a defined pre-mating period selected based on existing toxicokinetic information. However, even this protocol may be modified to address additional considerations to better delineate the impacts of postnatal exposure to PBT chemicals on health effects observed in offspring, as described below.

It is important to ensure that the sample size of a reproductive and developmental toxicity study provides power adequate to detect exposure-related effects. The OECD guidelines state that each test and control group should contain a sufficient number of animals to yield at least 20 pregnant females at or near parturition ([OECD, 2011](#)). Workshop participants suggested using outbred strains of rats or mice to optimize fertility, which reduces the overall number of animals needed to yield 20 litters. But, the number of animals required to detect a particular effect depends on the magnitude of the effect and the sensitivity of the assays used to detect it. So, the number of animals needed for adequate power may vary depending on the specific endpoints investigated.

In many animal studies of lactational exposure to PBT chemicals, dose response has not been well-defined. As discussed in [Section 1.1](#), a POD derived from a benchmark dose level is preferred over one derived using the NOAEL/LOAEL approach ([U.S. EPA, 2012a](#)). In order to maximize the potential for a study to produce dose-response data suitable for BMD modeling, at least three dose levels and a concurrent control should be used, as recommended by the OECD guidelines ([OECD, 2011](#)). The highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering. It may be necessary to conduct an initial dose range finding study in order to establish appropriate dosing conditions.

For reproductive and developmental data, there are additional reporting considerations beyond what is recommended by the OECD guideline; these are important to support BMD modeling. As described in U.S. EPA's *Benchmark Dose Technical Guidance* (2012a), data from reproductive and developmental toxicity studies in rodents are best modeled using nested models, which account for any intralitter correlation (i.e., the tendency for littermates to respond similarly to one another as compared to other litters in a dose group). If this correlation is not estimated, variance estimates (and thus the confidence limits on calculated BMDs) will generally be too small. In addition, these models often include provision for a litter specific covariate, such as initial dam weight, which may be correlated with the outcome of interest (but not with the treatment) and may help clarify the response pattern. In order to account for intralitter correlation, existing versions of nested models require the input of individual offspring data sorted by litter (Davis et al., 2009). Often, the results of reproductive and developmental toxicity studies are reported as summary statistics taken across litters, which do not provide the information necessary to evaluate intralitter correlation. To maximize the potential for developmental data to be analyzed using BMD modeling, researchers are encouraged to publish individual offspring data sorted by litter.

In order to extrapolate developmental dose-response data from animal studies to a human exposure scenario, reliable information on chemical kinetics in pregnancy and lactation is needed. It can be difficult to obtain data on chemical exposures to humans, and if human exposure has not yet occurred (i.e., in the case of an emerging chemical), these data may be impossible to obtain ethically. So, as discussed in Section 4.3, there may be uncertainty associated with some modeling parameters, especially those for humans. But, in animal studies, dosing amounts can be carefully controlled; and, often, levels of individual chemicals, constituent components of mixtures, and their metabolites can be accurately measured. Generally, uncertainty in animal parameters can be decreased by conducting the necessary research.

Workshop participants noted a preference for studies that measure both toxicological outcomes of exposure and kinetic parameters in the same animals; currently, these studies are not abundant in the literature. The precision and applicability of modeling approaches to support dose-response assessment (Section 6) would be greatly enhanced if reproductive and developmental toxicity studies collected data on offspring exposure. It would also be useful to have time-course data on the tissue distribution of chemicals in toxicologically relevant animal species to inform modeling efforts (e.g., tissue-specific and whole-body elimination rates, partition coefficients). These studies might employ single or repeated dosing of dams followed by analysis of the chemical in a variety of tissues harvested from dams and offspring at various time points (e.g., milk, urine, feces, blood, fat, liver, target tissue).

In rodent studies, during the lactational period, dams often groom their pups and consume their feces and urine, which results in recycling of the chemical dose and a larger dose to the dam than is reported in the study. This phenomenon was demonstrated by a cross-fostering study in which unexposed dams nursed pups that had been exposed to perfluorooctanoic acid (PFOA) *in utero* (Wolf et al., 2007). At the end of the study, large concentrations of PFOA were detected in the serum of these dams while no PFOA was found in the serum of unexposed dams that had nursed unexposed offspring. These results

underscore the utility of measuring chemical concentrations in milk or in offspring tissues as a routine component of developmental toxicology studies. Uncertainty related to the estimation of offspring exposure based on administered dose to the dam would be eliminated if lactational dose or internal dose to the offspring were measured in the same study observing a developmental effect of postnatal PBT chemical exposure. The availability of offspring exposure data would greatly simplify the extrapolation of dose-response data from that study to a human exposure scenario. Similarly, if studies provided data on fetal exposure at a given maternal dose, this would facilitate the extrapolation of dose-response information for developmental endpoints that occur as a result of prenatal exposure.

Measures of internal exposures to pups (i.e., in blood and/or other tissues) may be preferred over measurements of chemical concentrations in milk. Measurements in expressed milk may be misleading because milk is not an easy matrix to work with experimentally. For example, in laboratory animals, it may be necessary to use the exogenous hormone oxytocin to produce the sample ([Depeters and Hovey, 2009](#)). However, milk chemical concentrations may also be determined by measuring the chemical of interest in offspring stomach contents harvested at the same time as tissue and/or blood samples. In this way, measures of both lactational dose and internal dose to the offspring can be made available. Furthermore, these data could be used to gain a better understanding of chemical absorption in the offspring, a parameter that is often not explicitly measured ([Section 4.3](#)). In any case, the study design must include some control of litter size to provide adequate exposure to individual offspring such that offspring dose can be accurately measured using current analytical methods.

Given the time and financial resources required to conduct a study (or studies) incorporating all of the above-mentioned elements, it is likely that most risk assessment efforts will be based on dose-response data that do not contain all of these elements. If developmental effects of exposure to a PBT chemical have not been investigated at all, workshop participants suggested the conservative assumption that there is no safe level of exposure to a human mother that would fully protect against effects in an infant. This assumption may be especially warranted if studies in adult animals provide evidence of neurotoxicity, immunotoxicity or endocrine disruption. If studies have shown developmental effects of chemical exposure, including effects on behavior, endocrine function or the immune system, then dose-response data from these studies may be used in the modeling approaches presented in [Section 6](#). But, uncertainty resulting from suboptimal study designs must be transparently acknowledged when the results of the dose-response assessment are reported.

## **6. AVAILABLE APPROACHES**

This section provides a review of the full spectrum of modeling approaches discussed at the workshop, with specific examples provided for hexachlorobenzene (HCB). Also discussed are the advantages and disadvantages of each of the approaches.

Participants noted that breast milk PBT chemical concentration is influenced not only by long-term accumulation of the chemical in the body lipids of the mother, but also by current maternal exposures ([Lakind, 2007](#); [Miyata et al., 2006](#)). Thus, preferred modeling approaches should address all of the

factors that contribute to breast milk PBT chemical concentrations, including past and current maternal exposures.

When estimating exposures, the use of a validated chemical-specific PBPK model is the most robust approach, but these PBPK models are often unavailable for a particular chemical in the species of interest. A further disadvantage of these models is that they may not be transparent to many users developing a risk assessment or for potential stakeholders. Workshop participants suggested that the results of PBPK models be compared to the results of other approaches for a set of data-rich chemicals to see if there is an advantage to using PBPK. This exercise would help (1) to determine the utility of using more than one approach, and (2) to evaluate the overall benefit of PBPK models for this application. If information adequate for risk assessment can be achieved using simpler modeling approaches, then there might not be much benefit in collecting the data to support a chemical-specific PBPK model of lactational exposure. A systematic evaluation of differences in model output could help to establish the degree of confidence in results provided by simpler models. This may also help to identify specific biochemical characteristics that predict inaccurate results from simpler modeling approaches. Chemicals with these characteristics could be prioritized for PBPK model development. As a preliminary step toward meeting this objective, in this section, all of the suggested modeling approaches are illustrated for HCB.

Another step in this direction was made previously by the Oregon Department of Environmental Quality (Oregon DEQ) to support risk assessment guidance ([Farrer et al., 2010](#); [Oregon DEQ, 2010](#)). Using data for PCB 153, Farrer et al. ([2010](#)) compared models by Verner et al. ([2009](#)) and Redding et al. ([2008](#)) to the model from U.S. EPA's *Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions* ([U.S. EPA, 1998](#)) and the biotransfer method for estimating milk concentrations based on an average daily maternal dose. They concluded that the U.S. EPA model estimates for milk concentrations and infant lactational doses were sufficiently similar to the results of PBPK models, producing a conservative, health-protective estimate.

Relevant to all of the modeling approaches, participants noted that the extrapolation of doses from a laboratory animal study to human doses for a dose-response assessment of infant lactational exposure is problematic. Several exposure metrics might be useful. These include measures of average daily maternal exposure, maternal body burden, offspring lactational dose and offspring body burden. In the case of early life exposure that results in developmental effects, the dose-response relationship of interest is that between the health effect and the dose to the human or animal offspring. When using data from animal studies of PBT chemical exposure, dose extrapolation between animals and humans at the level of maternal exposure might be inadequate to account for differences in chemical exposures to animal offspring compared to human infants. Extrapolation between animals and humans in this context is complicated because animal dams and human mothers exposed to the same average daily dose of a PBT chemical may deliver different lactational doses to their respective offspring. For this reason, workshop participants suggested that it may be important to conduct an interspecies dose extrapolation

for PBT chemicals at the level of offspring lactational exposure as opposed to the level of maternal exposure.

Offspring exposure metrics that could be used for interspecies extrapolation include tissue dose, body burden and lactational dose. Workshop participants suggested that the ideal metric would be tissue dose: a measure of chemical concentration in the target tissue affected in offspring. These data were not available for any of the chemicals discussed at this workshop, and it was acknowledged that, in many cases, the target tissue may not be known, let alone the dose to that tissue. However, for highly lipophilic compounds, lipid-adjusted blood concentrations might be assumed to be similar to lipid-adjusted chemical concentrations in target tissues when the target tissue or dose to that tissue is not known ([Haddad et al., 2000b](#)). Therefore, lipid-adjusted blood concentrations might be used as a surrogate measure of target tissue dose or offspring body burden. However, it is important to note that, as discussed in [Section 4.3.3](#), the link between body burden and chemical concentration in blood is not always clear. Also, modeling offspring body burden for use as the exposure metric for interspecies extrapolation may be complicated because body burden increases rapidly in neonates due to increased exposure with the onset of nursing, but also may decrease due to factors including growth-related chemical dilution and changes in metabolic enzyme expression ([Hines, 2008](#)). Accurate estimation of offspring body burden over time requires knowledge of a chemical's half-life in the juvenile. Chemical half-lives in juveniles are often quite different from half-lives in adults ([Milbrath et al., 2009](#)), and juvenile-specific half-lives are rarely available in the literature.

Another exposure metric that may be used for interspecies extrapolation is offspring lactational dose as calculated from PBT chemical concentrations in milk and estimates of offspring milk consumption. One advantage of using this metric is that juvenile-specific chemical half-lives are not required. However, if this is used, interspecies scaling may be needed to account for pharmacokinetic differences between humans and animals ([Thompson et al., 2008](#)). Such scaling is not necessary if data are available to use target tissue dose or body burden as the exposure metric.

Once an offspring exposure metric is chosen for the interspecies extrapolation, it is also necessary to choose whether to use an average or peak value. Workshop participants argued in favor of using an estimate of average pup exposure. If the critical window of susceptibility for the relevant developmental effect is known, then the appropriate metric would be the average exposure during the critical window. If the precise critical window of susceptibility for the relevant developmental effect is unclear, then the appropriate metric would be the average over the exposure duration. The reasoning for this is that the use of the average exposure to animal offspring provides a more health-protective HED than peak exposure would; and, unless the critical window of susceptibility is known to coincide with the timing of peak exposure, it isn't clear that peak exposure was required to elicit the effect. However, there is some question regarding the appropriate definition of the "exposure duration" when target tissue dose or offspring body burden is used as the dose metric. Often, the exposure is averaged over the nursing duration. However, with PBT chemicals, offspring body burden accumulated as a result of lactational exposure persists beyond the nursing duration. And, a number of developmental processes also

continue to occur after weaning. Thus, it may be important to consider the potential for residual exposure beyond the nursing period to impact postnatal developmental endpoints.

Modeling approaches identified during the workshop for estimating lactational exposures in humans and animals are discussed below. In general, workshop participants agreed that PBPK methods are preferred *if* the appropriate data for parameterization are available for a chemical. The PBPK model discussion begins with a decision tree that allows a risk assessor to decide whether a PBPK approach is appropriate for a given chemical. If not, the selection of an alternative simpler method depends on the availability of reliable half-life data. Each of the approaches discussed during the workshop is applied below to HCB as a demonstration of the method and to allow comparisons among the results of different modeling approaches. All model predictions in this report use 1 ng/kg-day maternal dose, and all model outputs (including those from the PBPK models) are linear across doses. This relationship is expected at low environmental exposures for all lipophilic PBT chemicals where metabolic saturation is not expected.

Any model should be validated before it is used to support risk assessment. The types of data useful to evaluate model predictions of maternal body burden include chemical levels in maternal serum, fat and milk. To evaluate model predictions of offspring body burden, chemical levels in cord blood and in offspring serum and fat are useful. Measures of environmental exposure to the mother are also important for assessing the accuracy of the modeled relationship between this parameter and maternal body burden. Generally, administered dose to the mother is relatively easy to ascertain in an animal study, but it is often ill-defined in humans. Ideally, all of these body burden and exposure data would be gathered from mother-offspring pairs at different time points over a period of time longer than the half-life of the chemical. However, when data from individual human subject pairs are not available, population-based diet and serum data, such as those gathered by the FDA (<http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/TotalDietStudy>) and CDC (<http://www.cdc.gov/nchs/nhanes.htm>), respectively, may be compared to model output. This comparison would not serve as a model validation, per se, but may demonstrate whether or not the model is performing as expected in general.

## 6.1. PBPK Model Approach

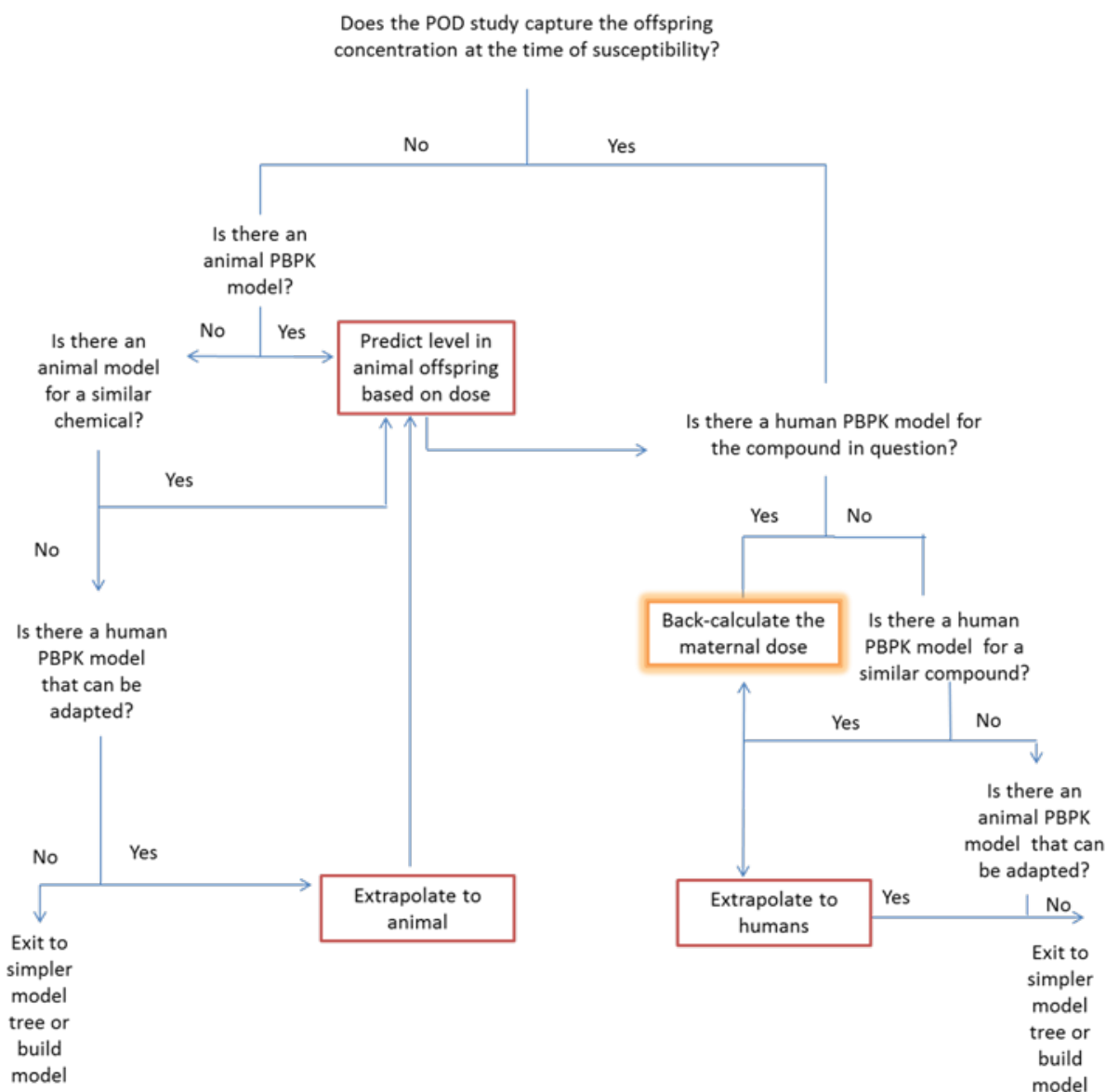
According to workshop participants, PBPK approaches may offer significant advantages over simpler modeling approaches for estimating offspring exposure to PBT chemicals in humans or laboratory animals. In particular, appropriately developed PBPK models have the ability to account for ongoing exposure, fat (and chemical) deposition in the mother before and during gestation, postpartum fat (and chemical) mobilization in the mother, and dose-dependent variations in chemical metabolism or half-life. Additionally, PBPK models account for physiological differences and pharmacokinetics, while the more simple toxicokinetic models typically only account for pharmacokinetic differences.



However, PBPK models that explicitly estimate infant lactational doses in humans are currently available for only a few of the many chemicals found in human milk [e.g., PCB 153 ([Redding et al., 2008](#)), perchlorate ([Clewell and Gearhart, 2002](#)), dioxin ([Lorber and Phillips, 2002](#))]. To effectively build a PBPK model, at a minimum, the partition coefficients from blood to different model compartments (e.g., fat, muscle, mammary tissue, liver) and metabolism constants for the chemical and species are needed. To estimate these parameters, serum and tissue chemical and metabolite concentrations are measured in the species of interest at multiple time points, preferably after known exposure doses. As discussed above, these measurements are also useful for model validation.

The flow chart below illustrates decision logic that can be applied to evaluate whether a PBPK model can be used.





**Figure 6-1. Decision Tree for Applying PBPK Approaches**

When a chemical-specific PBPK model is not available, workshop participants suggested that more general models developed by Verner et al. (2013; 2009) for PBT chemicals that distribute according to lipid solubility and have elimination half-lives in humans on the order of years may be considered. An important advantage of the models developed by Verner et al. (2013; 2009) is that the chemical-specific parameters can be estimated using only the elimination half-life and the octanol-water partition coefficient ( $K_{ow}$ ) of the compound.

The maternal and infant PBPK model presented in Verner et al. (2009) is intended to simulate the entire life-cycle of a woman up to age 55 years and includes nine maternal tissue compartments and five infant

tissue compartments. The oral maternal dose of a PBT chemical is modeled as being directly absorbed into the liver and is assumed to be fully bioavailable. First-order hepatic metabolism is included and assumed to represent all excretion in the absence of breastfeeding or childbirth. The elimination rate constant is derived for the chemical from the adult whole-body half-life. Chemical concentrations in each compartment are determined using partition coefficients, where estimates of the chemical-specific partition coefficients are based on  $K_{ow}$ . Physiological parameters (e.g., body weight and adiposity) are assumed to vary across time to capture changes over the life of a woman. The average daily maternal exposure to a particular chemical can be estimated by modeling its concentration in mother's blood, assuming constant daily dosing from birth. In turn, the chemical concentration in milk can be estimated based on a milk:blood partition coefficient. The infant lactational dose can be derived using estimates of infant daily milk intake.

The Verner et al. (2009) model assumes that the chemical essentially distributes in neutral lipids only. For many PBT chemicals, the assumption that all of the chemical is stored in lipid is sound. However, if there are data to suggest that a chemical may partition into both lipid and aqueous compartments, then another complex multi-compartment model that can accommodate this information would be useful. In addition, if distribution mechanisms such as protein binding are implicated and may significantly affect the overall kinetics of a chemical, then other models will need to be developed to accommodate these processes. Finally, the model accounts for transfer to the infant in a fairly simplistic way and does not account for variation in the fraction of fat in the milk over the course of the lactation duration or within a single feeding.

The Verner et al. (2009) model was used to predict milk concentrations, infant dose, and resulting ratios of infant lactational dose to 1 ng HCB/kg-day average daily maternal dose. Model parameters used are shown in Table 6-1; model parameters not shown below were the average population values used as described in Verner et al. (2009). The PBPK model results are shown in Table 6-2.

**Table 6-1. Model parameters used in Verner et al. ([2013](#); [2009](#)) simulations for HCB**

Model Parameter	Parameter Value	Used in HCB Model Execution		Reference
		Verner et al. ( <a href="#">2009</a> )	Verner et al. ( <a href="#">2013</a> )	
Maternal age at delivery	25 years	Yes	Yes	Mid assumption, see <a href="#">Table 3-14</a>
Pre-pregnancy weight	75 kg	Yes	Yes <sup>a</sup>	Mean, all races, 20 years and over ( <a href="#">Fryar et al., 2012</a> )
Gestational weight gain	12.5 kg	No	Yes	Assumption
Maternal height	163 cm	Yes	No	Mean, all races, 20-49 years ( <a href="#">Fryar et al., 2012</a> )
Gestational age	9 months	No	Yes	Assumption
Child weight at birth	3.4 kg	Yes	Yes	CDC ( <a href="#">2000</a> )
Child weight at 6 months of age	7.2 kg	Yes	Yes	CDC ( <a href="#">2000</a> )
Child weight at 12 months of age	9.6 kg	Yes	Yes	CDC ( <a href="#">2000</a> )
Child length at birth	49 cm	Yes	No	CDC ( <a href="#">2000</a> )
Child height at 6 months of age	65 cm	Yes	No	CDC ( <a href="#">2000</a> )
Child height at 12 months of age	74 cm	Yes	No	CDC ( <a href="#">2000</a> )
Child gender	Female	Yes	Yes	Assumption
HCB half-life	6 years	Yes	Yes	To-Figueras et al. ( <a href="#">2000</a> )

<sup>a</sup> Return to this value 6 months postpartum to match the Verner et al. ([2009](#)) simulations

**Table 6-2. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB Using the PBPK Model Published by Verner et al. (2009)**

Infant Nursing Duration	Ratio of Infant Lactational Dose to Average Daily Maternal Dose
1 month, Peak Infant Intake	28.1
1 month, Average Infant Intake	25.8
12 months <sup>3</sup> , Peak Infant Intake	28.4
12 months, Average Infant Intake	21.2

## 6.2. Toxicokinetic Modeling Approaches

In a less complex approach, the multi-compartment PBPK model is reduced to the consideration of only two-compartments representing the mass of maternal and fetal/child body lipids as a function of age (Verner et al., 2013). The infant lactational dose estimation is based on the lipid-adjusted chemical concentration in milk, infant daily milk intake, and milk lipid content (Arcus-Arth et al., 2005). As stated above, this approach may be appropriate for highly lipophilic chemicals that do not bind with protein and are not stored in aqueous compartments.

The Verner et al. (2013) model was executed for HCB. The model parameter values used were the same as implemented for the Verner et al. (2009) model; however, as this is a simpler model, fewer parameters were required and are indicated in Table 6-1, above. The ratios of infant lactational dose to average daily maternal dose for this two-compartment model are provided in Table 6-3.

**Table 6-3. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB Using the Two-Compartment Model Published by Verner et al. (2013)**

Infant Nursing Duration	Ratio of Infant Lactational Dose to Average Daily Maternal Dose
1 month, Peak Infant Intake	37.7
1 month, Average Infant Intake	34.3
12 months, Peak Infant Intake	38.8
12 months, Average Infant Intake	32.3

<sup>3</sup> Although the Centers for Disease Control and Prevention (CDC) estimate that only 27% of infants in the United States are breastfed for 12 months (CDC, 2013), both the American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend exclusive breastfeeding until 6 months of age and continued breastfeeding until 12 months (AAP, 2012; WHO, 2003). For this reason, the analyses presented here assume a breastfeeding duration of 12 months in humans.

Another approach is the time-varying body burden model described in the briefing packet and provided to the workshop participants in a spreadsheet ([ICF, 2013a, b](#)). This model is also based on maternal and infant body burden. But, unlike the Verner et al. ([2013](#); [2009](#)) models, it does not assume 100% storage in the lipid compartment. It does require an estimate of the fraction of the total body burden within the lipid compartment, and this may be set to 100% in the absence of any data. This model allows the maternal dose, the offspring body weight, and the milk ingestion rate to vary over time by using different point estimates for each measure over the course of the simulation. It also allows for an observation period after dosing ends. This is particularly important for application to laboratory animal exposures since the exact dosing protocol (e.g., dosing only on particular gestation days) can be reproduced in the model. Varying offspring body weight and milk ingestion rate allows the model to better approximate the chemical exposures of offspring during the neonatal period when these parameters are changing rapidly. Workshop participants commented on this approach, stating that it could be useful in risk assessment. However, it might be improved if it were implemented with a continuous simulation language rather than the larger time step approach used in the time-varying body burden model provided at the workshop.

Below, the model is applied to HCB using the same model parameters as those in the briefing packet, with a few important updates. Following recommendations from the workshop attendees, the fraction of body weight that is fat in the mother was updated to reflect a CDC report where the mean body fat percentage for 20-39 year old women is reported to be 38% ([Borrud et al., 2010](#)). Thus, the fraction of the mother's weight that is fat was increased from 0.3 to 0.4. The fraction of fat in human milk was increased from 0.03 to 0.04 ([U.S. EPA, 2011a](#)). In addition, the workshop participants strongly disagreed with the human half-life value for HCB that was used in the briefing packet (i.e., 215 days). The half-life estimated by Yesair et al. ([1986](#)) and used in the briefing packet may be based on the first phase of HCB elimination, as opposed to the slower second phase of elimination from non-blood (i.e., fat) compartments. As discussed in [Section 4](#), this two phase process has been described for other PBT chemicals, such as DDT. Additional studies have reported half-life values of about 3-5 years for HCB in humans ([Sala et al., 1999](#)) and in rhesus monkeys ([Yang et al., 1978](#)). To-Figueras ([2000](#)) estimated a whole-body half-life of 6 years based on analysis of feces and urine samples from 53 human subjects. For the purposes of this example, the estimate of 6 years from To-Figueras ([2000](#)) was used. Parameter updates for humans are shown in [Table 6-4](#). Using these parameters results in estimates of the ratio of infant lactational dose to average daily maternal dose as shown below in [Table 6-5](#).

An average daily maternal dose of 1 ng/kg-day was assumed for the purposes of this example; estimates of the ratio are independent of the specific average daily maternal dose used because the model is linear with respect to the dose.

**Table 6-4. Model Parameters and Their Values Used in the Human Time-Varying Body Burden Model (ICF, 2013a) after Workshop Participant Review**

Parameter (units)	Variable	Value	Note/source
<b>Human Parameters (not chemical-specific)</b>			
Maternal age at pregnancy (years)	Age	25	Martin et al. (2012)
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75	U.S. EPA (2011a)
Infant body weight, Month 1 (kg)	BW <sub>INF,t</sub>	4.8	U.S. EPA (2011a)
Infant body weight, Month 2-3 (kg)		5.9	
Infant body weight, Month 4-6 (kg)		7.4	
Infant body weight, Month 7-12 (kg)		9.2	
Infant ingestion rate, Month 1 (kg milk/day)	CR <sub>milk,t</sub>	0.51	U.S. EPA (2011a)
Infant ingestion rate, Month 2-3 (kg milk/day)		0.69	
Infant ingestion rate, Month 4-6 (kg milk/day)		0.77	
Infant ingestion rate, Month 7-12 (kg milk/day)		0.62	
Fraction of mother's weight that is fat (kg maternal fat/kg BW)	f <sub>fm</sub>	0.4	Borrud et al. (2010)
Fraction of fat in breast milk (dimensionless)	f <sub>mbm</sub>	0.04	U.S. EPA (2011a)
<b>Human Chemical-Specific Parameters</b>			
Fraction of ingested chemical that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1.00	Assumed
Fraction of ingested chemical that is absorbed by the mother (dimensionless)	f <sub>am</sub>	0.80	Schlummer et al. (1998)
Fraction of chemical that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	U.S. EPA (1998)
Biological elimination constant for chemical (day <sup>-1</sup> )	k <sub>elim</sub>	0.000317	Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans (days)	t <sub>1/2</sub>	2,190	To-Figueras (2000)

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost postpartum.

**Table 6-5. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB Using the Time-Varying Body Burden Model (ICF, 2013a)**

Infant Nursing Duration	Ratio of Infant Lactational Dose to Average Daily Maternal Dose
1 month, Peak Infant Intake	22.8
1 month, Average Infant Intake	22.7
12 months, Peak Infant Intake	24.7
12 months, Average Infant Intake	17.0

As an example, the time-varying body burden model (ICF, 2013a, b) was also used to estimate a human equivalent dose (HED) assuming equivalency between rats and humans based on four different metrics: offspring body burden averaged over the nursing period; peak offspring body burden; lactational dose to offspring averaged over the nursing period; and peak lactational dose to offspring. For the purposes of the example, it was assumed that dosing to the rat dam occurred from the time of mating until weaning of the pups at a level of 1 mg/kg-day. This value is used simply as an illustrative example to indicate the differences in HED across the different intake/internal dose equivalency assumptions. The parameters used for modeling can be found in [Table 3-8](#) (rat parameters) and [Table 6-4](#) (updated human parameters), respectively. [Table 6-6](#) shows the estimated HEDs for the different intakes/internal dose equivalency assumptions. These values were estimated by calculating the offspring lactational dose or offspring internal dose in the rat model (ICF, 2013b) and then back-calculating the average daily maternal dose in humans that gives the same infant lactational dose or infant internal dose (ICF, 2013a).

**Table 6-6. Estimates of Human Equivalent Dose Using the Time-Varying Body Burden Model (ICF, 2013a, b)**

Dose Metric Used to Extrapolate between Rat Offspring and Human Infant Exposures	Daily Dose Administered to Rat Dam (mg/kg-day)	Rat Offspring Lactational Dose (mg/kg-day) or Internal Dose (mg/kg)	Human Average Daily Maternal Dose (HED) (mg/kg-day)
Offspring Lactational Dose Averaged Over the Nursing Period	1	1.9 mg/kg-day	0.1
Offspring Body Burden Averaged Over the Nursing Period	1	12 mg/kg	0.0035
Peak Offspring Lactational Dose	1	4.9 mg/kg-day	0.2
Peak Offspring Body Burden	1	15 mg/kg	0.0026

According to workshop participants, the Verner et al. (2013) model and the time-varying body burden model (ICF, 2013a, b) are appropriate for very lipophilic chemicals and are most appropriate when there is no metabolism or when metabolism is relatively slow. For both of these models, model output is highly dependent on the value used for the half-life of the chemical, so care must be taken in calculation and selection of the half-life to use. The workshop attendees proposed a tiered approach to estimating half-life based on available data.

*For a first tier chemical, half-life values are available in the published literature for both animals and humans. Here, the advantage is that half-lives derived using experimental data may be more reliable than those estimated using QSAR or log  $K_{ow}$  as suggested below; but, as discussed in Section 4, even data-derived half-lives may be uncertain. Methods used to estimate half-life may vary among studies, and half-lives may be different for parent and metabolite compounds.*

*For a second tier chemical, there may be a literature value for half-life in animals, but not in humans or in humans, but not in animals. Similarly, for a third tier chemical, there are no animal or human half-lives reported in the literature. For missing half-life values, QSAR techniques might be developed to predict a half-life based on a chemical's structure. This approach would be preferred over the cruder approach suggested for fourth tier chemicals; but, currently, there is no database of QSAR data that could be used for this particular exercise.*

*Finally, for a fourth tier chemical, no half-life information or QSAR data exist in the literature. In this case, the log  $K_{ow}$  may be used to estimate the half-life. No general models for PBT chemicals were found after a limited search of the literature, but an approach similar to that of Lien (1975) for a series of probenecid analogs could be pursued.  $K_{ow}$  is available for most chemicals, and the approach used to estimate half-life from log  $K_{ow}$  would be fairly straightforward.<sup>4</sup> However, this approach is not necessarily accurate since it only takes into account fat solubility and does not consider metabolism.*

### 6.3. Combustor Emissions Model

The time-varying body burden model presented in Section 6.2 was developed using equations provided in the U.S. EPA's *Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions* (U.S. EPA, 1998) (hereafter "Combustor Emissions Model"). Unlike the time-varying body burden model, the Combustor Emissions Model assumes that the dose does not vary in time and continues for all times evaluated in the model. The overall equation is then:

$$BB_{MAT} = \frac{DI_{MAT}}{k_{elim}} (1 - e^{-k_{elim}T})$$

---

<sup>4</sup> See Seydel and Schaper (1981) for a discussion of elimination rate modeling.



Where:

- $BB_{MAT}$  = the time-dependent maternal body burden (ng/kg),  
 $DI_{mat,t}$  = the constant maternal dose (ng/kg-day),  
 $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ , and  
 $T$  = the evaluation time in days.

The maternal body burden can be used to estimate an infant intake rate assuming a specific fraction of fat in milk and an infant ingestion rate:

$$DI_{INF} = \frac{DI_{MAT} \times f_{mbm} \times CR_{milk,t} \times f_f}{k_{elim} \times BW_{INF,t} \times f_{fm}} (1 - e^{-k_{elim}T})$$

Where:

- $DI_{INF}$  = the time-dependent infant daily ingestion (ng/kg-day),  
 $DI_{MAT}$  = the constant maternal dose (ng/kg-day),  
 $f_{mbm}$  = the fraction of fat in milk (dimensionless),  
 $CR_{milk,t}$  = the average infant ingestion rate of milk (kg/day),  
 $f_f$  = the fraction of chemical that is stored in maternal fat (dimensionless),  
 $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$   
 $BW_{INF,t}$  = the average infant's body weight (kg),  
 $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW), and  
 $T$  = the evaluation time in days.

Assuming pregnancy at age 25, continuous dosing to the mother since birth, and average milk ingestion rates and infant body weights as in [Table 6-4 \(U.S. EPA, 2011a\)](#), this gives ratios of infant lactational dose to average daily maternal dose as shown in [Table 6-7](#) below.

**Table 6-7. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB using the Combustor Emissions Model**

Infant Nursing Duration	Ratio of Infant Lactational Dose to Average Daily Maternal Dose
1 month	28.5
12 months	22.8

This equation is an approximation and has limitations. Notably, the dosing assumed in the model (continuous) may not be able to reflect dosing protocols that end a few days before delivery/lactation, as is the case for many animal studies. The equation also does not include an absorption fraction applied to the maternal dose. As is, a conservative value of 1 (100% absorption) is assumed. But, if data are available to suggest a lower absorption rate for a given chemical, then an absorption parameter could be added to the equation.

## 6.4. Simple No-Elimination Method

Rogan and Ragan ([1994](#)) presented a very simple approach based on the assumption that lactation is the only PBT chemical elimination route for mothers. This represents a conservative, default assumption. However, as suggested by some workshop participants, for chemicals for which there are no reliable half-life data or where there is significant uncertainty in the reported log  $K_{ow}$ , this method may provide a first approximation or worst-case scenario that could be considered until these data are available. Examples of factors that are considered in this model include the average daily maternal PBT chemical exposure, age at parturition, and infant daily milk intake.

The starting point of the method is the average pup body burden (mg/kg bw) at the POD from the POD study. This value is set as the benchmark for the average human infant body burden during lactation (assumed to be a 12 month duration<sup>3</sup>). The dose to the infant over this time period (mg/kg-day) is calculated as:

$$DI_{INF} = \frac{BB_{INF}}{Age}$$

Where:

- $DI_{INF}$  = the infant daily ingestion (mg/kg-day),
- $BB_{INF}$  = the infant body burden (mg/kg), and
- Age = the age of the infant in days (12 months = 365 days).

Then, the chemical concentration in milk (mg/kg fat) is calculated as:

$$C_{\text{milk fat}} = \frac{DI_{\text{INF}} \times BW_{\text{INF}}}{CR_{\text{milk}} f_{\text{mbm}}}$$

Where:

- $C_{\text{milk fat}}$  = the concentration of chemical in the maternal milk fat (mg/kg milk fat),
- $DI_{\text{INF}}$  = the infant daily intake (mg/kg-day),
- $BW_{\text{INF}}$  = the average infant's body weight (kg),
- $CR_{\text{milk}}$  = the average infant ingestion rate of milk (kg/day), and
- $f_{\text{mbm}}$  = the fraction of fat in milk (dimensionless).

The total maternal body burden is then:

$$BB_{\text{MAT}} = C_{\text{milk fat}} \times f_{\text{fm}}$$

Where:

- $BB_{\text{MAT}}$  = the maternal body burden (mg/kg),
- $C_{\text{milk fat}}$  = the concentration of chemical in the maternal milk fat (mg/kg milk fat),
- $f_{\text{fm}}$  = the fraction of the mother's weight that is fat (kg fat/kg BW).

Finally, the average daily dose to the mother from continuous exposure (assumed she is 25 years of age at the time of first parturition ([Martin et al., 2012](#))) is calculated as:

$$DI_{\text{MAT}} = \frac{BB_{\text{MAT}}}{\text{Age}_{\text{MAT}}}$$

Where:

- $DI_{\text{MAT}}$  = the maternal daily intake (mg/kg-day),
- $BB_{\text{MAT}}$  = the maternal body burden (mg/kg), and
- $\text{Age}_{\text{MAT}}$  = the mother's age at parturition in days (25 years = 9,125 days).

**Table 6-8. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB using the No-Elimination Method**

Infant Nursing Duration	Ratio of Infant Lactational Dose to Average Daily Maternal Dose
1 month	97.0
12 months	76.9

## 6.5. Biotransfer Method

The biotransfer method, presented as the most data-poor option in the briefing packet, was not endorsed by the workshop attendees. In this method, the  $\log K_{ow}$  is used to estimate a transfer factor rather than a half-life. The biological implications of this transfer factor were considered unclear by workshop participants because of the lack of half-life information considered and the difficulty in dealing with metabolites. Instead, when  $\log K_{ow}$  is the only known parameter, workshop participants suggested using  $\log K_{ow}$  to estimate half-life (see previous discussion at the end of [Section 6.2](#)) and using a simple equation model based on half-life. However, for completeness and for comparison with the other methods, the ratio of infant lactational dose to average daily maternal dose is provided for HCB below.

**Table 6-9. Ratios of Infant Lactational Dose to Average Daily Maternal Dose for HCB using the Biotransfer Method**

Infant Nursing Duration	Ratio of Infant Lactational Dose to Average Daily Maternal Dose
1 month	79.6
12 months	63.4

## 6.6. Monte Carlo Methods to Account for Variability

The approaches outlined in [Sections 6.1](#) to [6.5](#) all employ different modeling methods to estimate the ratio of infant to maternal exposure. However, all of the methods use point estimates for each parameter. Typically, the exposure level and the values for certain physiological characteristics vary across a population group and with time. The parameter values associated with the models described above may vary significantly across human population groups. Some that may impact the determination of the infant lactational dose include average daily maternal exposure, body weight (maternal and infant), age at pregnancy, maternal adiposity, milk lipid content, infant milk intake, duration of lactation, parity of the mother, duration of previous breastfeeding episode(s), and years since previous breastfeeding.

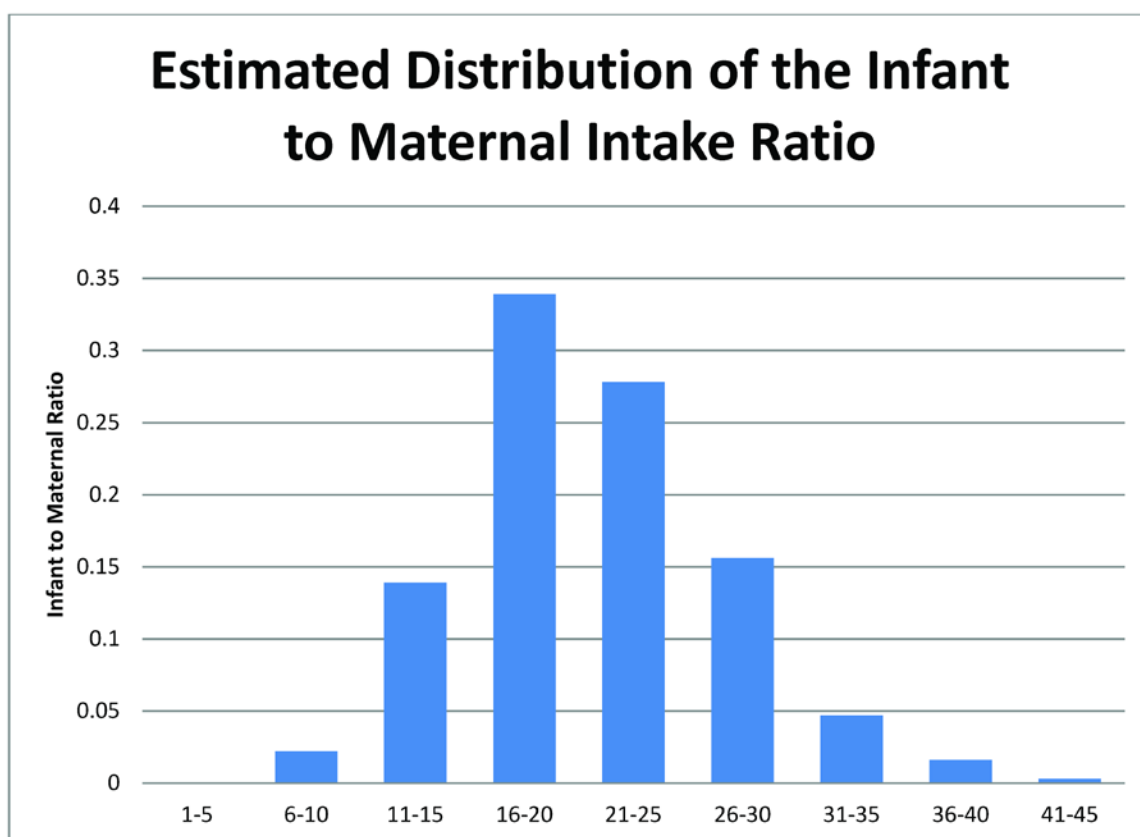
For parameters for which there are a range of values or estimates, average values are often used in modeling approaches as shown in the example approaches described above. However, when a parameter varies across a wide range, the model output using an average value may differ significantly from that using a 5<sup>th</sup> or 95<sup>th</sup> percentile value. It is necessary and important to account for the variability of a parameter and to assess the impact that this variability may have on model output and results in order to identify population groups most susceptible to lactational PBT chemical exposure. To this end, workshop participants suggested that stochastic modeling methods may be used to address concerns regarding variability in model parameters.

Population-based projections of an exposure distribution can be generated using Monte Carlo methods ([Bogen et al., 2009](#)). To perform this type of analysis, the distribution of a parameter must be developed to replace the simple point estimate values for all parameters that are expected to vary widely across the population, or to significantly affect the estimation of exposure, or both. This information allows the model to be run for numerous iterations, where the model generates a different estimate of the infant to maternal intake ratio at each iteration. To execute an iteration, each input distribution is “sampled”, meaning a specific input value is selected based on the shape of the distribution of the parameter. Then, the combination of inputs is used to estimate the infant to maternal ratio. The overall outcome of the model analysis is a set of model predictions comprised of the results of the different iterations. When taken together for the specific case described here, they give an indication of the likely distribution of lactational exposure in offspring across the population.

As an example, the inputs of the time-varying body burden model ([ICF, 2013a](#)) were used to perform a simple Monte Carlo analysis for HCB, assuming a half-life of 6 years. The age at pregnancy, duration of breastfeeding, fraction of fat in breast milk, and fraction of mother’s weight that is fat were all varied using distributions. The following assumptions were made to estimate the distributions:

- The CDC reports the fraction of pregnant women in different age ranges as of 2002: 3.0% were 15-17, 6.6% were 18-19, 51.5 % were 20-29, 36.2% were 20-39, and 2.6% were 40-54. These proportions were used to estimate the best fitting normal distribution; a distribution with a mean of 27 years and standard deviation of 6 years was selected ([Martin et al., 2012](#)).
- The duration of breastfeeding was not known, so a uniform distribution between 0 months and 1 year was used for this simple exercise.
- The distribution of the fraction of fat in breast milk was developed using the data in Arcus-Arth et al. ([2005](#)). The mean value of 0.04 and standard deviation of 0.05 were used.
- The fraction of the mother’s weight that is fat, as reported by Borrud et al. ([2010](#)), has a mean value of 38%, with a 5<sup>th</sup> percentile of 26% and a 95<sup>th</sup> percentile of 50%. This was approximated to a normal distribution with a mean of 0.4 and a standard deviation of 0.1.

The estimated probability density function for the ratio of infant lactational dose to average daily maternal dose is shown below. This distribution is estimated by taking the prediction of the ratio for 1,000 realizations and finding the proportions that fall within different ratio ranges. For the input distributions assumed in this example, the resulting distribution is skewed, with a mean of 25.8 and a 95<sup>th</sup> percentile of 38.9.



**Figure 6-2. Results from Exploratory Monte Carlo Analysis for HCB**

A further approach was suggested to expand the PBPK modeling work to include population PBPK modeling using the Markov chain Monte Carlo simulation approach. This approach has not been conducted here, but may be useful to consider for future modeling efforts.

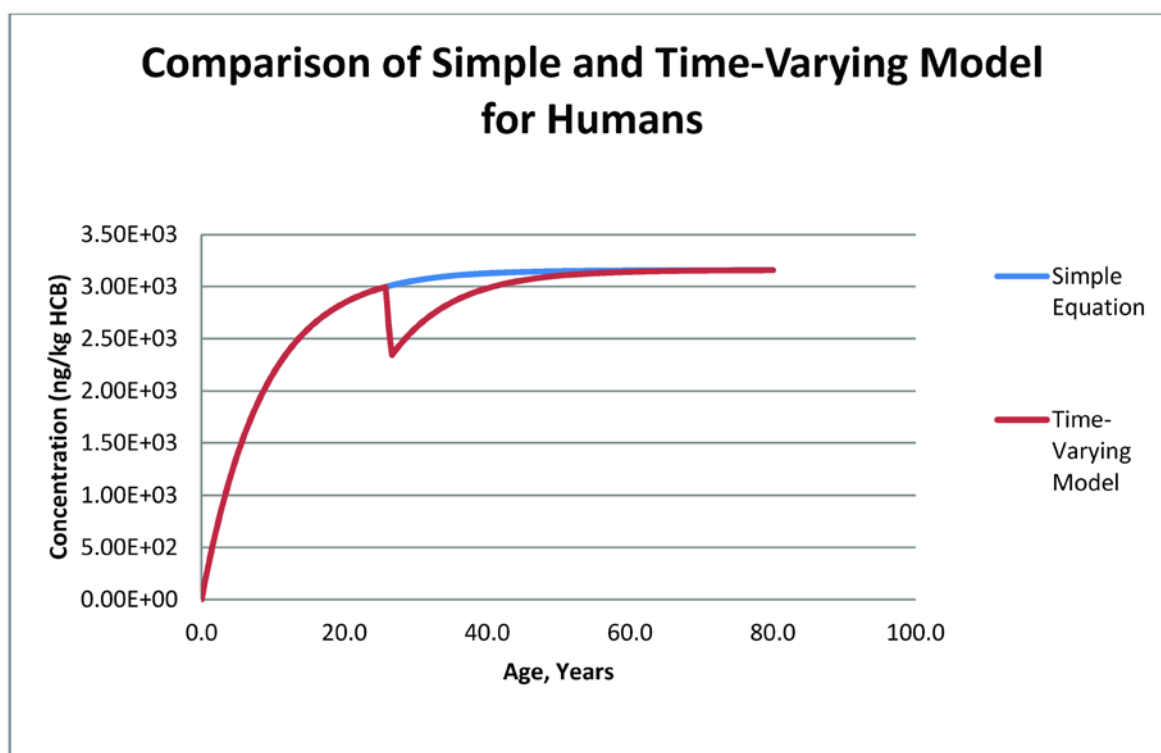
## 6.7. Selecting an Approach

Workshop attendees agreed that there is no one approach that is suitable for all chemicals. When available, chemical-specific PBPK models will tend to give more sophisticated predictions of maternal body burden and infant dose because they can incorporate metabolic processes and other non-linear

kinetics. However, simpler models require knowledge of fewer parameters and may be better suited for data-poor chemicals. The flow chart presented in [Section 6.1](#) may be helpful when deciding whether to use a PBPK or simpler model. As mentioned previously, comparison of multiple models can be useful for assessing the relative effectiveness of the various approaches.

Workshop participants suggested that an approach using the time-varying body burden model ([ICF, 2013a, b](#)) could be cost-effective and sufficiently accurate to achieve risk assessment goals. However, to increase confidence, it might be useful to conduct additional case studies and to compare PBPK simulations with those made using a first-order body burden model implemented with a continuous simulation language rather than the larger time step approach used in the time-varying body burden model provided at the workshop.

In addition, when comparing the Combustor Emissions Model to the time-varying body burden model, participants considered the inclusion of infant body burden and maternal elimination due to lactational transfer to be an advantage of the time-varying model. Also, the ability to accommodate a variety of dosing protocols is an advantage of the time-varying model that is critical for use with animal data. However, when applied to human exposure scenarios, these models produce very similar results, and the simplicity of the Combustor Emissions Model may be an advantage in risk communication. To compare the two approaches for HCB, the absorption fraction was set to 1, and the predictions from the Combustor Emissions Model were compared to the time-varying body burden model for HCB in humans, assuming pregnancy at age 25. A comparison plot is shown below using a half-life of 6 years. The simulations directly agree up until the beginning of breastfeeding. However, the time-varying model accounts for maternal transfer and enhanced elimination during the breastfeeding period.



**Figure 6-3. Comparison of Time-Varying Body Burden Model and Simple Equation for HCB**

Selection of modeling approaches for estimating the offspring lactational dose and the average daily maternal exposure depends on the PBT chemical under evaluation and the availability of data to inform parameter values for that chemical or similar chemicals. The most robust estimates will often result from using a model that includes as much chemical-specific information as possible. Of the approaches discussed here, PBPK modeling is the most robust approach, but validated chemical-specific PBPK models are not always available for a particular chemical in the species of interest. Simpler models, such as the time-varying body burden model, have fewer parameters and may be appropriate for estimating human infant lactational doses for a variety of highly lipophilic chemicals that do not appreciably bind with proteins and are not soluble in aqueous compartments. A simpler version of the time-varying body burden model, using equations from U.S. EPA's *Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions* (U.S. EPA, 1998), assumes no variation in dose over time. This model is capable of estimating infant intake rate based on maternal body burden, but it cannot easily be applied to animal studies where the dose varies in time. The simplest approach, presented by Rogan and Ragan (1994) is more conservative than the other methods, due to the assumption of no elimination of the chemical. Some workshop participants suggested that this model might be useful to establish a tentative relationship between average daily maternal exposure and the subsequent infant lactational dose that could be considered for emerging chemicals and others for



which data are limited until chemical-specific information (i.e., log  $K_{ow}$ ) is available. Stochastic modeling techniques, including Monte Carlo methods, may be used with any of these approaches to better characterize interindividual variability within certain populations. Each of the modeling approaches presented in this review have advantages and disadvantages for their use, and the selection of the most appropriate model for a given PBT chemical depends on factors like availability of data to inform modeling parameter values, tolerance for model uncertainty, available time and funding for conducting the analysis, and the application of the model(s) for the specific situation.

## 7. CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, workshop participants suggested a number of ways in which PBT chemical risk assessment could be improved. Specific improvements were suggested for three major components of the human health risk assessment process: hazard identification, dose-response assessment and exposure assessment. Issues important to hazard identification for PBT chemical exposure during the postnatal period were discussed in detail in this report ([Section 4.2](#)), and suggested approaches to closing some of the data gaps related to these issues were also provided ([Section 5](#)). The report also described toxicological study design features that could facilitate the use of neonatal animal dose-response data in human health risk assessment ([Section 5](#)). Finally, the report includes suggested modeling approaches to support both dose-response and exposure assessment for lactational exposures to PBT chemicals ([Section 6](#)).

Human development begins at conception and continues, in some aspects, into young adulthood with different developmental processes occurring at different times ([Makris et al., 2008](#)). In both humans and experimental animal models, PBT chemicals have been shown to affect developmental processes, which may lead to long-lasting health effects ([Mead, 2008](#)). Developmental processes are, in general, most sensitive to chemical disruption if exposure occurs when the process is active (i.e., during a critical and/or sensitive window) ([Makris et al., 2008](#)). Thus, the identification of hazards to development requires an understanding of the timing of sensitive windows of exposure. However, for many developmental endpoints, the precise timing of these sensitive windows remains unknown. As described in [Section 4.2](#), modeling approaches can be used with data from epidemiological studies to identify sensitive windows of exposure in humans ([Verner et al., 2010](#)). Sensitive windows of exposure can also be identified in animals through the use of experimental designs that examine the effects of exposure during specific periods of time ([Makris et al., 2008](#); [Holladay and Smialowicz, 2000](#)). Because the timing of developmental processes in humans and animals is often very different, it is important to understand this timing in all relevant species if animal data are to be used to support human health risk assessment.

Hazard identification for early life PBT chemical exposure may also be facilitated by advancements in the field of epigenetics. Validated epigenetic biomarkers of developmental effects may enable earlier detection of potential effects and their associations with perinatal chemical exposures in human populations.

Because of differences in rates of elimination of PBT chemicals, and thus, their bioaccumulation, between humans and laboratory animals, lactational exposure to a PBT chemical in offspring in an animal study may be very different compared to lactational exposure in a human infant whose mother was exposed to the same chemical at the same average daily dose as that administered to the animal dam. As discussed in [Section 6](#), workshop participants suggested a set of kinetic modeling approaches that can be used to estimate a human equivalent dose (HED) to improve the use of dose-response data from animal studies of early life PBT chemical exposure. Kinetic modeling approaches can also be used in exposure assessment to estimate infant lactational exposures to PBT chemicals based on information on the mother's exposure. This is particularly useful for conducting an exposure assessment in situations where data on chemical concentrations in environmental media are available, but breast milk chemical concentrations are not.

Workshop attendees agreed that there is no single modeling approach that is suitable for all assessments of all PBT chemicals. Of the approaches suggested in [Section 6](#), validated chemical-specific PBPK models would be the most robust. However, these models are often unavailable for a particular chemical of interest. Furthermore, these models may not be transparent to many users developing a risk assessment or for potential stakeholders. As discussed in [Section 6](#), in many cases, simpler modeling approaches may be adequate for risk assessment. Simpler approaches often require less data and may be more transparent, especially when the equations used to support them can be presented in a readily accessible format. Workshop participants suggested that the results of PBPK models be compared to the results of other approaches for a set of data-rich lipophilic PBT chemicals with different kinetic profiles. Systematic evaluation of differences in model output may facilitate the identification of chemicals for which the simpler modeling approaches are adequate for risk assessment and those for which chemical-specific PBPK models should be developed.

The accuracy of the exposure estimates produced by these modeling approaches depends on the availability of accurate data and parameter values for use in the models. For a given chemical, many of the parameters needed for the suggested modeling approaches may be variable and/or uncertain. [Section 4.3](#) includes suggested methods for using the modeling approaches with variable or uncertain parameters. However, the application of the modeling approaches both to dose-response and to exposure assessment would be improved by reducing uncertainty in critical modeling parameters. Human biomonitoring data may be useful in this context. Workshop participants highlighted the importance of measuring PBT chemical concentrations in human milk to support neonatal exposure assessment and to gain a better understanding of chemical partitioning between milk and serum, including how this parameter may vary among women ([LaKind et al., 2009](#)). To improve the use of animal data in the modeling approaches, workshop participants suggested that future toxicological studies collect measures of internal dose in the same animals monitored for toxicological outcomes.

For several of the modeling approaches suggested in [Section 6](#), model output is highly dependent on the value used for the half-life of the chemical. And, often, the data to support this parameter are inadequate, resulting in uncertainty. Estimates of chemical half-lives in humans would be better

supported by data from studies measuring PBT chemical concentrations in serum over time that (1) include estimates of ongoing exposure to the chemical from the environment, and (2) control for changes in body weight. For estimates of PBT chemical half-lives in laboratory animal species, it would be useful to have time-course data on tissue distribution of the chemicals to develop both tissue-specific and whole-body elimination rates.

Notably, there are many PBT chemicals beyond those that were specifically addressed at this workshop (i.e., HCB, mirex, DDT and chlordane), including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). Many of these chemicals share properties with the four PBT chemicals considered at the workshop, namely (1) their widespread presence in the environment and in human tissues, and (2) their lipophilicity, bioaccumulation, and potential for infant exposure to be higher than maternal exposure during the time period of lactation. The approaches described in this report may prove to be useful in conducting risk assessments for exposure during development for many lipophilic PBT chemicals.

In addition, there are lipophilic PBT chemicals in the environment that are not currently monitored. And, as new chemicals are synthesized or changes are made to the use and/or disposal of existing chemicals, there is the potential for more PBT chemicals to enter the environment. Importantly, this report provides strategies that may be useful for protecting children from possible health impacts of these emerging chemicals, for which very little data are likely to exist to support hazard identification or dose-response assessment.

Although lipophilic PBT chemicals were the specific focus of this workshop, infants may be exposed to many other types of chemicals present in the environment. These include perfluorinated chemicals (PFCs), perchlorates, heavy metals, phthalates, bisphenols, and many more. For some of these, breastfed infant exposure may be of interest ([Mead, 2008](#)). However, formula-fed infants may also be exposed to chemicals for which neonatal developmental endpoints have not been adequately investigated ([Lakind et al., 2005b](#)). And, both breastfed and formula-fed infants may also be exposed to chemicals via non-dietary sources (e.g., air, soil, dust). A better understanding of critical and/or sensitive windows of development in both humans and animals, as advocated in this report, would support the identification of neonatal health impacts of chemicals with wide-ranging biochemical properties and sources of exposure. But, future efforts are needed to fully consider data gaps, uncertainties, and appropriate modeling approaches to support risk assessment of neonatal exposure to chemicals with properties very different from those considered in this report.

Finally, as discussed in this report ([Section 4.1](#)), when developing risk assessments that include evaluations of lactational exposures to PBT chemicals, it is important to develop parallel risk communication strategies that consider the benefits of breastfeeding to infant and maternal health ([Lakind et al., 2004](#)). Currently, all available evidence suggests that, compared to formula-feeding, breastfeeding is most often the healthiest option for infants despite the presence of environmental chemicals in breast milk ([Mead, 2008](#)). While it is important to assess early life exposures as part of an overall risk assessment, it is important to reiterate that the goal of risk assessment efforts is not to

discourage breastfeeding, but to accurately account for all possible risk and, if indicated, to determine how to reduce chemical levels in breast milk by reducing maternal exposure.

## 8. REFERENCES

- [Aafjes, JH; Vels, JM; Schenck, E.](#) (1980). Fertility of rats with artificial oligozoospermia. *J Reprod Fertil* 58: 345-351.
- [AAP](#) (American Academy of Pediatrics). (2012). Breastfeeding and the use of human milk [Review]. *Pediatrics* 129: e827-e841. <http://dx.doi.org/10.1542/peds.2011-3552>
- [Abraham, K; Hille, A; Ende, M; Helge, H.](#) (1994). Intake and fecal excretion of PCDDs, PCDFs, HCB and PCBs (138, 153, 180) in a breast-fed and a formula-fed infant. *Chemosphere* 29: 2279-2286. [http://dx.doi.org/10.1016/0045-6535\(94\)90395-6](http://dx.doi.org/10.1016/0045-6535(94)90395-6)
- [Adams, J; Barone, S, Jr.; Lamantia, A; Philen, R; Rice, DC; Spear, L; Susser, E.](#) (2000). Workshop to identify critical windows of exposure for children's health: Neurobehavioral Work Group summary [Review]. *Environ Health Perspect* 108: 535-544.
- [Adamsson, A; Salonen, V; Paranko, J; Toppari, J.](#) (2009). Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) on steroidogenesis in the fetal rat testis and adrenal gland. *Reprod Toxicol* 28: 66-74. <http://dx.doi.org/10.1016/j.reprotox.2009.03.002>
- [Al-Hachim, GM; Al-Baker, A.](#) (1973). Effects of chlordane on conditioned avoidance response, brain seizure threshold and open-field performance of prenatally-treated mice. *Br J Pharmacol* 49: 311-315.
- [Al-Omar, M; Abdul-Jalil, FH; Al-Ogaily, NH; Tawfiq, SJ; Al-Bassomy, MA.](#) (1986). A follow-up study of maternal milk contamination with organochlorine insecticide residues. *Environ Pollut Ecol Biol* 42: 79-92. [http://dx.doi.org/10.1016/0143-1471\(86\)90046-2](http://dx.doi.org/10.1016/0143-1471(86)90046-2)
- [Al-Omar, MA; Tawfiq, SJ; Al-Ogaily, N.](#) (1985). Organochlorine residue levels in human milk from Baghdad. *Bull Environ Contam Toxicol* 35: 65-67. <http://dx.doi.org/10.1007/BF01636481>
- [Aldrich, FD; Holmes, JH.](#) (1969). Acute chlordane intoxication in a child: case report with toxicological data. *Arch Environ Occup Health* 19: 129-132.
- [Anderson, LM; Diwan, BA; Fear, NT; Roman, E.](#) (2000). Critical windows of exposure for children's health: Cancer in human epidemiological studies and neoplasms in experimental animal models [Review]. *Environ Health Perspect* 108: 573-594.
- [Ando, M.](#) (1978). Transfer of 2,4,5,2',4',5'-hexachlorobiphenyl and 2,2-bis-(p-chlorophenyl),1,1,1-trichloroethane (p,p'-DDT) from maternal to newborn and suckling rats. *Arch Toxicol* 41: 179-186.
- [Ando, M; Wakisaka, I.](#) (1978). [Transfer of 2,4,5,2',4',5'-hexachlorobiphenyl and p,p'-DDT to new born rats from the mother] [Abstract]. *Nippon Eiseigaku Zasshi* 33: 216.
- [Andrews, JE; Courtney, KD.](#) (1986). Hexachlorobenzene-induced renal maldevelopment in CD-1 mice and CD rats. In CR Morris; JRP Cabral (Eds.), *IARC Scientific Publications* (pp. 381-391). Lyon, France: International Agency for Research on Cancer. <http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=73&codcch=77>
- [Arcus-Arth, A; Krowech, G; Zeise, L.](#) (2005). Breast milk and lipid intake distributions for assessing cumulative exposure and risk. *J Expo Anal Environ Epidemiol* 15: 357-365. <http://dx.doi.org/10.1038/sj.jea.7500412>
- [Asakawa, F; Jitsunari, F; Shiraishi, H; Suna, S; Takeda, N; Kitamado, T.](#) (1996). Accumulation of chlordanes in adipose tissues of mice caused by long-term exposure of low level technical chlordane. *Bull Environ Contam Toxicol* 57: 909-916.
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (1994). Toxicological profile for chlordane. (RISKLIN/1995020003). Washington, DC: U.S. Department of Health and Human Services.

- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (1995). Toxicological profile for mirex (CASRN 2385-85-5) and chlordecone (CASRN 143-50-0). <http://www.atsdr.cdc.gov/toxprofiles/tp66.pdf>
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2002a). Toxicological profile for DDT, DDE, and DDD. Atlanta, GA. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=81&tid=20>
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2002b). Toxicological profile for hexachlorobenzene [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
- [Ayotte, P; Carrier, G; Dewailly, E.](#) (1996). Health risk assessment for inuit newborns exposed to dioxin-like compounds through breast feeding. *Chemosphere* 32: 531-542.
- [Ayotte, P; Dewailly, E; Bruneau, S; Careau, H; Vézina, A.](#) (1995). Arctic air pollution and human health: what effects should be expected [Review]. *Sci Total Environ* 160-161: 529-537. [http://dx.doi.org/10.1016/0048-9697\(95\)04387-G](http://dx.doi.org/10.1016/0048-9697(95)04387-G)
- [Ayotte, P; Muckle, G; Jacobson, JL; Jacobson, SW; Dewailly, E.](#) (2003). Assessment of pre- and postnatal exposure to polychlorinated biphenyls: Lessons from the Inuit Cohort Study. *Environ Health Perspect* 111: 1253-1258. <http://dx.doi.org/10.1289/ehp.6054>
- [Bailey, J; Knauf, V; Mueller, W; Hobson, W.](#) (1980). Transfer of hexachlorobenzene and polychlorinated biphenyls to nursing infant rhesus monkeys: enhanced toxicity. *Environ Res* 21: 190-196.
- [Barnett, JB; Barfield, L; Walls, R; Joyner, R; Owens, R; Soderberg, LSF.](#) (1987). The effect of in utero exposure to hexachlorobenzene on the developing immune response of BALB/c mice. *Toxicol Lett* 39: 263-274.
- [Barnett, JB; Blaylock, BL; Gandy, J; Menna, JH; Denton, R; Soderberg, LS.](#) (1990a). Long-term alteration of adult bone marrow colony formation by prenatal chlordane exposure. *Fundam Appl Toxicol* 14: 688-695. [http://dx.doi.org/10.1016/0272-0590\(90\)90294-T](http://dx.doi.org/10.1016/0272-0590(90)90294-T)
- [Barnett, JB; Blaylock, BL; Gandy, J; Menna, JH; Denton, R; Soderberg, LSF.](#) (1990b). Alteration of fetal liver colony formation by prenatal chlordane exposure. *Fundam Appl Toxicol* 15: 820-822. [http://dx.doi.org/10.1016/0272-0590\(90\)90198-S](http://dx.doi.org/10.1016/0272-0590(90)90198-S)
- [Barnett, JB; Holcomb, D; Menna, JH; Soderberg, LSF.](#) (1985a). The effect of prenatal chlordane exposure on specific anti-influenza cell-mediated immunity. *Toxicol Lett* 25: 229-238. [http://dx.doi.org/10.1016/0378-4274\(85\)90202-4](http://dx.doi.org/10.1016/0378-4274(85)90202-4)
- [Barnett, JB; Soderberg, LSF; Menna, JH.](#) (1985b). The effect of prenatal chlordane exposure on the delayed hypersensitivity response of BALB/c mice. *Toxicol Lett* 25: 173-183. [http://dx.doi.org/10.1016/0378-4274\(85\)90079-7](http://dx.doi.org/10.1016/0378-4274(85)90079-7)
- [Barnett, RW; D'Ercole, AJ; Cain, JD; Arthur, RD.](#) (1979). Organochlorine pesticide residues in human milk samples from women living in Northwest and Northeast Mississippi, 1973-75. *Pestic Monit J* 13: 47-51.
- [Basri Ustünbas, H; Adnan Oztürk, MA; Hasanoğlu, E; Doğan, M.](#) (1994). Organochlorine pesticide residues in human milk in Kayseri. *Hum Exp Toxicol* 13: 299-302. <http://dx.doi.org/10.1177/096032719401300501>
- [Bates, JH; Rincon, M; Irvin, CG.](#) (2009). Animal models of asthma [Review]. *Am J Physiol Lung Cell Mol Physiol* 297: L401-L410. <http://dx.doi.org/10.1152/ajplung.00027.2009>
- [Bates, MN; Hannah, DJ; Buckland, SJ; Taucher, JA; van Maanen, T.](#) (1994). Chlorinated organic contaminants in breast milk of New Zealand women. *Environ Health Perspect* 102: 211-217.
- [Belfiore, CJ; Yang, RS; Chubb, LS; Lohitnavy, M; Lohitnavy, OS; Andersen, ME.](#) (2007). Hepatic sequestration of chlordecone and hexafluoroacetone evaluated by pharmacokinetic modeling. *Toxicology* 234: 59-72. <http://dx.doi.org/10.1016/j.tox.2007.02.002>

- [Beretta, M; Dick, T.](#) (1994). Organochlorine compounds in human milk, Porto Alegre, Brazil. *Bull Environ Contam Toxicol* 53: 357-360. <http://dx.doi.org/10.1007/BF00197226>
- [Berlin, CM; Crase, BL; Fürst, P; Lakind, JS; Moy, G; Needham, LL; Pugh, LC; Tully, MR.](#) (2005a). Methodologic considerations for improving and facilitating human milk research. *J Toxicol Environ Health A* 68: 1803-1823. <http://dx.doi.org/10.1080/15287390500226755>
- [Berlin, CM; Lakind, JS; Fenton, SE; Wang, RY; Bates, MN; Brent, RL; Condon, M; Crase, BL; Dourson, ML; Ettinger, AS; Foos, B; Fürst, P; Giacoia, GP; Goldstein, DA; Haynes, SG; Hench, KD; Kacew, S; Koren, G; Lawrence, RA; Mason, A; McDiarmid, MA; Moy, G; Needham, LL; Paul, IM; Pugh, LC; Qian, Z; Salamone, L; Selevan, SG; Sonawane, B; Tarzian, AJ; Rose Tully, M; Uhl, K.](#) (2005b). Conclusions and recommendations of the expert panel: technical workshop on human milk surveillance and biomonitoring for environmental chemicals in the United States. *J Toxicol Environ Health A* 68: 1825-1831. <http://dx.doi.org/10.1080/15287390500226896>
- [Bhatia, R; Shiau, R; Petreas, M; Weintraub, JM; Farhang, L; Eskenazi, B.](#) (2005). Organochlorine pesticides and male genital anomalies in the child health and development studies. *Environ Health Perspect* 113: 220-224.
- [Birnbaum, LS; Fenton, SE.](#) (2003). Cancer and developmental exposure to endocrine disruptors [Review]. *Environ Health Perspect* 11: 389-394. <http://dx.doi.org/10.1289/ehp.5686>
- [Blanck, HM; Marcus, M; Tolbert, PE; Rubin, C; Henderson, AK; Hertzberg, VS; Zhang, RH; Cameron, L.](#) (2000). Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. *Epidemiology* 11: 641-647.
- [Blaylock, BL; Newsom, KK; Holladay, SD; Shipp, BK; Bartow, TA; Mehendale, HM.](#) (1995). Topical exposure to chlordane reduces the contact hypersensitivity response to oxazolone in BALB/c mice. *Toxicol Lett* 81: 205-211. [http://dx.doi.org/10.1016/0378-4274\(95\)03436-6](http://dx.doi.org/10.1016/0378-4274(95)03436-6)
- [Blaylock, BL; Soderberg, LS; Gandy, J; Menna, JH; Denton, R; Barnett, JB.](#) (1990). Cytotoxic T-lymphocyte and NK responses in mice treated prenatally with chlordane. *Toxicol Lett* 51: 41-49.
- [Blyler, G; Landreth, KS; Barnett, JB.](#) (1994). Gender-specific effects of prenatal chlordane exposure on myeloid cell development. *Fundam Appl Toxicol* 23: 188-193. <http://dx.doi.org/10.1093/toxsci/23.2.188>
- [Bodkin, NL; Alexander, TM; Ortmeyer, HK; Johnson, E; Hansen, BC.](#) (2003). Mortality and morbidity in laboratory-maintained Rhesus monkeys and effects of long-term dietary restriction. *J Gerontol A Biol Sci Med Sci* 58: B212-B219. <http://dx.doi.org/10.1093/gerona/58.3.B212>
- [Boersma, ER; Lanting, CI.](#) (2000). Environmental exposure to polychlorinated biphenyls (PCBs) and dioxins. Consequences for longterm neurological and cognitive development of the child lactation [Review]. *Adv Exp Med Biol* 478: 271-287. [http://dx.doi.org/10.1007/0-306-46830-1\\_25](http://dx.doi.org/10.1007/0-306-46830-1_25)
- [Bogen, KT; Cullen, AC; Frey, HC; Price, PS.](#) (2009). Probabilistic exposure analysis for chemical risk characterization [Review]. *Toxicol Sci* 109: 4-17. <http://dx.doi.org/10.1093/toxsci/kfp036>
- [Bond, CA; Woodham, DW; Ahrens, EH; Medley, JG.](#) (1975). The cumulation and disappearance of mirex residues. II. In milk and tissues of cows fed two concentrations of the insecticide in their diet. *Bull Environ Contam Toxicol* 14: 25-31. <http://dx.doi.org/10.1007/BF01685593>
- [Bordet, F; Mallet, J; Maurice, L; Borrel, S; Venant, A.](#) (1993). ORGANOCHLORINE PESTICIDE AND PCB CONGENER CONTENT OF FRENCH HUMAN MILK. *Bull Environ Contam Toxicol* 50: 425-432.
- [Borrud, LG; Flegal, KM; Looker, AC; Everhart, JE; Harris, TB; Shepherd, JA.](#) (2010). Body composition data for individuals 8 years of age and older: U.S. population, 1999–2004. *Vital Health Stat* 11 no. 250.
- [Bouchard, M; Bellinger, D; Wright, R; Weisskopf, M.](#) (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. *Pediatrics* 125: e1270-e1277. <http://dx.doi.org/10.1542/peds.2009-3058>



- [Bouchard, MF; Chevrier, J; Harley, KG; Kogut, K; Vedar, M; Calderon, N; Trujillo, C; Johnson, C; Bradman, A; Barr, DB; Eskenazi, B.](#) (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect* 119: 1189-1195. <http://dx.doi.org/10.1289/ehp.1003185>
- [Bouwman, H; Becker, PJ; Cooppan, RM; Reinecke, AJ.](#) (1992). Transfer of DDT used in malaria control to infants via breast milk. *Bull World Health Organ* 70: 241-250.
- [Bowen-Jones, A; Thompson, C; Drewett, RF.](#) (1982). Milk flow and sucking rates during breast-feeding. *Dev Med Child Neurol* 24: 626-633. <http://dx.doi.org/10.1111/j.1469-8749.1982.tb13673.x>
- [Brown, RP; Delp, MD; Lindstedt, SL; Rhomberg, LR; Beliles, RP.](#) (1997). Physiological parameter values for physiologically based pharmacokinetic models [Review]. *Toxicol Ind Health* 13: 407-484.
- [BUA](#) (GDCh Advisory Committee on Existing Chemicals). (1993). Hexachlorobenzene. (BUA Report 119). Stuttgart, Germany: S. Hirzel Verlag. <http://www.hirzel.de/fachbuch/naturwissenschaften/chemie-und-biologie/bua-reports/view/titel/1287.html>
- [Buck Louis, GM; Gray, LE, Jr.; Marcus, M; Ojeda, SR; Pescovitz, OH; Witchel, SF; Sippell, W; Abbott, DH; Soto, A; Tyl, RW; Bourguignon, JP; Skakkebaek, NE; Swan, SH; Golub, MS; Wabitsch, M; Toppari, J; Euling, SY.](#) (2008). Environmental factors and puberty timing: expert panel research needs. *Pediatrics* 121 Suppl 3: S192-207. <http://dx.doi.org/10.1542/peds.1813E>
- [Burse, VW; Head, SL; McClure, PC; Korver, MP; Alley, CC; Phillips, DL; Needham, LL; Rowley, DL; Kahn, SE.](#) (1989). Partitioning of mirex between adipose tissue and serum. *J Agric Food Chem* 37: 692-699. <http://dx.doi.org/10.1021/jf00087a025>
- [Bush, B; Snow, J; Conner, S; Rueckert, L; Han, Y; Dymerski, P; Hilker, D.](#) (1983). Mirex in human milk in upstate New York. *Arch Environ Contam Toxicol* 12: 739-746. <http://dx.doi.org/10.1007/BF01060759>
- [Bush, B; Snow, J; Connor, S; Koblitz, R.](#) (1985). Polychlorinated biphenyl congeners (PCBs), p,p'-DDE and hexachlorobenzene in human milk in three areas of upstate New York. *Arch Environ Contam Toxicol* 14: 443-450. <http://dx.doi.org/10.1007/BF01055530>
- [Bush, B; Snow, J; Koblitz, R.](#) (1984). Polychlorobiphenyl (PCB) congeners, p,p'-DDE, and hexachlorobenzene in maternal and fetal cord blood from mothers in Upstate New York. *Arch Environ Contam Toxicol* 13: 517-527. <http://dx.doi.org/10.1007/BF01056331>
- [Byczkowski, JZ; Kinkead, ER; Leahy, HF; Randall, GM; Fisher, JW.](#) (1994). Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically based model. *Toxicol Appl Pharmacol* 125: 228-236. <http://dx.doi.org/10.1006/taap.1994.1068>
- [Byczkowski, JZ; Lipscomb, JC.](#) (2001). Physiologically based pharmacokinetic modeling of the lactational transfer of methylmercury. *Risk Anal* 21: 869-882.
- [Byrd, RA; Kimmel, CA; Morris, MD; Holson, JF; Young, JF.](#) (1981). Altered pattern of prenatal toxicity in rats due to different treatment schedules with mirex. *Toxicol Appl Pharmacol* 60: 213-219. [http://dx.doi.org/10.1016/0041-008X\(91\)90225-4](http://dx.doi.org/10.1016/0041-008X(91)90225-4)
- [Byrd, RA; Young, JF; Kimmel, CA; Morris, MD; Holson, JF.](#) (1982). Computer simulation of mirex pharmacokinetics in the rat. *Toxicol Appl Pharmacol* 66: 182-192. [http://dx.doi.org/10.1016/0041-008X\(82\)90283-6](http://dx.doi.org/10.1016/0041-008X(82)90283-6)
- [Cassidy, RA; Vorhees, CV; Minnema, DJ; Hastings, L.](#) (1994). The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. *Toxicol Appl Pharmacol* 126: 326-337. <http://dx.doi.org/10.1006/taap.1994.1123>
- [CDC.](#) (2000). Birth to 36 months: Girls: Length-for-age and weight-for-age percentiles. In *Vital and Health Statistics* (pp. 36). Washington, DC: National Center for Health Statistics. [www.cdc.gov/growthcharts/data/set1clinical/cj411018.pdf](http://www.cdc.gov/growthcharts/data/set1clinical/cj411018.pdf)



- [CDC](#) (Centers for Disease Control and Prevention). (2009). Fourth national report on human exposure to environmental chemicals. Atlanta, GA. <http://www.cdc.gov/exposurereport/>
- [CDC](#) (Centers for Disease Control and Prevention). (2013). Breastfeeding report card : United States 2013. Atlanta, GA.
- [Chambers, J; Yarbrough, J.](#) (1979). Disposition and excretion of mirex, 2, 8-dihydromirex, and 5, 10-dihydromirex by adult rats. *Fed Proc* 38: 266.
- [Chambers, JE; Case, RS; Alley, EG; Yarbrough, JD.](#) (1982). Short-term fate of mirex and 2,8-dihydromirex in rats. *J Agric Food Chem* 30: 878-882. <http://dx.doi.org/10.1021/jf00113a020>
- [Chan, HM; Berti, PR; Receveur, O; Kuhnlein, HV.](#) (1997). Evaluation of the population distribution of dietary contaminant exposure in an Arctic population using Monte Carlo statistics. *Environ Health Perspect* 105: 316-321. <http://dx.doi.org/10.2307/3433269>
- [Chen, J; Gao, J.](#) (1993). The Chinese total diet study in 1990. Part I. Chemical contaminants. *J AOAC Int* 76: 1193-1205.
- [Chernoff, N; Kavlock, RJ.](#) (1982). An in vivo teratology screen utilizing pregnant mice. *J Toxicol Environ Health* 10: 541-550. <http://dx.doi.org/10.1080/15287398209530275>
- [Chernoff, N; Linder, RE; Scotti, TM; Rogers, EH; Carver, BD; Kavlock, RJ.](#) (1979a). Fetotoxicity and cataractogenicity of mirex in rats and mice with notes on kepone. *Environ Res* 18: 257-269. [http://dx.doi.org/10.1016/0013-9351\(79\)90102-6](http://dx.doi.org/10.1016/0013-9351(79)90102-6)
- [Chernoff, N; Stevens, J; Rogers, E.](#) (1979b). Perinatal toxicology of mirex administered in the diet: I. viability, growth, cataractogenicity and tissue levels. *Toxicol Lett* 4: 263-268. [http://dx.doi.org/10.1016/0378-4274\(79\)90063-8](http://dx.doi.org/10.1016/0378-4274(79)90063-8)
- [Chevrier, J; Dewailly, E; Ayotte, P; Mauriège, P; Després, JP; Tremblay, A.](#) (2000). Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *Int J Obes (Lond)* 24: 1272-1278.
- [Chu, I; Villeneuve, DC; Secours, VE; Valli, VE; Becking, GC.](#) (1981). Effects of photomirex and mirex on reproduction in the rat. *Toxicol Appl Pharmacol* 60: 549-556. [http://dx.doi.org/10.1016/0041-008X\(81\)90341-0](http://dx.doi.org/10.1016/0041-008X(81)90341-0)
- [Clewell, HJ; Gentry, PR; Covington, TR; Sarangapani, R; Teeguarden, JG.](#) (2004). Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol Sci* 79: 381-393. <http://dx.doi.org/10.1093/toxsci/kfh109>
- [Clewell, RA; Gearhart, JM.](#) (2002). Pharmacokinetics of toxic chemicals in breast milk: use of PBPK models to predict infant exposure. *Environ Health Perspect* 110: A333-A337.
- [Clewell, RA; Merrill, EA; Yu, KO; Mahle, DA; Sterner, TR; Fisher, JW; Gearhart, JM.](#) (2003). Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. *Toxicol Sci* 74: 416-436. <http://dx.doi.org/10.1093/toxsci/kfg147>
- [Cohen Hubal, EA; Moya, J; Selevan, SG.](#) (2008). A lifestage approach to assessing children's exposure [Review]. *Birth Defects Res B Dev Reprod Toxicol* 83: 522-529. <http://dx.doi.org/10.1002/bdrb.20173>
- [Conde, C; Maluenda, C; Arrabal, C.](#) (1993). Hexachlorobenzene (HCB) in human milk in Spain from 1984 to 1991. *Bull Environ Contam Toxicol* 51: 827-831. <http://dx.doi.org/10.1007/BF00198277>
- [Cookson, WOCM; Moffatt, MF.](#) (1997). Asthma: An epidemic in the absence of infection? [Comment]. *Science* 275: 41-42. <http://dx.doi.org/10.1126/science.275.5296.41>

- Courtney, KD; Andrews, JE. (1985). Neonatal and maternal body burdens of hexachlorobenzene (HCB) in mice: gestational exposure and lactational transfer. *Toxicol Sci* 5: 265-277. [http://dx.doi.org/10.1016/0272-0590\(85\)90074-0](http://dx.doi.org/10.1016/0272-0590(85)90074-0)
- Courtney, KD; Andrews, JE; Grady, MA; Ebron, MT. (1984). Postnatal effects of hexachlorobenzene (HCB) on cardiac lactic dehydrogenase (LDH) and creatine kinase (CK) isozymes in CD-1 mice. *Toxicol Lett* 22: 223-228.
- Courtney, KD; Andrews, JE; Svendsgaard, DJ. (1979). Hexachlorobenzene (HCB) deposition in maternal and fetal tissue of rat and mouse: I. Chemical quantification of HCB in tissues. *Environ Res* 19: 1-13.
- Courtney, KD; Copeland, MF; Robbins, A. (1976). The effects of pentachloronitrobenzene, hexachlorobenzene, and related compounds on fetal development. *Toxicol Appl Pharmacol* 35: 239-256.
- Craan, AG; Haines, DA. (1998). Twenty-Five Years of Surveillance for Contaminants in Human Breast Milk. *Arch Environ Contam Toxicol* 35: 702-710. <http://dx.doi.org/10.1007/s002449900434>
- Craig, GR; Ogilvie, DM. (1974). Alteration of t-maze performance in mice exposed to DDT during pregnancy and lactation. *Environ Physiol Biochem* 4: 189-199.
- Cranmer, JM; Cranmer, MF; Goad, PT. (1984). Prenatal chlordane exposure: Effects on plasma corticosterone concentrations over the lifespan of mice. *Environ Res* 35: 204-210. [http://dx.doi.org/10.1016/0013-9351\(84\)90128-2](http://dx.doi.org/10.1016/0013-9351(84)90128-2)
- Cranmer, JS; Avery, DL; Grady, RR; Kitay, JL. (1978). Postnatal endocrine dysfunction resulting from prenatal exposure to carbofuran, diazinon or chlordane. *J Environ Pathol Toxicol* 2: 357-369.
- Cripps, DJ. (1990). Transplacental and mammary absorption of hexachlorobenzene: experimental pembe yara porphyria in neonates. *Mol Aspects Med* 11: 81-82.
- Cripps, DJ; Peters, HA; Gocmen, A; Dogramici, I. (1984). Porphyria turcica due to hexachlorobenzene: a 20 to 30 year follow-up study on 204 patients. *Br J Dermatol* 111: 413-422.
- Curley, A; Garrettson, LK. (1969). Acute chlordane poisoning: Clinical and chemical studies. *Arch Environ Occup Health* 18: 211-215.
- Dallaire, F; Dewailly, E; Muckle, G; Vézina, C; Jacobson, SW; Jacobson, JL; P, A. (2004). Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik. *Environ Health Perspect* 112: 1359-1364. <http://dx.doi.org/10.1289/ehp.7255>
- Damgaard, IN; Skakkebaek, NE; Toppari, J; Virtanen, HE; Shen, H; Schramm, KW; Petersen, JH; Jensen, TK; Main, KM. (2006). Persistent pesticides in human breast milk and cryptorchidism. *Environ Health Perspect* 114: 1133-1138.
- Davies, D; Mes, J. (1987). Comparison of the residue levels of some organochlorine compounds in breast milk of the general and indigenous Canadian populations. *Bull Environ Contam Toxicol* 39: 743-749.
- Davis, A; Gift, JS; Woodall, GM; Narotsky, MG; Fourman, GL. (2009). The role of developmental toxicity studies in acute exposure assessments: analysis of single-day vs. multiple-day exposure regimens. *Regul Toxicol Pharmacol* 54: 134-142. <http://dx.doi.org/10.1016/j.yrtph.2009.03.006>
- De Bruijn, J; Busser, F; Seinen, W; Hermens, J. (1989). Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the slow-stirring method. *Environ Toxicol Chem* 8: 499-512. <http://dx.doi.org/10.1002/etc.5620080607>
- de Escobar, GM; Ares, S; Berbel, P; Obregón, MJ; del Rey, FE. (2008). The changing role of maternal thyroid hormone in fetal brain development [Review]. *Semin Perinatol* 32: 380-386. <http://dx.doi.org/10.1053/j.semperi.2008.09.002>

- [Dearth, MA; Hites, RA.](#) (1991a). Chlordane accumulation in people. *Environ Sci Technol* 25: 1279-1285. <http://dx.doi.org/10.1021/es00019a008>
- [Dearth, MA; Hites, RA.](#) (1991b). Depuration rates of chlordane compounds from rat fat. *Environ Sci Technol* 25: 1125-1128.
- [Dekant, W.](#) (2009). The role of biotransformation and bioactivation in toxicity [Review]. *EXS* 99: 57-86. [http://dx.doi.org/10.1007/978-3-7643-8336-7\\_3](http://dx.doi.org/10.1007/978-3-7643-8336-7_3)
- [Depeters, EJ; Hovey, RC.](#) (2009). Methods for collecting milk from mice. *J Mammary Gland Biol Neoplasia* 14: 397-400. <http://dx.doi.org/10.1007/s10911-009-9158-0>
- [Desaulniers, D; Cooke, GM; Leingartner, K; Soumano, K; Cole, J; Yang, J; Wade, M; Yagminas, A.](#) (2005). Effects of postnatal exposure to a mixture of polychlorinated biphenyls, p,p'-dichlorodiphenyltrichloroethane, and p,p'-dichlorodiphenyldichloroethene in prepubertal and adult female Sprague-Dawley rats. *Int J Toxicol* 24: 111-127. <http://dx.doi.org/10.1080/10915810590936382>
- [Deutch, B; Dyerberg, J; Pedersen, HS; Asmund, G; Moller, P; Hansen, JC.](#) (2006). Dietary composition and contaminants in north Greenland, in the 1970s and 2004. *Sci Total Environ* 370: 372-381. <http://dx.doi.org/10.1016/j.scitotenv.2006.07.015>
- [Deutch, B; Pedersen, HS; Asmund, G; Hansen, JC.](#) (2007). Contaminants, diet, plasma fatty acids and smoking in Greenland 1999-2005. *Sci Total Environ* 372: 486-496. <http://dx.doi.org/10.1016/j.scitotenv.2006.10.043>
- [Devanathan, G; Subramanian, A; Someya, M; Sudaryanto, A; Isobe, T; Takahashi, S; Chakraborty, P; Tanabe, S.](#) (2009). Persistent organochlorines in human breast milk from major metropolitan cities in India. *Environ Pollut* 157: 148-154. <http://dx.doi.org/10.1016/j.envpol.2008.07.01>
- [Dewailly, E; Ayotte, P; Bruneau, S; Gingras, S; Belles-Isles, M; Roy, R.](#) (2000). Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. *Environ Health Perspect* 108: 205-211. <http://dx.doi.org/10.1289/ehp.00108205>
- [Dewailly, E; Ayotte, P; Bruneau, S; Laliberte, C; Muir, DC; Norstrom, RJ.](#) (1993). Inuit exposure to organochlorines through the aquatic food chain in arctic quebec. *Environ Health Perspect* 101: 618-620.
- [Dewailly, E; Nantel, A; Laliberté, C; Weber, J, -P; Gingras, S; Meyer, F.](#) (1991). Organochlorines in the breast milk in Québec: A provincial survey. Poster presented at Conference on Measuring, Understanding, and Predicting Exposures in the 21st Century, November 18-22, 1991, Atlanta, GA.
- [Dietert, RR; Etzel, RA; Chen, D; Halonen, M; Holladay, SD; Jarabek, AM; Landreth, K; Peden, DB; Pinkerton, K; Smialowicz, RJ; Zoetis, T.](#) (2000). Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary [Review]. *Environ Health Perspect* 108 Suppl 3: 483-490.
- [Dietert, RR; Zelikoff, JT.](#) (2008). Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma [Review]. *Birth Defects Res B Dev Reprod Toxicol* 83: 547-560. <http://dx.doi.org/10.1002/bdrb.20170>
- [DNHW](#) (Department of National Health and Welfare (Canada)). (1987). Nutrient value of some common foods. Ottawa, Canada: Department of National Health and Welfare.
- [Dorough, HW; Ivie, GW.](#) (1974). Fate of mirex-14C during and after a 28-day feeding period to a lactating cow. *J Environ Qual* 3: 65-67.
- [Dyer, JS; Rosenfeld, CR.](#) (2011). Metabolic imprinting by prenatal, perinatal, and postnatal overnutrition: A review [Review]. *Seminars in Reproductive Medicine* 29: 266-276. <http://dx.doi.org/10.1055/s-0031-1275521>

- [Engel, SM; Berkowitz, GS; Barr, DB; Teitelbaum, SL; Siskind, J; Meisel, SJ; Wetmur, JG; Wolff, MS.](#) (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol* 165: 1397-1404. <http://dx.doi.org/10.1093/aje/kwm029>
- [Ennaceur, S; Gandoura, N; Driss, MR.](#) (2007). Organochlorine pesticide residues in human milk of mothers living in northern Tunisia. *Bull Environ Contam Toxicol* 78: 325-329. <http://dx.doi.org/10.1007/s00128-007-9185-8>
- [Eriksson, P; Ahlbom, J; Fredriksson, A.](#) (1992). Exposure to DDT during a defined period in neonatal life induces permanent changes in brain muscarinic receptors and behaviour in adult mice. *Brain Res* 582: 277-281. [http://dx.doi.org/10.1016/0006-8993\(92\)90144-X](http://dx.doi.org/10.1016/0006-8993(92)90144-X)
- [Eskenazi, B; Marks, AR; Bradman, A; Fenster, L; Johnson, C; Barr, DB; Jewell, NP.](#) (2006). In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. *Pediatrics* 118: 233-241. <http://dx.doi.org/10.1542/peds.2005-3117>
- [Eskenazi, B; Marks, AR; Bradman, A; Harley, K; Barr, DB; Johnson, C; Morga, N; Jewell, NP.](#) (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect* 115: 792-798. <http://dx.doi.org/10.1289/ehp.9828>
- [Fahim, MS; Bennett, R; Hall, DG.](#) (1970). Effect of DDT on the nursing neonate [Letter]. *Nature* 228: 1222-1223. <http://dx.doi.org/10.1038/2281222a0>
- [Falcó, G; Llobet, JM; Bocio, A; Domingo, JL.](#) (2008). Exposure to hexachlorobenzene through fish and seafood consumption in Catalonia, Spain. *Sci Total Environ* 389: 289-295. <http://dx.doi.org/10.1016/j.scitotenv.2007.09.025>
- [Fang, SC; Fallin, E; Freed, VH.](#) (1977). Maternal transfer of 14<sup>1</sup>-DDT via placenta and milk and its metabolism in infant rats. *Arch Environ Contam Toxicol* 5: 427-536. <http://dx.doi.org/10.1007/BF02220922>
- [Farrer, D; Poulsen, Mi; Davoli, D; Bailey, M; Moffett, D; Fowler, D; Welsh, C; Ayotte, P; Verner, MA; Muckle, G; Yang, R; Haddad, S.](#) (2010). Comparison of estimated PCB-153 concentrations in human milk using various pharmacokinetic models. *Toxicologist* 114: 892.
- [FDA](#) (U.S. Food and Drug Administration). (1989). Food and Drug Administration pesticide program: Residues in foods-1988 [Review]. *J Assoc Offic Anal Chem* 72: 133A-152A.
- [FDA](#) (U.S. Food and Drug Administration). (1990). Food and Drug Administration pesticide program: Residues in foods 1989. *J Assoc Offic Anal Chem* 73: 127A-146A.
- [FDA](#) (U.S. Food and Drug Administration). (1994). Pesticide program: Residue monitoring 1993. *J AOAC Int* 77: 161A-185A.
- [FDA, US.](#) (2011). Pesticide Residue Monitoring Program FY 2004-2006. U.S. FDA. <http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Pesticides/ResidueMonitoringReports/ucm125183.htm>
- [Fedorov, S.](#) (2008). GetData Graph Digitizer (Version 2.24) [Computer Program]. Russia: GetData-graph-digitizer.com. Retrieved from <http://www.getdata-graph-digitizer.com/index.php>
- [Feldman, HA; McMahon, TA.](#) (1983). The 3/4 mass exponent for energy metabolism is not a statistical artifact. *Respir Physiol* 52: 149-163. [http://dx.doi.org/10.1016/0034-5687\(83\)90002-6](http://dx.doi.org/10.1016/0034-5687(83)90002-6)
- [Fenster, L; Eskenazi, B; Anderson, M; Bradman, A; Hubbard, A; Barr, DB.](#) (2007). In utero exposure to DDT and performance on the Brazelton neonatal behavioral assessment scale. *Neurotoxicology* 28: 471-477. <http://dx.doi.org/10.1016/j.neuro.2006.12.009>

- [Fenton, SE; Condon, M; Ettinger, AS; Lakind, JS; Mason, A; Mcdiarmid, M; Qian, Z; Selevan, SG.](#) (2005). Collection and use of exposure data from human milk biomonitoring in the United States [Review]. *J Toxicol Environ Health A* 68: 1691-1712. <http://dx.doi.org/10.1080/15287390500225708>
- [Fiserova-Bergerova, V; Diaz, ML.](#) (1986). Determination and prediction of tissue-gas partition coefficients. *Int Arch Occup Environ Health* 58: 75-87. <http://dx.doi.org/10.1007/BF00378543>
- [Fisher, JW; Whittaker, TA; Taylor, DH; Clewell, HJ, III; Andersen, ME.](#) (1990). Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol* 102: 497-513.
- [Fitzgerald, EF; Hwang, SA; Deres, DA; Bush, B; Cook, K; Worswick, P.](#) (2001). The association between local fish consumption and DDE, mirex, and HCB concentrations in the breast milk of Mohawk women at Akwesasne. *J Expo Anal Environ Epidemiol* 11: 381-388. <http://dx.doi.org/10.1038/sj.jea.7500180>
- [Franchi, E; Focardi, S.](#) (1991). Polychlorinated biphenyl congeners, hexachlorobenzene and DDTs in human milk in central Italy. *Sci Total Environ* 102: 223-228.
- [Frank, R; Braun, HE; Thorpe, B.](#) (1993). Comparison of DDE and PCB residues in the general diet and in human blood--Ontario 1986-87. *Bull Environ Contam Toxicol* 51: 146-152. <http://dx.doi.org/10.1007/BF00201013>
- [Frank, R; Rasper, J; Smout, MS; Braun, HE.](#) (1988). Organochlorine residues in adipose tissues blood and milk from Ontario, Canada residents 1976-1985. *Can J Public Health* 79: 150-158.
- [Fried, GF; Marrow, GS; Gordon, CH.](#) (1969). Comparative excretion and retention of DDT analogs by dairy cows. *J Dairy Sci* 52: 1800-1805. [http://dx.doi.org/10.3168/jds.S0022-0302\(69\)86844-X](http://dx.doi.org/10.3168/jds.S0022-0302(69)86844-X)
- [Fries, GF; Marrow, GS.](#) (1974). Hexachlorobenzene excretion into milk of cows. *J Anim Sci* 39: 155.
- [Fryar, CD; Gu, Q; Ogden, CL.](#) (2012). Anthropometric reference data for children and adults: United States, 2007-2010. In *Vital Health Statistics*. Washington, DC: National Center for Health Statistics.
- [Fujii, Y; Haraguchi, K; Harada, KH; Hitomi, T; Inoue, K; Itoh, Y; Watanabe, T; Takenaka, K; Uehara, S; Yang, HR; Kim, MY; Moon, CS; Kim, HS; Wang, P; Liu, A; Hung, NN; Koizumi, A.](#) (2011). Detection of dicofol and related pesticides in human breast milk from China, Korea and Japan. *Chemosphere* 82: 25-31. <http://dx.doi.org/10.1016/j.chemosphere.2010.10.036>
- [Fürst, P; Fürst, C; Wilmers, K.](#) (1994). Human milk as a bioindicator for body burden of PCDDs, PCDFs, organochlorine pesticides, and PCBs. *Environ Health Perspect* 102: 187-193.
- [Gaines, TB; Kimbrough, RD.](#) (1970). Oral toxicity of mirex in adults and suckling rats: With notes on the ultrastructure of liver changes. *Arch Environ Health* 21: 7-14.
- [Galassi, S; Bettinetti, R; Neri, MC; Falandysz, J; Kotecka, W; King, I; Lo, S; Klingmueller, D; Schulte-Oehlmann, U.](#) (2008). pp'DDE contamination of the blood and diet in central European populations. *Sci Total Environ* 390: 45-52. <http://dx.doi.org/10.1016/j.scitotenv.2007.09.029>
- [Gallenberg, LA; Vodick, MJ.](#) (1987). Potential mechanisms for redistribution of polychlorinated biphenyls during pregnancy and lactation. *Xenobiotica* 17: 299-310. <http://dx.doi.org/10.3109/00498258709043940>
- [Galván-Portillo, M; Jiménez-Gutiérrez, C; Torres-Sánchez, L; López-Carrillo, L.](#) (2002). Food consumption and adipose tissue DDT levels in Mexican women. *Cad Saude Publica* 18: 447-452.
- [Gascon, M; Vrijheid, M; Martínez, D; Forn, J; Grimalt, JO; Torrent, M; Sunyer, J.](#) (2011). Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. *Environ Int* 37: 605-611. <http://dx.doi.org/10.1016/j.envint.2010.12.005>
- [Gentry, PR; Covington, TR; Clewell, HJ, III.](#) (2003). Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. *Regul Toxicol Pharmacol* 38: 1-16. [http://dx.doi.org/10.1016/S0273-2300\(03\)00047-3](http://dx.doi.org/10.1016/S0273-2300(03)00047-3)

- [Geyer, H; Scheunert, I; Korte, F.](#) (1986). Bioconcentration potential of organic environmental chemicals in humans. *Regul Toxicol Pharmacol* 6: 313-347. [http://dx.doi.org/10.1016/0273-2300\(86\)90002-4](http://dx.doi.org/10.1016/0273-2300(86)90002-4)
- [Gibaldi, M; Perrier, D.](#) (1982). Pharmacokinetics. In M Gibaldi; D Perrier (Eds.), (2nd ed.). New York, NY: Marcel Dekker.
- [Gibson, JR; Ivie, GW; Dorough, HW.](#) (1972). Fate of mirex and its major photodecomposition product in rats. *J Agric Food Chem* 20: 1246-1248.
- [Gingell, R; Wallcave, L.](#) (1974). Species differences in the acute toxicity and tissue distribution of DDT in mice and hamsters. *Toxicol Appl Pharmacol* 28: 385-394.
- [Ginsberg, G; Hattis, D; Sonawane, B.](#) (2004). Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants [Review]. *Toxicol Appl Pharmacol* 198: 164-183. <http://dx.doi.org/10.1016/j.taap.2003.10.010>
- [Gladen, BC; Ragan, NB; Rogan, WJ.](#) (2000). Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. *J Pediatr* 136: 490-496. <http://dx.doi.org/10.1067/mpd.2000.103505>
- [Gladen, BC; Rogan, WJ.](#) (1991). Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. *J Paediatr Child Health* 119: 58-63.
- [Gladen, BC; Rogan, WJ; Hardy, P; Thullen, J; Tingelstad, J; Tully, M.](#) (1988). Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. *J Pediatr* 113: 991-995. [http://dx.doi.org/10.1016/S0022-3476\(88\)80569-9](http://dx.doi.org/10.1016/S0022-3476(88)80569-9)
- [Glinioer, D.](#) (2001). Potential consequences of maternal hypothyroidism on the offspring: evidence and implications [Review]. *Horm Res* 55: 109-114. <http://dx.doi.org/49981>
- [Gocmen, A; Peters, HA; Cripps, DJ; Bryan, GT; Morris, CR.](#) (1989). Hexachlorobenzene episode in Turkey. *Biomed Environ Sci* 2: 36-43.
- [Goldey, ES; Fisher, JW; Taylor, DH.](#) (1990). Maternal transfer of hexachlorobenzene in the rat [Abstract]. *Neurotoxicol Teratol* 12: 562-563. [http://dx.doi.org/10.1016/0892-0362\(90\)90029-C](http://dx.doi.org/10.1016/0892-0362(90)90029-C)
- [Goldey, ES; Taylor, DH.](#) (1992). Developmental neurotoxicity following premating maternal exposure to hexachlorobenzene in rats. *Neurotoxicol Teratol* 14: 15-21.
- [Görs, S; Kucia, M; Langhammer, M; Junghans, P; Metges, CC.](#) (2009). Technical note: Milk composition in mice--methodological aspects and effects of mouse strain and lactation day. *J Dairy Sci* 92: 632-637. <http://dx.doi.org/10.3168/jds.2008-1563>
- [Grabowski, CT.](#) (1981). Plasma proteins and colloid osmotic pressure of blood of rat fetuses prenatally exposed to Mirex. *J Toxicol Environ Health* 7: 705-714. <http://dx.doi.org/10.1080/15287398109530013>
- [Grabowski, CT.](#) (1983). Persistent cardiovascular problems in newborn rats prenatally exposed to sub-teratogenic doses of the pesticide, mirex. In AW Hayes; RC Schnell; TS Miya (Eds.), *Developments in the science and practice of toxicology: Proceedings of the Third International Congress on Toxicology held in San Diego, California, USA, August 28-September 3, 1983* (pp. 537-540). New York, NY: Elsevier Science.
- [Grabowski, CT; Payne, DB.](#) (1980). An electrocardiographic study of cardiovascular problems in mirex-fed rat fetuses. *Teratology* 22: 167-177. <http://dx.doi.org/10.1002/tera.1420220205>
- [Grabowski, CT; Payne, DB.](#) (1983a). The causes of perinatal death induced by prenatal exposure of rats to the pesticide, mirex. Part I: Pre-parturition observations of the cardiovascular system. *Teratology* 27: 7-11. <http://dx.doi.org/10.1002/tera.1420270103>
- [Grabowski, CT; Payne, DB.](#) (1983b). The Causes Of Perinatal Death Induced By Prenatal Exposure Of Rats To The Pesticide, Mirex. Part II: Postnatal Observations. *J Toxicol Environ Health* 11: 301-316.



- [Grandjean, P; Harari, R; Barr, DB; Debes, F.](#) (2006). Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. *Pediatrics* 117: e546-e556.  
<http://dx.doi.org/10.1542/peds.2005-1781>
- [Gray, LE, Jr; Price, M; Lambright, C; Wolf, C; Hotchkiss, A; Parks, L; Ostby, J.](#) (1999). Environmental antiandrogens: the malformation pattern varies with the mechanism of antiandrogenic action. *Biol Reprod* 60: 201.
- [Greizerstein, HB; Stinson, C; Mendola, P; Buck, GM; Kostyniak, PJ; Vena, JE.](#) (1999). Comparison of PCB congeners and pesticide levels between serum and milk from lactating women. *Environ Res* 80: 280-286.  
<http://dx.doi.org/10.1006/enrs.1999.3956>
- [Greve, PA.](#) (1986). Environmental and human exposure to hexachlorobenzene in the Netherlands. In *Hexachlorobenzene: Proceedings of an international symposium, 24-25 June 1985. Lyon, France: International Agency for Research on Cancer.*
- [Greve, PA; Van Zoonen, P.](#) (1990). Organochlorine pesticides and PCBs in tissues from Dutch citizens (1968–1986). *Int J Environ Anal Chem* 38: 265-277. <http://dx.doi.org/10.1080/03067319008026932>
- [Gunderson, EL.](#) (1988). FDA Total Diet Study, April 1982–April 1984, dietary intakes of pesticides, selected elements, and other chemicals. *J Assoc Offic Anal Chem* 71: 1200-1209.
- [Haddad, S; Charest-Tardif, G; Krishnan, K.](#) (2000a). Physiologically based modeling of the maximal effect of metabolic interactions on the kinetics of components of complex chemical mixtures. *J Toxicol Environ Health A* 61: 209-223. <http://dx.doi.org/10.1080/00984100050131350>
- [Haddad, S; Poulin, P; Krishnan, K.](#) (2000b). Relative lipid content as the sole mechanistic determinant of the adipose tissue: Blood partition coefficients of highly lipophilic organic chemicals. *Chemosphere* 40: 839-843.  
[http://dx.doi.org/10.1016/S0045-6535\(99\)00279-9](http://dx.doi.org/10.1016/S0045-6535(99)00279-9)
- [Hansch, C; Leo, A; Hoekman, D.](#) (1995a). Exploring QSAR: Hydrophobic, electronic, and steric constants. In *ACS Professional Reference Book. Washington, DC: American Chemical Society.*
- [Hansch, C; Leo, A; Hoekman, D.](#) (1995b). Dibutyl phthalate. In *SR Heller (Ed.), Exploring QSAR Hydrophobic, electronic, and steric constants (pp. 144). Washington, DC: American Chemical Society.*
- [Haraguchi, K; Koizumi, A; Inoue, K; Harada, KH; Hitomi, T; Minata, M; Tanabe, M; Kato, Y; Nishimura, E; Yamamoto, Y; Watanabe, T; Takenaka, K; Uehara, S; Yang, HR; Kim, MY; Moon, CS; Kim, HS; Wang, P; Liu, A; Hung, NN.](#) (2009). Levels and regional trends of persistent organochlorines and polybrominated diphenyl ethers in Asian breast milk demonstrate POPs signatures unique to individual countries. *Environ Int* 35: 1072-1079. <http://dx.doi.org/10.1016/j.envint.2009.06.003>
- [Hardell, E; Carlberg, M; Nordstrom, M; Van Bavel, B.](#) (2010). Time trends of persistent organic pollutants in Sweden during 1993-2007 and relation to age, gender, body mass index, breast-feeding and parity. *Sci Total Environ* 408: 4412-4419. <http://dx.doi.org/10.1016/j.scitotenv.2010.06.029>
- [Hart, MM; Whang-Peng, J; Sieber, SM; Fabro, S; Adamson, RH.](#) (1972). Distribution and effects of DDT in the pregnant rabbit. *Xenobiotica* 2: 567-574. <http://dx.doi.org/10.3109/00498257209111084>
- [Hayes, WJ.](#) (1976). Dosage relationships associated with DDT in milk. *Toxicol Appl Pharmacol* 38: 19-28.  
[http://dx.doi.org/10.1016/0041-008X\(76\)90156-3](http://dx.doi.org/10.1016/0041-008X(76)90156-3)
- [Hernik, A; Góralczyk, K; Struciński, P; Czaja, K; Kucharska, A; Korcz, W; Snopczyński, T; Ludwicki, JK.](#) (2011). Polybrominated diphenyl ethers, polychlorinated biphenyls and organochlorine pesticides in human milk as markers of environmental exposure to these compounds. *Ann Agric Environ Med* 18: 113-118.
- [Heusner, AA.](#) (1982). Energy metabolism and body size I Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? *Respir Physiol Neurobiol* 48: 10-12.

- [Hines, RN.](#) (2008). The ontogeny of drug metabolism enzymes and implications for adverse drug events [Review]. *Pharmacol Ther* 118: 250-267. <http://dx.doi.org/10.1016/j.pharmthera.2008.02.005>
- [Ho, SM.](#) (2010). Environmental epigenetics of asthma: an update [Review]. *J Allergy Clin Immunol* 126: 453-465. <http://dx.doi.org/10.1016/j.jaci.2010.07.030>
- [Holladay, SD; Smialowicz, RJ.](#) (2000). Development of the murine and human immune system: Differential effects of immunotoxicants depend on time of exposure [Review]. *Environ Health Perspect* 108: 463-473.
- [Hooper, K; Hopper, K; Petreas, MX; She, J; Visita, P; Winkler, J; McKinney, M; Mok, M; Sy, F; Garcha, J; Gill, M; Stephens, RD; Semenova, G; Sharmanov, T; Chuvakova, T; Hopper, K.](#) (1997). Analysis of breast milk to assess exposure to chlorinated contaminants in Kazakhstan: PCBs and organochlorine pesticides in southern Kazakhstan. *Environ Health Perspect* 105: 1250-1254.
- [Hui, LL; Hedley, AJ; Kypke, K; Cowling, BJ; Nelson, EA; Wong, TW; van Leeuwen, FX; Malisch, R.](#) (2008). DDT levels in human milk in Hong Kong, 2001-02. *Chemosphere* 73: 50-55. <http://dx.doi.org/10.1016/j.chemosphere.2008.05.045>
- [Iatropoulos, MJ; Bailey, J; Adams, HP; Coulston, F; Hobson, W.](#) (1978). Response of nursing infant rhesus to clophen A-30 or hexachlorobenzene given to their lactating mothers. *Environ Res* 16: 38-47.
- [ICF.](#) (2013a). Time-varying human body burden model.
- [ICF.](#) (2013b). Time-varying rodent body burden model.
- [Ingebrigtsen, K; Nafstad, I.](#) (1983). Distribution and elimination of 14C-hexachlorobenzene after single oral exposure in the male rat. *Acta Pharmacol Toxicol* 52: 254-260. <http://dx.doi.org/10.1111/j.1600-0773.1983.tb01096.x>
- [Ingebrigtsen, K; Skaare, JU; Nafstad, I; Grave, K; Forde, M.](#) (1986). Metabolism of hexachlorobenzene (HCB) in the isolated perfused rat liver. *Vascul Pharmacol* 17: 19-24.
- [Ingle, L.](#) (1952). Chronic oral toxicity of chlordan to rats. *Arch Environ Occup Health* 6(4): 357-367.
- [IPCS.](#) (1996). Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. In *Environmental Health Criteria* (pp. 116). Geneva, Switzerland: World Health Organization. <http://www.inchem.org/documents/ehc/ehc/ehc180.htm>
- [IPCS.](#) (1997). Hexachlorobenzene. In *Environmental Health Criteria* (pp. 82). Geneva, Switzerland: World Health Organization. <http://www.inchem.org/documents/ehc/ehc/ehc195.htm>
- [Ivie, GW; Gibson, JR; Bryant, HE; Begin, JJ; Barnett, JR; Dorrough, HW.](#) (1974). Accumulation, distribution, and excretion of mirex-14C in animals exposed for long periods to the insecticide in the diet. *J Agric Food Chem* 22: 646-653. <http://dx.doi.org/10.1021/jf60194a054>
- [Jacobson, JL; Jacobson, SW.](#) (2002). Breast-feeding and gender as moderators of teratogenic effects on cognitive development. *Neurotoxicol Teratol* 24: 349-358. [http://dx.doi.org/10.1016/S0892-0362\(02\)00197-6](http://dx.doi.org/10.1016/S0892-0362(02)00197-6)
- [Jensen, AA.](#) (1983). Chemical contaminants in human milk [Review]. *Residue Rev* 89: 1-128.
- [Johansen, HR; Becher, G; Polder, A; Skaare, JU.](#) (1994). Congener-specific determination of polychlorinated biphenyls and organochlorine pesticides in human milk from Norwegian mothers living in Oslo. *J Toxicol Environ Health A* 42: 157-171. <http://dx.doi.org/10.1080/15287399409531870>
- [Johnson, MS; Thomson, SC; Speakman, JR.](#) (2001). Limits to sustained energy intake. I. Lactation in the laboratory mouse *Mus musculus*. *J Exp Biol* 204: 1925-1935.



- Joubert, BR; Håberg, SE; Nilsen, RM; Wang, X; Vollset, SE; Murphy, SK; Huang, Z; Hoyo, C; Midttun, Ø; Cupul-Uicab, LA; Ueland, PM; Wu, MC; Nystad, W; Bell, DA; Peddada, SD; London, SJ. (2012). 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 120: 1425-1431. <http://dx.doi.org/10.1289/ehp.1205412>
- Kalra, RL; Chawla, RP; Joia, BS; Tiwana, MS. (1986). Excretion of DDT residues into milk of the Indian buffalo, *Bubalus bubalis* (L.) after oral and dermal exposure. *Pestic Sci* 17: 128-134. <http://dx.doi.org/10.1002/ps.2780170209>
- Kapoor, SK; Kalra, RL. (1993). Comparative excretion of DDT analogues into milk of Indian buffalo, *Bubalus bubalis* L. following oral administration. *Pestic Sci* 37: 261-266. <http://dx.doi.org/10.1002/ps.2780370305>
- Karmaus, W; Dekoning, EP; Kruse, H; Witten, J; Osius, N. (2001). Early childhood determinants of organochlorine concentrations in school-aged children. *Pediatr Res* 50: 331-336. <http://dx.doi.org/10.1203/00006450-200109000-00007>
- Kashyap, R; Iyer, LR; Singh, MM. (1994). Evaluation of daily dietary intake of dichloro-diphenyl-trichloroethane (DDT) and benzene hexachloride (BHC) in India. *Arch Environ Health* 49: 63-66. <http://dx.doi.org/10.1080/00039896.1994.9934417>
- Kavlock, R; Chernoff, N; Rogers, E; Whitehouse, D. (1980). Comparative tissue distribution of mirex and chlordecone in fetal and neonatal rats. *Pestic Biochem Physiol* 14: 227-235. [http://dx.doi.org/10.1016/0048-3575\(80\)90029-2](http://dx.doi.org/10.1016/0048-3575(80)90029-2)
- Kearney, JP; Cole, DC; Ferron, LA; Weber, JP. (1999). Blood PCB, p,p'-DDE, and mirex levels in Great Lakes fish and waterfowl consumers in two Ontario communities. *Environ Res* 80: S138-S149. <http://dx.doi.org/10.1006/enrs.1998.3927>
- Kelce, WR; Stone, CR; Laws, SC; Gray, LE; Kemppainen, JA; Wilson, EM. (1995). Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature* 375: 581-585. <http://dx.doi.org/10.1038/375581a0>
- Khera, K; Villeneuve, D; Terry, G; Panopio, L; Nash, L; Trivett, G. (1976). Mirex: A teratogenicity, dominant lethal and tissue distribution study in rats. *Food Cosmet Toxicol* 14: 25-29. [http://dx.doi.org/10.1016/S0015-6264\(76\)80359-8](http://dx.doi.org/10.1016/S0015-6264(76)80359-8)
- Khera, KS. (1974). Teratogenicity and dominant lethal studies on hexachlorobenzene in rats. *Food Chem Toxicol* 12: 471-477.
- Kim, S; Choi, K; Ji, K; Seo, J; Kho, Y; Park, J; Kim, S; Park, S; Hwang, I; Jeon, J; Yang, H; Giesy, JP. (2011). Trans-Placental Transfer of Thirteen Perfluorinated Compounds and Relations with Fetal Thyroid Hormones. *Environ Sci Technol* 45: 7465-7472. <http://dx.doi.org/10.1021/es202408a>
- Kitchin, KT; Linder, RE; Scotti, TM; Walsh, D; Curley, AO; Svendsgaard, D. (1982). Offspring mortality and maternal lung pathology in female rats fed hexachlorobenzene. *Toxicology* 23: 33-39.
- Knight, CH; Docherty, AH; Peaker, M. (1984). Milk yield in rats in relation to activity and size of the mammary secretory cell population. *J Dairy Res* 51: 29-35.
- Kodric-Smit, M; Smit, Z; Olie, K. (1980). Organochlorine contaminants in human milk from Slavonia Province, Yugoslavia, 1978. *Pestic Monit J* 14: 1-2.
- Konishi, Y; Kuwabara, K; Hori, S. (2001). Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in Osaka, Japan. *Arch Environ Contam Toxicol* 40: 571-578. <http://dx.doi.org/10.1007/s002440010212>
- Koopman-Elseboom, C; Morse, DC; Weisglas-Kuperus, N; Lutkeschipholt, IJ; Van der Paauw, CG; Tuinstra, LGMT; Brouwer, A; Sauer, PJJ. (1994). Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatr Res* 36: 468-473. <http://dx.doi.org/10.1203/00006450-199410000-00009>

- [Koopman-Esseboom, C; Weisglas-Kuperus, N; de Ridder, MAJ; Van der Paauw, CG; Tuinstra, LGMT; Sauer, PJJ.](#) (1996). Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *Pediatrics* 97: 700-706.
- [Kosatsky, T; Przybysz, R; Shatenstein, B; Weber, JP; Armstrong, B.](#) (1999). Contaminant exposure in Montrealers of Asian origin fishing the St. Lawrence River: Exploratory assessment. *Environ Res* 80: S159-S165. <http://dx.doi.org/10.1006/enrs.1998.3911>
- [Koss, G; Koransky, W.](#) (1975). Studies on the toxicology of hexachlorobenzene: I pharmacokinetics. *Arch Toxicol* 34: 203-212. <http://dx.doi.org/10.1007/BF00353283>
- [Koss, G; Reuter, A; Koransky, W.](#) (1986). Excretion of metabolites of hexachlorobenzene in the rat and in man. In CR Morris; JRP Cabral (Eds.), (pp. 261-266). Lyon, France: International Agency for Research on Cancer.
- [Koss, G; Seubert, S; Seubert, A; Koransky, W; Ippen, H.](#) (1978). Studies on the toxicology of hexachlorobenzene: III observations in a long-term experiment. *Arch Toxicol* 40: 285-294. <http://dx.doi.org/10.1007/BF00310334>
- [Koss, G; Seubert, S; Seubert, A; Seidel, J; Koransky, W; Ippen, H.](#) (1983). Studies on the toxicology of hexachlorobenzene. V. Different phases of porphyria during and after treatment. *Arch Toxicol* 52: 13-22.
- [Kostyniak, PJ; Stinson, C; Greizerstein, HB; Vena, J; Buck, G; Mendola, P.](#) (1999). Relation of Lake Ontario fish consumption, lifetime lactation, and parity to breast milk polychlorobiphenyl and pesticide concentrations. *Environ Res* 80: S166-S174. <http://dx.doi.org/10.1006/enrs.1998.3939>
- [Krauthacker, B; Votava-Raić, A; Herceg Romanić, S; Tjesić-Drinković, D; Tjesić-Drinković, D; Reiner, E.](#) (2009). Persistent organochlorine compounds in human milk collected in Croatia over two decades. *Arch Environ Contam Toxicol* 57: 616-622. <http://dx.doi.org/10.1007/s00244-009-9301-3>
- [Kreuzer, PE; Csanády, GA; Baur, C; Kessler, W; Pöpke, O; Greim, H; Filser, JG.](#) (1997). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and congeners in infants. A toxicokinetic model of human lifetime body burden by TCDD with special emphasis on its uptake by nutrition. *Arch Toxicol* 71: 383-400.
- [Kumar, A; Dayal, P; Shukla, G; Singh, G; Joseph, PE.](#) (2006). DDT and HCH residue load in mother's breast milk: a survey of lactating mother's from remote villages in Agra region. *Environ Int* 32: 248-251. <http://dx.doi.org/10.1016/j.envint.2005.08.019>
- [Kunisue, T; Muraoka, M; Ohtake, M; Sudaryanto, A; Minh, NH; Ueno, D; Higaki, Y; Ochi, M; Tsydenova, O; Kamikawa, S; Tonegi, T; Nakamura, Y; Shimomura, H; Nagayama, J; Tanabe, S.](#) (2006). Contamination status of persistent organochlorines in human breast milk from Japan: recent levels and temporal trend. *Chemosphere* 64: 1601-1608. <http://dx.doi.org/10.1016/j.chemosphere.2005.11.010>
- [Kunisue, T; Someya, M; Kayama, F; Jin, Y; Tanabe, S.](#) (2004). Persistent organochlorines in human breast milk collected from primiparae in Dalian and Shenyang, China. *Environ Pollut* 131: 381-392. <http://dx.doi.org/10.1016/j.envpol.2004.03.008>
- [Lackmann, GM.](#) (2002). Polychlorinated biphenyls and hexachlorobenzene in full-term neonates. Reference values updated. *Biol Neonate* 81: 82-85.
- [Lackmann, GM; Angerer, J; Salzberger, U; Tollner, U.](#) (1999). Influence of maternal age and duration of pregnancy on serum concentrations of polychlorinated biphenyls and hexachlorobenzene in full-term neonates. *Biol Neonate* 76: 214-219. <http://dx.doi.org/10.1159/000014161>
- [Lakind, JS.](#) (2005). Preface: workshop on human milk surveillance and research on environmental chemicals in the United States. *J Toxicol Environ Health A* 68: 1681. <http://dx.doi.org/10.1080/15287390500225534>
- [Lakind, JS.](#) (2007). Recent global trends and physiologic origins of dioxins and furans in human milk [Review]. *J Expo Sci Environ Epidemiol* 17: 510-524. <http://dx.doi.org/10.1038/sj.jes.7500543>

- Lakind, JS; Amina Wilkins, A; Berlin, CM. (2004). Environmental chemicals in human milk: a review of levels, infant exposures and health, and guidance for future research [Review]. *Toxicol Appl Pharmacol* 198: 184-208. <http://dx.doi.org/10.1016/j.taap.2003.08.021>
- Lakind, JS; Berlin, CM; Bates, MN. (2005a). Overview: technical workshop on human milk surveillance and biomonitoring for environmental chemicals in the United States. *J Toxicol Environ Health A* 68: 1683-1689. <http://dx.doi.org/10.1080/15287390500225617>
- LaKind, JS; Berlin, CM; Mattison, DR. (2008). The heart of the matter on breastmilk and environmental chemicals: Essential points for healthcare providers and new parents [Review]. *Breastfeed Med* 3: 251-259. <http://dx.doi.org/10.1089/bfm.2008.0121>
- LaKind, JS; Berlin, CM; Sjödin, A; Turner, W; Wang, RY; Needham, LL; Paul, IM; Stokes, JL; Naiman, DQ; Patterson, DG. (2009). Do human milk concentrations of persistent organic chemicals really decline during lactation? Chemical concentrations during lactation and milk/serum partitioning. *Environ Health Perspect* 117: 1625-1631. <http://dx.doi.org/10.1289/ehp.0900876>
- Lakind, JS; Brent, RL; Dourson, ML; Kacew, S; Koren, G; Sonawane, B; Tarzian, AJ; Uhl, K. (2005b). Human milk biomonitoring data: Interpretation and risk assessment issues [Review]. *J Toxicol Environ Health A* 68: 1713-1769. <http://dx.doi.org/10.1080/15287390500225724>
- Larsen, BR; Turrio-Baldassarri, L; Nilsson, T; Iacovella, N; Di Domenico, A; Montagna, M; Facchetti, S. (1994). Toxic PCB congeners and organochlorine pesticides in Italian human milk. *Ecotoxicol Environ Saf* 28: 1-13.
- Lee, SK; Hamer, D; Bedwell, CL; Lohitnavy, M; Yang, RSH. (2009). Effect of PCBs on the lactational transfer of methyl mercury in mice: PBPK modeling. *Environ Toxicol Pharmacol* 27: 75-83. <http://dx.doi.org/10.1016/j.etap.2008.08.014>
- Lee, SK; Ou, YC; Yang, RSH. (2002). Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in nonpregnant mice, lactating mice, and suckling pups. *Toxicol Sci* 65: 26-34. <http://dx.doi.org/10.1093/toxsci/65.1.26>
- Lemasters, GK; Perreault, SD; Hales, BF; Hatch, M; Hirshfield, AN; Hughes, CL; Kimmel, GL; Lamb, JC; Pryor, JL; Rubin, C; Seed, JG. (2000). Workshop to identify critical windows of exposure for children's health: reproductive health in children and adolescents work group summary [Review]. *Environ Health Perspect* 108 Suppl 3: 505-509.
- Leng, JH; Kayama, F; Wang, PY; Nakamura, M; Nakata, T; Wang, Y. (2009). Levels of persistent organic pollutants in human milk in two Chinese coastal cities, Tianjin and Yantai: Influence of fish consumption. *Chemosphere* 75: 634-639. <http://dx.doi.org/10.1016/j.chemosphere.2009.01.008>
- Li, L; Choi, JY; Lee, KM; Sung, H; Park, SK; Oze, I; Pan, KF; You, WC; Chen, YX; Fang, JY; Matsuo, K; Kim, WH; Yuasa, Y; Kang, D. (2012). DNA methylation in peripheral blood: A potential biomarker for cancer molecular epidemiology [Review]. *J Epidemiol* 22: 384-394. <http://dx.doi.org/10.2188/jea.JE20120003>
- Liddle, JA; Smrek, AL; Adams, SR; Kimbrough, RD. (1977). Pharmacodynamics of Mirex in Goats: Effect of Reproduction and Lactation. *Toxicol Appl Pharmacol* 41: 138.
- Lien, EJ. (1975). Structure absorption-distribution relationships: Significance for drug design. In EJ Ariëns (Ed.), *Drug Design* (pp. 81132). New York, NY: Academic Press.
- Liguori, A; Puglianiello, A; Germani, D; Deodati, A; Peschiaroli, E; Cianfarani, S. (2010). Epigenetic changes predisposing to type 2 diabetes in intrauterine growth retardation. *Front Endocrinol (Lausanne)* 1: 5. <http://dx.doi.org/10.3389/fendo.2010.00005>
- Lilienthal, H; Benthe, C; Heinzow, B; Winneke, G. (1996). Impairment of Schedule-Controlled Behavior by Preand Postnatal Exposure to Hexachlorobenzene in Rats. *Arch Toxicol* 70: 174-181.

- Ling, C; Del Guerra, S; Lupi, R; Rönn, T; Granhall, C; Luthman, H; Masiello, P; Marchetti, P; Groop, L; Del Prato, S. (2008). Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia* 51: 615-622. <http://dx.doi.org/10.1007/s00125-007-0916-5>
- Lora, RP; Marteache, AH; Villar, LMP; Gimenez, RL; Villarejo, MJ; Perez, JL. (1979). Plaquicidas organoclorados en leches humanas espanolas. *Revista Espanola de Pediatria* 35: 93.
- Lorber, M; Phillips, L. (2002). Infant exposure to dioxin-like compounds in breast milk. *Environ Health Perspect* 110: A325-A332.
- Lovelady, CA; Whitehead, RA; Mccrory, MA; Nommsen-Rivers, LA; Mabury, S; Dewey, KG. (1999). Weight change during lactation does not alter the concentrations of chlorinated organic contaminants in breast milk of women with low exposure. *J Hum Lact* 15: 307-315.
- Lu, Y; Lohitnavy, M; Reddy, MB; Lohitnavy, O; Ashley, A; Yang, RS. (2006). An updated physiologically based pharmacokinetic model for hexachlorobenzene: incorporation of pathophysiological states following partial hepatectomy and hexachlorobenzene treatment. *Toxicol Sci* 91: 29-41. <http://dx.doi.org/10.1093/toxsci/kfj133>
- Lutter, C; Iyengar, V; Barnes, R; Chuvakova, T; Kazbekova, G; Sharmanov, T. (1998). Breast milk contamination in Kazakhstan: Implications for infant feeding. *Chemosphere* 37: 1761-1772. [http://dx.doi.org/10.1016/S0045-6535\(98\)00241-0](http://dx.doi.org/10.1016/S0045-6535(98)00241-0)
- Lutz, RJ; Dedrick, RL; Matthews, HB; Eling, TE; Anderson, MW. (1977). A preliminary pharmacokinetic model for several chlorinated biphenyls in the rat. *Drug Metab Dispos* 5: 386-396.
- Madden, AB; Makarewicz, JC. (1996). Salmonine consumption as a source of mirex in human breast milk near Rochester, New York. *J Great Lakes Res* 22: 810-817. [http://dx.doi.org/10.1016/S0380-1330\(96\)71002-X](http://dx.doi.org/10.1016/S0380-1330(96)71002-X)
- Makris, SL; Thompson, CM; Euling, SY; Selevan, SG; Sonawane, B. (2008). A lifestage-specific approach to hazard and dose-response characterization for children's health risk assessment [Review]. *Birth Defects Res B Dev Reprod Toxicol* 83: 530-546. <http://dx.doi.org/10.1002/bdrb.20176>
- Marchitti, SA; Lakind, JS; Naiman, DQ; Berlin, CM; Kenneke, JF. (In Press) Improving Infant Exposure and Health Risk Estimates: Using Serum Data to Predict Polybrominated Diphenyl Ether Concentrations in Breast Milk. *Environ Sci Technol*. <http://dx.doi.org/10.1021/es305229d>
- Marks, AR; Harley, K; Bradman, A; Kogut, K; Barr, DB; Johnson, C; Calderon, N; Eskenazi, B. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect* 118: 1768-1774. <http://dx.doi.org/10.1289/ehp.1002056>
- Martin, J; Hamilton, B; Ventura, S; Osterman, M; Wilson, E; Mathews, T. (2012). Births: Final data for 2010. National Vital Statistics Reports, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, Centers for Disease Control and Prevention, National Center for Health Statistics.
- Matsumoto, H; Murakami, Y; Kuwabara, K; Tanaka, R; Kashimoto, T. (1987). Average daily intake of pesticides and polychlorinated biphenyls in total diet samples in Osaka, Japan. *Bull Environ Contam Toxicol* 38: 954-958. <http://dx.doi.org/10.1007/BF01609079>
- Mattison, DR; Wohlleb, J; To, T; Lamb, Y; Fatah, S; Brewster, MA; Walls, RC; Selevan, SG. (1992). Pesticide concentrations in Arkansas breast milk. *J Ark Med Soc* 88: 553-557.
- Mckim, J; Schmieder, P; Veith, G. (1985). Absorption dynamics of organic chemical transport across trout gills as related to octanol-water partition coefficient. *Toxicol Appl Pharmacol* 77: 1-10.
- McLachlan, MS. (1993). Digestive tract absorption of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in a nursing infant. *Toxicol Appl Pharmacol* 123: 68-72. <http://dx.doi.org/10.1006/taap.1993.1222>
- Mead, MN. (2008). Contaminants in human milk: weighing the risks against the benefits of breastfeeding. *Environ Health Perspect* 116: A427-A434.

- Mehendale, HM; Fishbein, L; Fields, M; Matthews, HB. (1972). Fate of mirex-14 C in the rat and plants. Bull Environ Contam Toxicol 8: 200-207. <http://dx.doi.org/10.1007/BF01839512>
- Mendoza, CE; Collins, B; Shields, JB; Laver, GW. (1977). Hexachlorobenzene residues and effects on esterase activities in pre-weanling rats after a reciprocal transfer between HCB-treated and control dams. Arch Toxicol 38: 191-199.
- Mendoza, CE; Collins, BT; Shields, JB; Laver, GW. (1978). Effects of hexachlorobenzene or hexabromobenzene on body and organ weights of preweanling rats after a reciprocal transfer between the treated and control dams. J Agric Food Chem 26: 941-945.
- Menna, JH; Barnett, JB; Soderberg, LS. (1985). Influenza type A virus infection of mice exposed in utero to chlordane; survival and antibody studies. Toxicol Lett 24: 45-52. [http://dx.doi.org/10.1016/0378-4274\(85\)90138-9](http://dx.doi.org/10.1016/0378-4274(85)90138-9)
- Merry, BJ; Holehan, AM. (1979). Onset of puberty and duration of fertility in rats fed a restricted diet. J Reprod Fertil 57: 253-259.
- Mes, J; Davies, DJ. (1979). Presence of polychlorinated biphenyl and organochlorine pesticide residues and the absence of polychlorinated terphenyls in Canadian human milk samples. Bull Environ Contam Toxicol 21: 381-387. <http://dx.doi.org/10.1007/BF01685440>
- Mes, J; Davies, DJ; Doucet, J; Weber, D; McMullen, E. (1993). Levels of chlorinated hydrocarbon residues in Canadian human breast milk and their relationship to some characteristics of the donors. Food Addit Contam 10: 429-441. <http://dx.doi.org/10.1080/02652039309374166>
- Mes, J; Davies, DJ; Miles, W. (1978). Traces of mirex in some Canadian human milk samples. Bull Environ Contam Toxicol 19: 564-570. <http://dx.doi.org/10.1007/BF01685842>
- Mes, J; Davies, DJ; Turton, D; Sun, WF. (1986). Levels and trends of chlorinated hydrocarbon contaminants in the breast milk of Canadian women. Food Addit Contam 3: 313-322. <http://dx.doi.org/10.1080/02652038609373598>
- Mes, J; Doyle, JA; Adams, BR; Davies, DJ; Turton, D. (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. Arch Environ Contam Toxicol 13: 217-223. <http://dx.doi.org/10.1007/BF01055879>
- Metcalf, RL. (1995). Insect control technology. In J Kroschwitz; M Howe-Grant (Eds.), Kirk-Othmer encyclopedia of chemical technology: Volume 14: Imaging technology to lanthanides (4th ed., pp. 524-602). New York, NY: John Wiley & Sons.
- Milbrath, MO; Wenger, Y; Chang, CW; Emond, C; Garabrant, D; Gillespie, BW; Jolliet, O. (2009). Apparent half-lives of dioxins, furans, and polychlorinated biphenyls as a function of age, body fat, smoking status, and breast-feeding. Environ Health Perspect 117: 417-425. <http://dx.doi.org/10.1289/ehp.11781>
- Miller, M; Marty, M. (2010). Impact of environmental chemicals on lung development [Review]. Environ Health Perspect 118: 1155-1164. <http://dx.doi.org/10.1289/ehp.0901856>
- Miyata, H; Takenaka, T; Nakao, T; Aozasa, O; Ohta, S; Fujimine, Y; Fukui, S. (2006). Investigation of the main source of halogenated environmental pollutants in human breast milk (The Third Report)—influence by fasting. Organohalogen Compounds 68: 145-148.
- Miyazaki, T; Akiyama, K; Kaneko, S; Horii, S; Yamagishi, T. (1980). Chlordane residues in human milk. Bull Environ Contam Toxicol 25: 518-523. <http://dx.doi.org/10.1007/BF01985565>
- Monheit, BM; Luke, BG. (1990). Pesticides in breast milk—a public health perspective. Community Health Stud 14: 269-273.

- [Morales, E; Sunyer, J; Castro-Giner, F; Estivill, X; Julvez, J; Ribas-Fitó, N; Torrent, M; Grimalt, JO; de Cid, R.](#) (2008). Influence of glutathione s-transferase polymorphisms on cognitive functioning effects induced by p,p'-ddt among preschoolers. *Environ Health Perspect* 116: 1581-1585. <http://dx.doi.org/10.1289/ehp.11303>
- [Mussalo-Rauhamaa, H; Pyysalo, H; Antervo, K.](#) (1988). Relation between the content of organochlorine compounds in Finnish human milk and characteristics of the mothers. *J Toxicol Environ Health* 25: 1-19. <http://dx.doi.org/10.1080/15287398809531185>
- [Nagayama, J; Okamura, K; Iida, T; Hirakawa, H; Matsueda, T; Tsuji, H; Hasegawa, M; Sato, K; Ma, HY; Yanagawa, T; Igarashi, H; Fukushige, J; Watanabe, T.](#) (1998). Postnatal exposure to chlorinated dioxins and related chemicals on thyroid hormone status in Japanese breast-fed infants. *Chemosphere* 37: 1789-1793. [http://dx.doi.org/10.1016/S0045-6535\(98\)00244-6](http://dx.doi.org/10.1016/S0045-6535(98)00244-6)
- [Nair, A; Pillai, MK.](#) (1989). Monitoring of hexachlorobenzene residues in Delhi and Faridabad, India. *Bull Environ Contam Toxicol* 42: 682-686. <http://dx.doi.org/10.1007/BF01700388>
- [Nakai, K; Nakamura, T; Murata, K; Satoh, H.](#) (2009). Tohoku study of child development and exposure assessment [Review]. *Nippon Eiseigaku Zasshi* 64: 749-758.
- [Nakashima, Y; Ohsawa, S; Umegaki, K; Ikegami, S.](#) (1997). Hexachlorobenzene accumulated by dams during pregnancy is transferred to suckling rats during early lactation. *J Nutr* 127: 648-654.
- [Nakata, H; Kawazoe, M; Arizono, K; Abe, S; Kitano, T; Shimada, H; Li, W; Ding, X.](#) (2002). Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from china: status of contamination, historical trend, and human dietary exposure. *Arch Environ Contam Toxicol* 43: 473-480. <http://dx.doi.org/10.1007/s00244-002-1254-8>
- [Narotsky, MG; Kavlock, RJ.](#) (1995). A multidisciplinary approach to toxicological screening: II. Developmental toxicity. *J Toxicol Environ Health* 45: 145-171. <http://dx.doi.org/10.1080/15287399509531987>
- [Nasir, K; Bilo, YY; Al-Shuraiki, Y.](#) (1998). Residues of chlorinated hydrocarbon insecticides in human milk of Jordanian women. *Environ Pollut* 99: 141-148. [http://dx.doi.org/10.1016/S0269-7491\(97\)00195-4](http://dx.doi.org/10.1016/S0269-7491(97)00195-4)
- [Needham, LL; Grandjean, P; Heinzow, B; Jørgensen, PJ; Nielsen, F; Patterson, DG; Sjödin, A; Turner, WE; Weihe, P.](#) (2011). Partition of environmental chemicals between maternal and fetal blood and tissues. *Environ Sci Technol* 45: 1121-1126. <http://dx.doi.org/10.1021/es1019614>
- [Newsome, WH; Davies, D; Doucet, J.](#) (1995). PCB and organochlorine pesticides in Canadian human milk-1992. *Chemosphere* 30: 2143-2153. [http://dx.doi.org/10.1016/0045-6535\(95\)00086-N](http://dx.doi.org/10.1016/0045-6535(95)00086-N)
- [Newsome, WH; Ryan, JJ.](#) (1999). Toxaphene and other chlorinated compounds in human milk from northern and southern Canada: A comparison. *Chemosphere* 39: 519-526. [http://dx.doi.org/10.1016/S0045-6535\(98\)00600-6](http://dx.doi.org/10.1016/S0045-6535(98)00600-6)
- [Newton, KG; Greene, NC.](#) (1972). Organochlorine pesticide residue levels in human milk--Victoria, Australia--1970. *Pestic Monit J* 6: 4-8.
- [Niessen, KH; Ramolla, J; Binder, M; Gruegmann, G; Hofmann, U.](#) (1984). Chlorinated hydrocarbons in adipose tissue of infants and toddlers: Inventory and studies on their association with intake of mothers milk. *Eur J Pediatr* 142: 238-243. <http://dx.doi.org/10.1007/BF00540242>
- [NLM](#) (National Institutes of Health, National Library of Medicine). (2011). Hazardous Substances Data Bank (HSDB). Bethesda, MD. <http://toxnet.nlm.nih.gov>
- [Noren, K.](#) (1983). Levels of organochlorine contaminants in human milk in relation to the dietary habits of the mothers. *Acta Paediatr Scand* 72: 811-816. <http://dx.doi.org/10.1111/j.1651-2227.1983.tb09821.x>
- [Noren, K.](#) (1993). Contemporary and retrospective investigations of human milk in the trend studies of organochlorine contaminants in Sweden. *Sci Total Environ* 139-140: 347-355.



- [Norén, K.](#) (1983). Some aspects of the determination of organochlorine contaminants in human milk. Arch Environ Contam Toxicol 12: 277-283. <http://dx.doi.org/10.1007/BF01059403>
- [Norén, K.](#) (1988). Changes in the levels of organochlorine pesticides, polychlorinated biphenyls, dibenzo--dioxins and dibenzofurans in human milk from Stockholm, 1972–1985. Chemosphere 17: 39-49. [http://dx.doi.org/10.1016/0045-6535\(88\)90042-2](http://dx.doi.org/10.1016/0045-6535(88)90042-2)
- [Norén, K; Meironyté, D.](#) (2000). Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. Chemosphere 40: 1111-1123. [http://dx.doi.org/10.1016/S0045-6535\(99\)00360-4](http://dx.doi.org/10.1016/S0045-6535(99)00360-4)
- [NRC](#) (National Research Council). (1983). Risk assessment in the federal government: Managing the process. Washington, DC: National Academies Press. [http://www.nap.edu/openbook.php?record\\_id=366&page=R1](http://www.nap.edu/openbook.php?record_id=366&page=R1)
- [NRC](#) (National Research Council). (2000). Scientific frontiers in developmental toxicology and risk assessment. Washington, DC: National Academy Press. [http://www.nap.edu/catalog.php?record\\_id=9871#description](http://www.nap.edu/catalog.php?record_id=9871#description)
- [NRC](#) (National Research Council). (2009). Science and decisions: Advancing risk assessment. Washington, DC: National Academies Press. <http://www.nap.edu/catalog/12209.html>
- [Ntow, WJ.](#) (2001). Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. Arch Environ Contam Toxicol 40: 557-563.
- [NTP](#) (National Toxicology Program). (2011a). Report on carcinogens: Twelfth edition (12th ed.). Research Triangle Park, NC. <http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf>
- [NTP](#) (National Toxicology Program). (2011b). Specifications for the conduct of studies to evaluate the reproductive and developmental toxicity of chemical, biological and physical agents in laboratory animals.
- [O'Flaherty, EJ; Scott, W; Schreiner, C; Beliles, RP.](#) (1992). A physiologically based kinetic model of rat and mouse gestation: disposition of a weak acid. Toxicol Appl Pharmacol 112: 245-256. [http://dx.doi.org/10.1016/0041-008X\(92\)90194-W](http://dx.doi.org/10.1016/0041-008X(92)90194-W)
- [OECD.](#) (2011). Test No. 443: Extended One-Generation Reproductive Toxicity Study. Paris, France: Organisation for Economic Co-operation and Development. <http://dx.doi.org/10.1787/9789264122550-en>
- [Ohno, Y; Kawanishi, T; Takahashi, A; Nakaura, S; Kawashima, K; Tanaka, S; Takanaka, A; Omori, Y; Sekita, H; Uchiyama, M.](#) (1986). Comparisons of the toxicokinetic parameters in rats determined for low and high dose of "gamma"-chlordane. J Toxicol Sci 11: 111-123.
- [Olanoff, LS; Bristow, WJ; Colcolough, J, Jr; Reigart, JR.](#) (1983). Acute chlordane intoxication. Clin Toxicol 20: 291-306.
- [Oliver, BG; Cleland, GB; Sonstegard, RA.](#) (1993). PCB congeners and organochlorine pesticides in the blood of Lake Ontario fish consumers [Abstract]. In Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop (pp. 77). Oliver, BG; Cleland, GB; Sonstegard, RA.
- [Oregon DEQ](#) (Oregon Department of Environmental Quality). (2010). Human health risk assessment guidance. Portland, OR. <http://www.deq.state.or.us/lq/pubs/docs/cu/HumanHealthRiskAssessmentGuidance.pdf>
- [Palanza, P; Parmigiani, S; Liu, H; vom Saal, FS.](#) (1999). Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. Pharmacol Biochem Behav 64: 665-672.
- [Palanza, P; Parmigiani, S; vom Saal, FS.](#) (2001). Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'-DDT, and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. Horm Behav 40: 252-265. <http://dx.doi.org/10.1006/hbeh.2001.1697>

- [Pan, IJ; Daniels, JL; Goldman, BD; Herring, AH; Siega-Riz, AM; Rogan, WJ.](#) (2009). Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: An analysis of the pregnancy, infection, and nutrition babies study. *Environ Health Perspect* 117: 488-494. <http://dx.doi.org/10.1289/ehp.0800063>
- [Patandin, S; Lanting, CI; Mulder, PGH; Boersma, ER; Sauer, PJ; Weisglas-Kuperus, N.](#) (1999). Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. *J Pediatr* 134: 33-41. [http://dx.doi.org/10.1016/S0022-3476\(99\)70369-0](http://dx.doi.org/10.1016/S0022-3476(99)70369-0)
- [Peden, DB.](#) (2000). Development of atopy and asthma: candidate environmental influences and important periods of exposure [Review]. *Environ Health Perspect* 108: 475-482.
- [Perera, F; Tang, WY; Herbstam, J; Tand, D; Levin, L; Miller, R; Ho, SM.](#) (2009). Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. *PLoS ONE* 4: e4488. <http://dx.doi.org/10.1371/journal.pone.0004488>
- [Peters, HA; Johnson, SA; Cam, S; Müftü, Y; Oral, S; Ergene, T.](#) (1966). Hexachlorobenzene-induced porphyria: effect of chelation on the disease, porphyrin and metal metabolism. *Am J Med Sci* 251: 314-322.
- [Peters, HA; Cripps, DJ; Gocmen, A; Bryan, GT; Erturk, E; Dogramaci, I; Morris, CR.](#) (1982a). An Epidemiologic Study of Populations Previously Exposed to Hexachlorobenzene. In *Proceedings of the second NCI/EPA/NIOSH Collaborative Workshop, Progress on Joint Environmental and Occupational Cancer Studies* (pp. 194-199). (Niosh/00187945). Bethesda, MD: National Cancer Institute.
- [Peters, HA; Gocmen, A; Cripps, DJ; Bryan, GT; Dogramaci, I.](#) (1982b). Epidemiology of hexachlorobenzene-induced porphyria in Turkey: clinical and laboratory follow-up after 25 years. *Arch Neurol* 39: 744-749.
- [Pittman, KA; Wiener, M; Treble, DH.](#) (1976). Mirex kinetics in the Rhesus monkey. II. Pharmacokinetic model. *Drug Metab Dispos* 4: 288-295.
- [Plagemann, A; Harder, T; Schellong, K; Schulz, S; Stupin, JH.](#) (2012). Early postnatal life as a critical time window for determination of long-term metabolic health. *Best Pract Res Clin Endocrinol Metab* 26: 641-653. <http://dx.doi.org/10.1016/j.beem.2012.03.008>
- [Pluim, HJ; de Vijlder, JIM; Olie, K; Kok, JH; Vulsma, T; van Tijn, DA; van der Slikke, JW; Koppe, JG.](#) (1993). Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. *Environ Health Perspect* 101: 504-508.
- [Pohl, HR; Tylanda, CA.](#) (2000). Breast-feeding exposure of infants to selected pesticides: a public health viewpoint [Review]. *Toxicol Ind Health* 16: 65-77. <http://dx.doi.org/10.1177/074823370001600203>
- [Poiger, H; Schlatter, C.](#) (1986). Pharmacokinetics of 2,3,7,8-TCDD in man. *Chemosphere* 15: 1489-1494. [http://dx.doi.org/10.1016/0045-6535\(86\)90429-7](http://dx.doi.org/10.1016/0045-6535(86)90429-7)
- [Polder, A; Becher, G; Savinova, TN; Skaare, JU.](#) (1998). Dioxins, PCBs and some chlorinated pesticides in human milk from the Kola Peninsula, Russia. *Chemosphere* 37: 1795-1806.
- [Poulin, P; Krishnan, K.](#) (1995). An algorithm for predicting tissue: Blood partition coefficients of organic chemicals from n-octanol: Water partition coefficient data. *J Toxicol Environ Health* 46: 117-129. <http://dx.doi.org/10.1080/15287399509532021>
- [Poulin, P; Krishnan, K.](#) (1996). A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. *Toxicol Appl Pharmacol* 136: 126-130. <http://dx.doi.org/10.1006/taap.1996.0015>
- [Prachar, V; Veningerova, M; Uhnak, J.](#) (1993). Levels of polychlorinated biphenyls and some other organochlorine compounds in breast milk samples in Bratislava. *Sci Total Environ* 0: 237-242.



- [Price, K; Haddad, S; Krishnan, K.](#) (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol Environ Health A* 66: 417-433.  
<http://dx.doi.org/10.1080/15287390306450>
- [Quinsey, PM; Donohue, DC; Ahokas, JT.](#) (1995). Persistence of organochlorines in breast milk of women in Victoria, Australia. *Food Chem Toxicol* 33: 49-56.
- [Quinsey, PM; Donohue, DC; Cumming, FJ; Ahokas, JT.](#) (1996). The importance of measured intake in assessing exposure of breast-fed infants to organochlorines. *Eur J Clin Nutr* 50: 438-442.
- [Rauh, VA; Garfinkel, R; Perera, FP; Andrews, HF; Hoepner, L; Barr, DB; Whitehead, R; Tang, D; Whyatt, RW.](#) (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics* 118: E1845-E1859. <http://dx.doi.org/10.1542/peds.2006-0338>
- [Raum, E; Küpper-Nybelen, J; Lamerz, A; Hebebrand, J; Herpertz-Dahlmann, B; Brenner, H.](#) (2011). Tobacco smoke exposure before, during, and after pregnancy and risk of overweight at age 6. *Obesity (Silver Spring)* 19: 2411-2417. <http://dx.doi.org/10.1038/oby.2011.129>
- [Rawn, DF; Cao, XL; Doucet, J; Davies, DJ; Sun, WF; Dabeka, RW; Newsome, WH.](#) (2004). Canadian Total Diet Study in 1998: pesticide levels in foods from Whitehorse, Yukon, Canada, and corresponding dietary intake estimates. *Food Addit Contam* 21: 232-250. <http://dx.doi.org/10.1080/02652030310001655470>
- [Redding, LE; Sohn, MD; Mckone, TE; Chen, JW; Wang, SL; Hsieh, DP; Yang, RS.](#) (2008). Population physiologically based pharmacokinetic modeling for the human lactational transfer of PCB-153 with consideration of worldwide human biomonitoring results. *Environ Health Perspect* 116: 1629-1635.  
<http://dx.doi.org/10.1289/ehp.11519>
- [Reiter, L.](#) (1977). Behavioral toxicology: effects of early postnatal exposure to neurotoxins on development of locomotor activity in the rat. *J Occup Med* 19: 201-204.
- [Renner, G.](#) (1988). Hexachlorobenzene and its metabolism. *Toxicol Environ Chem* 18: 51-78.
- [Ribas-Fito, N; Cardo, E; Sala, M; Eulalia De Muga, M; Mazon, C; Verdu, A; Kogevinas, M; Grimalt, JO; Sunyer, J.](#) (2003). Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants. *Pediatrics* 111: e580-e585.
- [Ribas-Fitó, N; Grimalt, JO; Marco, E; Sala, M; Mazón, C; Sunyer, J.](#) (2005). Breastfeeding and concentrations of HCB and p,p'-DDE at the age of 1 year. *Environ Res* 98: 8-13.  
<http://dx.doi.org/10.1016/j.envres.2004.05.017>
- [Rice, D; Barone, S.](#) (2000). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models [Review]. *Environ Health Perspect* 108: 511-533.  
<http://dx.doi.org/10.1289/ehp.00108s3511>
- [Ritter, R; Scheringer, M; MacLeod, M; Moeckel, C; Jones, KC; Hungerbühler, K.](#) (2011). Intrinsic human elimination half-lives of polychlorinated biphenyls derived from the temporal evolution of cross-sectional biomonitoring data from the United Kingdom. *Environ Health Perspect* 119: 225-231.  
<http://dx.doi.org/10.1289/ehp.1002211>
- [Ritter, R; Scheringer, M; Macleod, M; Schenker, U; Hungerbühler, K.](#) (2009). A multi-individual pharmacokinetic model framework for interpreting time trends of persistent chemicals in human populations: application to a postban situation. *Environ Health Perspect* 117: 1280-1286. <http://dx.doi.org/10.1289/ehp.0900648>
- [Rogan, WJ; Gladen, BC.](#) (1991). PCBs, DDE, and child development at 18 and 24 months. *Ann Epidemiol* 1: 407-413.
- [Rogan, WJ; Ragan, NB.](#) (1994). Chemical contaminants, pharmacokinetics, and the lactating mother [Review]. *Environ Health Perspect* 102: 89-95.

- [Rogers, EH; Hunter, ES; Rosen, MB; Rogers, JM; Lau, C; Hartig, PC; Francis, BM; Chernoff, N.](#) (2003). Lack of evidence for intergenerational reproductive effects due to prenatal and postnatal undernutrition in the female CD-1 mouse. *Reprod Toxicol* 17: 519-525.
- [Rogers, JM; Grabowski, CT.](#) (1983). Mirex-induced fetal cataracts: Lens growth, histology and cation balance, and relationship to edema. *Teratology* 27: 343-349. <http://dx.doi.org/10.1002/tera.1420270308>
- [Rogers, JM; Grabowski, CT.](#) (1984). Postnatal mirex cataractogenesis in rats: Lens cation balance, growth and histology. *Exp Eye Res* 39: 563-573. [http://dx.doi.org/10.1016/0014-4835\(84\)90056-3](http://dx.doi.org/10.1016/0014-4835(84)90056-3)
- [Rush, GF; Smith, JH; Maita, K; Bleavins, M; Aulerich, RJ; Ringer, RK; Hook, JB.](#) (1983). Perinatal hexachlorobenzene toxicity in the mink. *Environ Res* 31: 116-124.
- [Sala, M; Sunyer, J; Otero, R; Santiago-Silva, M; Camps, C; Grimalt, J.](#) (1999). Organochlorine in the serum of inhabitants living near an electrochemical factory. *Occup Environ Med* 56: 152-158.
- [Saltelli, A; Ratto, M; Andres, T; Campolongo, F; Cariboni, J; Gatelli, D; Saisana, M; Tarantola, S.](#) (2008). Global sensitivity analysis: The primer. West Sussex, UK: John Wiley & Sons.
- [Savage, EP; Keefe, TJ; Tessari, JD.](#) (1982). Pesticides in human breast milk. In Banbury report 11: Environmental factors in human growth and development (pp. 77-84). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- [Savage, EP; Keefe, TJ; Tessari, JD; Wheeler, HW; Applehans, FM; Goes, EA; Ford, SA.](#) (1981). National study of chlorinated hydrocarbon insecticide residues in human milk, USA: I. Geographic distribution of dieldrin, heptachlor, heptachlor epoxide, chlordane, oxychlordane, and mirex. *Am J Epidemiol* 113: 413-422.
- [Schantz, SL; Jacobson, JL; Humphrey, HE; Jacobson, SW; Welch, R; Gasior, D.](#) (1994). Determinants of polychlorinated biphenyls (PCBs) in the sera of mothers and children from Michigan farms with PCB-contaminated silos. *Arch Environ Health* 49: 452-458. <http://dx.doi.org/10.1080/00039896.1994.9955000>
- [Schechter, A; Fürst, P; Krüger, C; Meemken, HA; Groebel, W; Constable, JD.](#) (1989). Levels of polychlorinated dibenzofurans, dibenzodioxins, PCBS, DDT and DDE, hexachlorobenzene, dieldrin, hexachlorocyclohexanes and oxychlordane in human breast milk from the United States, Thailand, Vietnam, and Germany. *Chemosphere* 18: 445-454. [http://dx.doi.org/10.1016/0045-6535\(89\)90154-9](http://dx.doi.org/10.1016/0045-6535(89)90154-9)
- [Schlummer, M; Moser, GA; McLachlan, MS.](#) (1998). Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. *Toxicol Appl Pharmacol* 152: 128-137. <http://dx.doi.org/10.1006/taap.1998.8487>
- [Schoeters, G; Den Hond, E; Dhooze, W; van Larebeke, N; Leijts, M.](#) (2008). Endocrine disruptors and abnormalities of pubertal development [Review]. *Basic Clin Pharmacol Toxicol* 102: 168-175. <http://dx.doi.org/10.1111/j.1742-7843.2007.00180.x>
- [Schoula, R; Hajslova, J; Bencko, V; Poustka, J; Holadova, K; Vizek, V.](#) (1996). Occurrence of persistent organochlorine contaminants in human milk collected in several regions of Czech Republic. *Chemosphere* 33: 1485-1494. [http://dx.doi.org/10.1016/0045-6535\(96\)00287-1](http://dx.doi.org/10.1016/0045-6535(96)00287-1)
- [Scotti, TM; Chernoff, N; Linder, R; McElroy, WK.](#) (1981). Histopathologic lens changes in mirex-exposed rats. *Toxicol Lett* 9: 289-294. [http://dx.doi.org/10.1016/0378-4274\(81\)90163-6](http://dx.doi.org/10.1016/0378-4274(81)90163-6)
- [Selevan, SG; Kimmel, CA; Mendola, P.](#) (2000). Identifying critical windows of exposure for children's health [Review]. *Environ Health Perspect* 108: 451-455.
- [Seydel, JK; Schaper, KJ.](#) (1981). Quantitative structure-pharmacokinetic relationships and drug design [Review]. *Pharmacol Ther* 15: 131-182.
- [Simpson, CD; Wilcock, RJ; Smith, TJ; Wilkins, AL; Langdon, AG.](#) (1995). Determination of octanol-water partition coefficients for the major components of technical chlordane. *Bull Environ Contam Toxicol* 55: 149-153.

- [Sivarajan, M; Amory, DW; Lindbloom, LE; Schwettmann, RS.](#) (1975). Systemic and regional blood-flow changes during spinal anesthesia in the rhesus monkey. *Anesthesiology* 43: 78-88.
- [Sjödin, A; LaKind, JS; Patterson, DG, Jr.; Needham, LL; Wang, R; Paul, IM; Stokes, J; Berlin, CM.](#) (2005). Current concentrations and changes in concentrations of PBDEs, persistent pesticides, and PCBs in human milk. *Organohalogen Compounds* 67: 1745-1748.
- [Skaare, JU; Polder, A.](#) (1990). Polychlorinated biphenyls and organochlorine pesticides in milk of Norwegian women during lactation. *Arch Environ Contam Toxicol* 19: 640-645. <http://dx.doi.org/10.1007/BF01183978>
- [Smith, AH.](#) (1987). Infant exposure assessment for breast milk dioxins and furans derived from waste incineration emissions. *Risk Anal* 7: 347-353. <http://dx.doi.org/10.1111/j.1539-6924.1987.tb00470.x>
- [Smrek, AL; Adams, SR; Liddle, JA; Kimbrough, RD.](#) (1977). Pharmacokinetics of mirex in goats. 1. Effects on reproduction and lactation. *J Agric Food Chem* 25: 1321-1325. <http://dx.doi.org/10.1021/jf60214a011>
- [Smrek, AL; Adams, SR; Liddle, JA; Kimbrough, RD.](#) (1978). Pharmacokinetics of mirex in goats. 2. Residue tissue levels, trans-placental passage during recovery. *J Agric Food Chem* 26: 945-947. <http://dx.doi.org/10.1021/jf60218a006>
- [Soine, PJ; Blanke, RV; Guzelian, PS; Schwartz, CC.](#) (1982). Preferential binding of chlordecone to the protein and high density lipoprotein fractions of plasma from humans and other species. *J Toxicol Environ Health* 9: 107-118. <http://dx.doi.org/10.1080/15287398209530146>
- [Soine, PJ; Blanke, RV; Schwartz, CC.](#) (1984). Isolation of chlordecone binding proteins from pig liver cytosol. *J Toxicol Environ Health* 14: 305-317. <http://dx.doi.org/10.1080/15287398409530582>
- [Sonawane, BR.](#) (1995). Chemical contaminants in human milk: An overview [Review]. *Environ Health Perspect* 103: 197-205. <http://dx.doi.org/10.1289/ehp.95103s6197>
- [Spyker-Cranmer, JM; Barnett, JB; Avery, DL; Cranmer, MF.](#) (1982). Immunoteratolgy of chlordane: Cell-mediated and humoral immune responses in adult mice exposed in utero. *Toxicol Appl Pharmacol* 62: 402-408. [http://dx.doi.org/10.1016/0041-008X\(82\)90141-7](http://dx.doi.org/10.1016/0041-008X(82)90141-7)
- [Stacey, CI; Perriman, WS; Whitney, S.](#) (1985). Organochlorine pesticide residue levels in human milk: Western Australia, 1979-1980. *Arch Environ Health* 40: 102-108.
- [Stevens, MF; Ebell, GF; Psaila-Savona, P.](#) (1993). Organochlorine pesticides in Western Australian nursing mothers. *Med J Aust* 158: 238-241.
- [Strassman, SC; Kutz, FW.](#) (1977). Insecticide residues in human milk from Arkansas and Mississippi, 1973-74. *Pestic Monit J* 10: 130-133.
- [Stuetz, W; Prapamontol, T; Erhardt, JG; Classen, HG.](#) (2001). Organochlorine pesticide residues in human milk of a Hmong hill tribe living in Northern Thailand. *Sci Total Environ* 273: 53-60.
- [Subramanian, A; Ohtake, M; Kunisue, T; Tanabe, S.](#) (2007). High levels of organochlorines in mothers' milk from Chennai (Madras) city, India. *Chemosphere* 68: 928-939. <http://dx.doi.org/10.1016/j.chemosphere.2007.01.041>
- [Sunyer, J; Torrent, M; Garcia-Esteban, R; Ribas-Fitó, N; Carrizo, D; Romieu, I; Antó, JM; Grimalt, JO.](#) (2006). Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. *Clin Exp Allergy* 36: 1236-1241. <http://dx.doi.org/10.1111/j.1365-2222.2006.02560.x>
- [Szyrwińska, K; Lulek, J.](#) (2007). Exposure to specific polychlorinated biphenyls and some chlorinated pesticides via breast milk in Poland. *Chemosphere* 66: 1895-1903. <http://dx.doi.org/10.1016/j.chemosphere.2006.08.010>
- [Taguchi, S; Yakushiji, T.](#) (1988). Influence of termite treatment in the home on the chlordane concentration in human milk. *Arch Environ Contam Toxicol* 17: 65-71.

- [Takahashi, KL; Aoyama, H; Hojo, H; Shimizu, N; Kikuta, M; Kushida, H; Kawakatsu, H; Ebino, K; Teramoto, S.](#) (2001). Prenatal developmental toxicity studies of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT) in rats and rabbits. *Congenit Anom* 41: 321-328. <http://dx.doi.org/10.1111/j.1741-4520.2001.tb00842.x>
- [Takahashi, W; Saidin, D; Takei, G; Wong, L.](#) (1981). Organochloride pesticide residues in human milk in Hawaii, 1979-80. *Bull Environ Contam Toxicol* 27: 506-511. <http://dx.doi.org/10.1007/bf01611056>
- [Takei, GH; Kauahikaua, SM; Leong, GH.](#) (1983). Analysis of human milk samples collected in Hawaii for residues of organochlorine pesticides and polychlorobiphenyls. *Bull Environ Contam Toxicol* 30: 606-613. <http://dx.doi.org/10.1007/BF01610182>
- [Taylor, DH; Goldey, E.](#) (1992). Assessment of the behavioral and neurotoxic effects of hexachlorobenzene (HCB) in the developing rat. (AAMRL-TR-90-076). Oxford, OH: Miami University, Department of Zoology.
- [Taylor, DH; Goldey, ES; Fisher, JW.](#) (1991). Lactational transfer of hexachlorobenzene. *Toxicologist* 11: 236.
- [Telford, HS; Guthrie, JE.](#) (1945). Transmission of the toxicity of DDT through the milk of white rats and goats. *Science* 102: 647.
- [ten Tusscher, GW; Steerenberg, PA; van Loveren, H; Vos, JG; von Dem Borne, AE; Westra, M; van Der Slikke, JW; Olie, K; Pluim, HJ; Koppe, JG.](#) (2003). Persistent hematologic and immunologic disturbances in 8-year-old Dutch children associated with perinatal dioxin exposure. *Environ Health Perspect* 111: 1519-1523.
- [The Jackson Laboratory.](#) (2012). Mouse phenome database. Available online at <http://phenome.jax.org/db/q?rtn=meas/catlister&req=Dreproductionqqq245>
- [Theus, SA; Tabor, DR; Soderberg, LS; Barnett, JB.](#) (1992). Macrophage tumoricidal mechanisms are selectively altered by prenatal chlordane exposure. *Agents and Actions* 37: 140-146.
- [Thiering, E; Brüske, I; Kratzsch, J; Thiery, J; Sausenthaler, S; Meisinger, C; Koletzko, S; Bauer, CP; Schaaf, B; von Berg, A; Berdel, D; Lehmann, I; Herbarth, O; Krämer, U; Wichmann, HE; Heinrich, J; Giniplus; Groups, LIS.](#) (2011). Prenatal and postnatal tobacco smoke exposure and development of insulin resistance in 10 year old children. *Int J Hyg Environ Health* 214: 361-368. <http://dx.doi.org/10.1016/j.ijheh.2011.04.004>
- [Thompson, CM; Sonawane, B; Barton, HA; DeWoskin, RS; Lipscomb, JC; Schlosser, P; Chiu, WA; Krishnan, K.](#) (2008). Approaches for applications of physiologically based pharmacokinetic models in risk assessment [Review]. *J Toxicol Environ Health B Crit Rev* 11: 519-547. <http://dx.doi.org/10.1080/10937400701724337>
- [Timson, BF; Coffman, JL.](#) (1984). Body composition by hydrostatic weighing at total lung capacity and residual volume. *Med Sci Sports Exerc* 16: 411-414.
- [To-Figueras, J; Barrot, C; Sala, M; Otero, R; Silva, M; Ozalla, MD; Herrero, C; Corbella, J; Grimalt, J; Sunyer, J.](#) (2000). Excretion of hexachlorobenzene and metabolites in feces in a highly exposed human population. *Environ Health Perspect* 108: 595-598.
- [Tojo, Y; Wariishi, M; Suzuki, Y; Nishiyama, K.](#) (1986). Quantitation of chlordane residues in mothers' milk. *Arch Environ Contam Toxicol* 15: 327-332.
- [Torres-Sanchez, L; Rothenberg, SJ; Schnaas, L; Cebrian, ME; Osorio, E; Hernandez, MD; Garcia-Hernandez, RM; del Rio-Garcia, C; Wolff, MS; Lopez-Carrillo, L.](#) (2007). In utero p,p'-DDE exposure and infant neurodevelopment: A perinatal cohort in Mexico. *Environ Health Perspect* 115: 435-439. <http://dx.doi.org/10.1289/ehp.9566>
- [Torres-Sánchez, L; Schnaas, L; Cebrián, ME; Hernández, M; Valencia, EO; García Hernández, RM; López-Carrillo, L.](#) (2009). Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and neurodevelopment: a follow-up from 12 to 30 months of age. *Neurotoxicology* 30: 1162-1165. <http://dx.doi.org/10.1016/j.neuro.2009.08.010>
- [Travis, CC; Arms, AD.](#) (1988). Bioconcentration of organics in beef, milk, and vegetation. *Environ Sci Technol* 22: 271-274. <http://dx.doi.org/10.1021/es00168a005>

- U.S. EPA (U.S. Environmental Protection Agency). (1988). Recommendations for and documentation of biological values for use in risk assessment [EPA Report]. (EPA/600/6-87/008). Cincinnati, OH. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855>
- U.S. EPA (U.S. Environmental Protection Agency). (1998). Methodology for assessing health risks associated with multiple pathways of exposure to combustor emissions [EPA Report]. (EPA 600/R-98/137). Washington, DC. <http://cfpub.epa.gov/ncea/CFM/recordisplay.cfm?deid=55525>
- U.S. EPA (U.S. Environmental Protection Agency). (2000). Supplementary guidance for conducting health risk assessment of chemical mixtures [EPA Report]. (EPA/630/R-00/002). Washington, DC. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533>
- U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes [EPA Report]. (EPA/630/P-02/002F). Washington, DC: Risk Assessment Forum, U.S. Environmental Protection Agency. <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=51717>
- U.S. EPA (U.S. Environmental Protection Agency). (2003a). Framework for cumulative risk assessment [EPA Report]. (EPA/630/P-02/001F). Washington, DC. <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30004TJH.txt>
- U.S. EPA (U.S. Environmental Protection Agency). (2003b). IRIS Toxicological Review of Mirex (External Review Draft). (NCEA-C-1343). US Environmental Protection Agency.
- U.S. EPA (U.S. Environmental Protection Agency). (2009). Risk and technology review (RTR) risk assessment methodologies: For review by the EPA's Science Advisory Board, case studies, MACT I petroleum refining sources, Portland cement manufacturing [EPA Report]. (EPA-452/R-09-006).
- U.S. EPA (U.S. Environmental Protection Agency). (2011a). Exposure factors handbook 2011 edition (final) [EPA Report]. (EPA/600/R-09/052F). <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252>
- U.S. EPA (U.S. Environmental Protection Agency). (2011b). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose [EPA Report]. (EPA/100/R11/0001). Washington, DC. <http://www.epa.gov/raf/publications/interspecies-extrapolation.htm>
- U.S. EPA (U.S. Environmental Protection Agency). (2012a). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: Risk Assessment Forum. [http://www.epa.gov/raf/publications/pdfs/benchmark\\_dose\\_guidance.pdf](http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf)
- U.S. EPA. (2012b). EPA's reanalysis of key issues related to dioxin toxicity and response to NAS comments (CAS No. 1746-01-6) [EPA Report]. (EPA/600/R-10/038F). Washington, DC.
- Valvi, D; Mendez, MA; Martinez, D; Grimalt, JO; Torrent, M; Sunyer, J; Vrijheid, M. (2012). Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: a prospective birth cohort study. *Environ Health Perspect* 120: 451-457. <http://dx.doi.org/10.1289/ehp.1103862>
- Van Oostdam, JC; Dewailly, E; Gilman, A; Hansen, JC; Odland, JO; Chashchin, V; Berner, J; Butler-Walker, J; Lagerkvist, BJ; Olafsdottir, K; Soininen, L; Bjerregard, P; Klopov, V; Weber, JP. (2004). Circumpolar maternal blood contaminant survey, 1994-1997 organochlorine compounds. *Sci Total Environ* 330: 55-70. <http://dx.doi.org/10.1016/j.scitotenv.2004.02.028>
- van Tuyl, M; Blommaart, PE; de Boer, PA; Wert, SE; Ruijter, JM; Islam, S; Schnitzer, J; Ellison, AR; Tibboel, D; Moorman, AF; Lamers, WH. (2004). Prenatal exposure to thyroid hormone is necessary for normal postnatal development of murine heart and lungs. *Dev Biol* 272: 104-117. <http://dx.doi.org/10.1016/j.ydbio.2004.03.042>
- Vargesson, N. (2009). Thalidomide-induced limb defects: resolving a 50-year-old puzzle [Review]. *Bioessays* 31: 1327-1336. <http://dx.doi.org/10.1002/bies.200900103>

- [Vaz, R; Slorach, SA; Hofvander, Y.](#) (1993). Organochlorine contaminants in Swedish human milk: Studies conducted at the national food administration 1981-1990. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 10: 407-418. <http://dx.doi.org/10.1080/02652039309374164>
- [Veeramachaneni, DN; Palmer, JS; Amann, RP; Pau, KY.](#) (2007). Sequelae in male rabbits following developmental exposure to p,p'-DDT or a mixture of p,p'-DDT and vinclozolin: cryptorchidism, germ cell atypia, and sexual dysfunction. Reprod Toxicol 23: 353-365. <http://dx.doi.org/10.1016/j.reprotox.2007.01.001>
- [Veith, GD; Defoe, DL; Bergstedt, BV.](#) (1979). Measuring and estimating the bioconcentration factor of chemicals in fish. J Fish Res Board Can 36: 1040-1048. <http://dx.doi.org/10.1139/f79-146>
- [Verner, MA; Ayotte, P; Muckle, G; Charbonneau, M; Haddad, S.](#) (2009). A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. Environ Health Perspect 117: 481-487.
- [Verner, MA; Charbonneau, M; López-Carrillo, L; Haddad, S.](#) (2008). Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: A new tool in breast cancer epidemiologic studies. Environ Health Perspect 116: 886-892. <http://dx.doi.org/10.1289/ehp.10917>
- [Verner, MA; Plusquellec, P; Muckle, G; Ayotte, P; Dewailly, E; Jacobson, SW; Jacobson, JL; Charbonneau, M; Haddad, S.](#) (2010). Alteration of infant attention and activity by polychlorinated biphenyls: unravelling critical windows of susceptibility using physiologically based pharmacokinetic modeling. Neurotoxicology 31: 424-431. <http://dx.doi.org/10.1016/j.neuro.2010.05.011>
- [Verner, MA; Sonneborn, D; Lancz, K; Muckle, G; Ayotte, P; Dewailly, É; Kocan, A; Palkovicová, L; Trnovec, T; Haddad, S; Hertz-Picciotto, I; Eggesbø, M.](#) (2013). Toxicokinetic modeling of persistent organic pollutant levels in blood from birth to 45 months of age in longitudinal birth cohort studies. Environ Health Perspect 121: 131-137. <http://dx.doi.org/10.1289/ehp.1205552>
- [Waliszewski, SM; Pardio Sedas, VT; Chantiri-P, JN; Infanzon-R, RM; Rivera, J.](#) (1996). Organochlorine pesticide residues in human breast milk from tropical areas in Mexico. Bull Environ Contam Toxicol 57: 22-28. <http://dx.doi.org/10.1007/s001289900150>
- [Webb, SJ; Monk, CS; Nelson, CA.](#) (2001). Mechanisms of postnatal neurobiological development: Implications for human development. Dev Neuropsychol 19: 147-171. [http://dx.doi.org/10.1207/S15326942DN1902\\_2](http://dx.doi.org/10.1207/S15326942DN1902_2)
- [Weisenberg, E; Arad, I; Grauer, F; Sahm, Z.](#) (1985). Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch Environ Contam Toxicol 14: 517-522. <http://dx.doi.org/10.1007/BF01055379>
- [Welch, RM; Findlay, JW.](#) (1981). Excretion of drugs in human breast milk [Review]. Drug Metab Rev 12: 261-277. <http://dx.doi.org/10.3109/03602538108994032>
- [WHO](#) (World Health Organization). (1981). FAO/WHO: Joint FAO/WHO food and animal feed contamination monitoring programme. Summary of data received from collaborating centres - 1977 to 1980. Part B - Contaminants.
- [WHO](#) (World Health Organization). (2003). Global strategy for infant and young child feeding.
- [WHO](#) (World Health Organization). (2005). Frequently asked questions on DDT use for disease vector control. Geneva, Switzerland. [http://www.chem.unep.ch/DDT/documents/WHO\\_FAQonDDT.pdf](http://www.chem.unep.ch/DDT/documents/WHO_FAQonDDT.pdf)
- [WHO](#) (World Health Organization). (2006). Environmental health criteria 236. Principles and methods for assessing autoimmunity associated with exposure to chemicals [WHO EHC]. Geneva: WHO Press, World Health Organization. <http://www.inchem.org/documents/ehc/ehc/ehc236.pdf>
- [WHO](#). (2011). DDT in indoor residual spraying: Human health aspects [WHO EHC]. In Environmental Health Criteria. (Environmental health criteria: 241). Geneva, Switzerland. <http://www.who.int/ipcs/publications/ehc/ehc241.pdf>



- Wickstrom, K; Pyysalo, H; Siimes, MA. (1983). Levels of chlordane, hexachlorobenzene, PCB and DDT compounds in Finnish human milk in 1982. *Bull Environ Contam Toxicol* 31: 251-256.  
<http://dx.doi.org/10.1007/BF01608694>
- Wiener, M; Pittman, KA; Stein, V. (1976). Mirex kinetics in the rhesus monkey. I. Disposition and excretion. *Drug Metab Dispos* 4: 281-287.
- Williams, GR. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone [Review]. *J Neuroendocrinol* 20: 784-794. <http://dx.doi.org/10.1111/j.1365-2826.2008.01733.x>
- Witt, JM; Whiting, FM; Brown, WH; Stull, JW. (1966). Contamination of milk from different routes of animal exposure to DDT. *J Dairy Sci* 49: 370-380. [http://dx.doi.org/10.3168/jds.S0022-0302\(66\)87877-3](http://dx.doi.org/10.3168/jds.S0022-0302(66)87877-3)
- Wolf, CJ; Fenton, SE; Schmid, JE; Calafat, AM; Kuklennyik, Z; Bryant, XA; Thibodeaux, J; Das, KP; White, SS; Lau, CS; Abbott, BD. (2007). Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol Sci* 95: 462-473. <http://dx.doi.org/10.1093/toxsci/kfl159>
- Wolfe, GW; Pepperl, SG. (2005). Hexachlorobenzene: Reproductive Assessment by Continuous Breeding When Administered to Sprague-Dawley Rats by Oral Gavage, Volumes 1 and 2. (TherImmune No. 7244-206). Gaithersburg, MD: TherImmune Research Corporation.
- Wolff, MS. (1999). Letter to the Editor. *Arch Environ Contam Toxicol* 36: 504-504.  
<http://dx.doi.org/10.1007/PL00006624>
- Wolff, MS; Zeleniuch-Jacquotte, A; Dubin, N; Toniolo, P. (2000). Risk of breast cancer and organochlorine exposure. *Cancer Epidemiol Biomarkers Prev* 9: 271-277.
- WPPR. (1992). Report of the Working Party on Pesticide Residues, 1988-1990: The thirty-fourth report of the Steering Group on Chemical Aspects of Food Surveillance. In Food Surveillance Paper No 34. London, UK: Her Majesty's Stationery Office.
- Yamasaki, K; Okuda, H; Takeuchi, T; Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. *Toxicol Lett* 189: 14-20.  
<http://dx.doi.org/10.1016/j.toxlet.2009.04.023>
- Yang, RSH; Coulston, F; Golberg, L. (1975). Binding of hexachlorobenzene to erythrocytes: Species variation. *Life Sci* 17: 545-549. [http://dx.doi.org/10.1016/0024-3205\(75\)90088-0](http://dx.doi.org/10.1016/0024-3205(75)90088-0)
- Yang, RSH; Pittman, KA; Rourke, DR; Stein, VB. (1978). Pharmacokinetics and metabolism of hexachlorobenzene in the rat and the rhesus monkey. *J Agric Food Chem* 26: 1076-1083.  
<http://dx.doi.org/10.1021/jf60219a008>
- Yesair, DW; Feder, PI; Chin, AE; Naber, SJ; Kuiper-Goodman, T; Siegel Scott, C; Robinson, PE. (1986). Development, evaluation and use of a pharmacokinetic model for hexachlorobenzene. In CR Morris; JRP Cabral (Eds.), IARC scientific publications no 77 (pp. 297-318). Lyon, France: International Agency for Research on Cancer.
- Yess, NJ. (1991). FDA monitoring program. Residues in foods--1990. *J Assoc Offic Anal Chem* 74: 121A-141A.
- Yess, NJ. (1992). US Food and Drug Administration pesticide program residues in foods 1991. *J AOAC Int* 75: 135A-157A.
- You, L; Casanova, M; Archibeque-Engle, S; Sar, M; Fan, LQ; Heck, HA. (1998). Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to p,p'-DDE. *Toxicol Sci* 45: 162-173.
- You, L; Gazi, E; Archibeque-Engle, S; Casanova, M; Conolly, RB; Heck, HA. (1999). Transplacental and lactational transfer of p,p'-DDE in Sprague-Dawley rats. *Toxicol Appl Pharmacol* 157: 134-144.

- Young, JG; Eskenazi, B; Gladstone, EA; Bradman, A; Pedersen, L; Johnson, C; Barr, DB; Furlong, CE; Holland, NT. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology* 26: 199-209. <http://dx.doi.org/10.1016/j.neuro.2004.10.004>
- Zhou, P; Wu, Y; Yin, S; Li, J; Zhao, Y; Zhang, L; Chen, H; Liu, Y; Yang, X; Li, X. (2011). National survey of the levels of persistent organochlorine pesticides in the breast milk of mothers in China. *Environ Pollut* 159: 524-531. <http://dx.doi.org/10.1016/j.envpol.2010.10.014>
- Zietz, BP; Hoopmann, M; Funcke, M; Huppmann, R; Suchenwirth, R; Gierden, E. (2008). Long-term biomonitoring of polychlorinated biphenyls and organochlorine pesticides in human milk from mothers living in northern Germany. *Int J Hyg Environ Health* 211: 624-638. <http://dx.doi.org/10.1016/j.ijheh.2008.04.001>
- Zivkovic, D; Hadziselimovic, F. (2009). Development of Sertoli cells during mini-puberty in normal and cryptorchid testes. *Urol Int* 82: 89-91. <http://dx.doi.org/10.1159/000176032>
- Zoeller, RT; Rovet, J. (2004). Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings [Review]. *J Neuroendocrinol* 16: 809-818. <http://dx.doi.org/10.1111/j.1365-2826.2004.01243.x>





# Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk

## Appendices – Workshop Summary Report

October 2013

### Prepared for

U.S. Environmental Protection Agency  
Office of Research and Development  
National Center for Environmental Assessment  
109 T.W. Alexander Drive  
Durham, NC 27711

### Prepared by

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713



## Contents

<b>Appendix A.</b>	<b>Charge Questions for Workshop Participants .....</b>	<b>A-1</b>
<b>Appendix B.</b>	<b>Original Briefing Packets, as Distributed to Workshop Attendees .....</b>	<b>B-1</b>
B.1.	Chlordane Briefing Packet .....	B-1
B.1.1.	Introduction.....	B-3
B.1.2.	Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-3
B.1.3.	Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-11
B.1.4.	Developmental Effects of Chlordane.....	B-11
B.1.5.	Human Equivalent Dose (HED) Estimation .....	B-25
B.1.6.	Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-47
B.1.7.	References.....	B-56
B.2.	DDT Briefing Packet .....	B-61
B.2.1.	Introduction.....	B-65
B.2.2.	Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-66
B.2.3.	Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-79
B.2.4.	Developmental Effects of DDT .....	B-82
B.2.5.	Human Equivalent Dose (HED) Estimation .....	B-105
B.2.6.	Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-130
B.2.7.	References.....	B-134
B.3.	Hexachlorobenzene Briefing Packet.....	B-140
B.3.1.	Introduction.....	B-142
B.3.2.	Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-144
B.3.3.	Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-149
B.3.4.	Developmental Effects of HCB.....	B-153
B.3.5.	Human Equivalent Dose (HED) Estimation.....	B-168
B.3.6.	Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-191
B.3.7.	References.....	B-199
B.4.	Mirex Briefing Packet.....	B-206
B.4.1.	Introduction.....	B-210
B.4.2.	Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-210
B.4.3.	Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-221
B.4.4.	Developmental Effects of Mirex.....	B-222
B.4.5.	Human Equivalent Dose (HED) Estimation .....	B-238
B.4.6.	Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-261
B.4.7.	References.....	B-273
<b>Appendix C.</b>	<b>Workshop Charge and Instructions for Attendees .....</b>	<b>C-1</b>
C.1.	Charge for Chemical Breakout Groups .....	C-1
C.2.	Common Issues and Data Gaps: Group Brainstorming and Approaches Breakout Groups .....	C-2

---

## Appendix A. Charge Questions for Workshop Participants

1. For the chemical discussed in this briefing packet, please describe at least one approach that could be used to estimate the quantitative relationship between infant dose from milk and continuous daily maternal dose for both humans and any relevant laboratory species (i.e., species in which health effects have been observed as a result of postnatal exposure to this chemical). Please describe the approach(es) in detail and provide a rationale for each step. You may draw from the example approaches presented in the briefing packet; however, the briefing packet is primarily meant to serve as a source of information to facilitate the development of science-based innovative approaches. Thus, the approach(es) proposed in response to this question may deviate substantially from the approaches presented in the briefing packet.
2. Using the quantitative relationship(s) identified in response to Charge Question 1, how could an extrapolation be performed from a dose that produces effects as a result of postnatal exposure in animal studies to a continuous daily maternal exposure that would be expected to produce the same effects in human infants exposed through breast milk?
3. What data gaps exist that, if filled, would significantly improve the ability to use the approach(es) identified in response to Charge Question 1 to assess risk to breastfeeding infants?
4. Identify any problems, complications, or uncertainties encountered in the development of the approach(es) identified in response to Charge Question 1. Which of these are expected to be chemical-specific? Which of these may be expected to be common to PBT chemicals in general? How might these problems, complications, or uncertainties affect your confidence in using the approach(es) in risk assessment?
5. Considering the examples and characteristics given in the table below, how might the approach(es) identified in response to Charge Question 1 be adapted for application to estimation of risk to breastfeeding infants from exposure to PBT chemicals in general, including those with biochemical properties different from those of the chemical discussed in this briefing packet? Please identify additional data gaps, problems, complications, or uncertainties that would be important to address if the approach(es) were applied to other PBT chemicals.



---

## Appendix B. Original Briefing Packets, as Distributed to Workshop Attendees

---

### B.1. Chlordane Briefing Packet



## Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk

### Workshop Briefing Packet: Chlordane

#### Prepared for

National Center for Environmental Assessment,  
U.S. EPA  
109 T.W. Alexander Drive  
Durham, NC 27711

#### Prepared by

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713

#### Notice

Although the research described in this briefing packet has been supported by the U. S. Environmental Protection Agency through EP-C-09-009 to ICF International, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

## Contents B.1

<b>Appendix B. Original Briefing Packets, as Distributed to Workshop Attendees .....</b>	<b>B-1</b>
B.1. Chlordane Briefing Packet .....	B-1
B.1.1. Introduction.....	B-3
B.1.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-3
B.1.3. Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-11
B.1.4. Developmental Effects of Chlordane.....	B-11
B.1.5. Human Equivalent Dose (HED) Estimation .....	B-25
B.1.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-47
B.1.7. References.....	B-56

## Figures

Figure B-1. Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to Chlordane.....	B-24
Figure B-2. Exposure-Response Array Showing Developmental Effects in Animals after Gestational or Gestational and Lactational Exposure to Chlordane.....	B-25
Figure B-3. Process diagram for performing cross-species extrapolation .....	B-27
Figure B-4. Maternal and Infant POP Model (Verner et al., 2009) .....	B-35
Figure B-5. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day .....	B-41
Figure B-6. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Cassidy et al., 1994) and an Administered Dose of 0.1 mg/kg-day (POD) .....	B-44

## Tables

Table B-1. Summary of Breast Milk Concentration Studies.....	B-7
Table B-2. Concentration of Oxychlordane in Breast Milk and Maternal Blood (Mes et al., 1984) .....	B-8
Table B-3. Concentrations of Chlordane in Human Tissues Other than Breast Milk .....	B-9
Table B-4. Developmental Effects from Gestational and/or Lactational Exposure to Chlordane .....	B-14
Table B-5. Available Pharmacokinetic Parameters for Chlordane in Humans and Rats .....	B-30
Table B-6. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model.....	B-40
Table B-7. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model.....	B-41
Table B-8. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat.....	B-43
Table B-9. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model.....	B-44
Table B-10. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-47
Table B-11. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method .....	B-47
Table B-12. Potential Dose Metrics and Methods for HED Estimation .....	B-48
Table Att. B.1-1. Concentrations of Chlordane in Human Milk (whole milk, ppb) .....	B-51
Table Att. B.1-2. Concentrations of Chlordane in Human Milk (milk fat, ppb).....	B-53

### **B.1.1. Introduction**

Chlordane is a synthetic compound that was used in the United States from 1948 to 1988 ([ATSDR, 1994](#)). It was initially used as an insecticide on agricultural crops, lawns, and gardens, and as a fumigating agent against pests such as grubs, ants, chiggers, termites, and various worms ([NLM, 2011](#); [ATSDR, 1994](#)). In 1978, EPA banned the use of chlordane on food crops and phased out most other uses over the next 5 years. From 1983 to 1988, the only use of chlordane was to control termites in buildings through application to soils and around building foundations. As of 1992, chlordane was produced at one facility in Memphis, Tennessee for export to other countries ([ATSDR, 1994](#)). By 2003, chlordane production and use had ceased across all of North America ([NLM, 2011](#)). Chlordane is still used in other countries around the world as a pesticide for termite control ([NLM, 2011](#)).

Technical-grade chlordane is a mixture of about 10 major constituents, including trans-chlordane, cis-chlordane,  $\beta$ -chlordane, heptachlor, and trans-nonachlor ([ATSDR, 1994](#)). Technical-grade chlordane contains approximately 60–70% organochlorine compounds ([NLM, 2011](#)). Chlordane and the other chemicals that are the subjects of these briefing packets are classified as persistent, bioaccumulative, and toxic (PBT) compounds. These and other PBT chemicals may be transferred from mothers to infants through breast milk and may pose risks to the breastfeeding infants. The purpose of these briefing packets and the associated workshop is to stimulate ideas and discussion regarding innovative approaches to the assessment of risk to breastfeeding infants from chlordane and the other PBT chemicals in breast milk.

In this briefing packet, the available literature on gestational and lactational exposure to chlordane was reviewed for key findings and data to help estimate the maternal dose and breastfeeding infant dose of chlordane in humans. Selected references are summarized and evaluated in this briefing packet in five sections: maternal dose and breastfeeding infant dose of chlordane in humans; maternal dose and nursing offspring dose in animals; developmental effects of chlordane in humans and animals; human and animal kinetic models; and a final section, which discusses the application of kinetic models to derive an example human equivalent dose (HED).

These briefing packets are intended to stimulate ideas and provide material for discussion regarding innovative approaches to the assessment of risk to breastfeeding infants from chlordane and other PBT chemicals in breast milk. The information in this and other briefing packets is provided to help workshop participants discuss and formulate answers to the workshop charge questions. Areas where there exists uncertainty, data gaps, or where there is a lack of understanding in the current literature are highlighted to foster further discussion.

---

### **B.1.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans**

This section describes how the general population of women may be exposed to chlordane. It also includes information on the fate and transport of chlordane in the body, excretion of the compound through breast milk, and the potential for infant exposure. Exposure to chlordane may vary widely and is



at least partially dependent on social factors (e.g., dietary choices) and environmental factors (e.g., amount of residual chlordane in the local environment). Concentrations of chlordane have been measured in various human tissues, including breast milk and blood plasma. Some attempts have been made, as reported in the literature, to correlate measured body burdens of chlordane with dietary and environmental exposures. Researchers have also attempted to correlate body burden in mothers with intake by breastfeeding infants. However, the relationships between maternal exposure to chlordane, levels of intake from environmental and dietary sources, maternal body burden, and infant dose in humans are not well described in the literature.

---

#### **B.1.2.1. Mothers' Exposure to Chlordane**

Ingestion of chlordane-contaminated food is considered to be the most common source of chlordane exposure, but inhalation of chlordane by individuals living in homes that were treated with chlordane for termite control also represents a significant, but less common, exposure pathway ([ATSDR, 1994](#)). Chlordane persists in indoor air, so homes that were treated with chlordane for termite control still might represent sources of chlordane exposure for some populations ([ATSDR, 1994](#)). Inhalation exposure in treated homes represents the highest potential exposure to chlordane, though it is less common than ingestion ([ATSDR, 1994](#)). The extensive use of chlordane on farmlands in the 1960s and 1970s resulted in soil and water contamination that remains today, which has subsequently resulted in contamination of the food supply (including fish and shellfish) ([ATSDR, 1994](#)). Dietary exposure data are most common for chlordane, and the contribution of inhalation exposures is not described well in the literature. Thus, the contribution of inhalation exposures to total body burden may be significant in certain areas, and may contribute to infant exposures through breast milk, but the contribution from inhalation is not currently quantified in the literature.

Three studies of dietary exposure to chlordane provide insufficient evidence for a correlation between dietary intake and chlordane concentration in tissues ([Chan et al., 1997](#); [Mussalo-Rauhamaa et al., 1988](#); [Al-Omar et al., 1986](#)). Two of the three studies ([Mussalo-Rauhamaa et al., 1988](#); [Al-Omar et al., 1986](#)) did not establish a correlation between chlordane concentration in human tissue and chlordane concentration in the diet. Chan et al. ([1997](#)) measured concentrations of chlordane in various food sources and collected data on eating habits based on dietary recall to estimate daily chlordane intake. The authors inferred exposure distributions across the populations based on these data in lieu of tissue sampling ([Chan et al., 1997](#)). Without actual measurements of chlordane concentrations in human tissue, a correlation between dietary exposure and chlordane levels in the tissues of mothers could not be assumed. Additionally, confounding exposures via inhalation are a possibility in all three studies, which may preclude the ability to determine a correlation between dietary exposure and measured chlordane concentrations in tissues.

Taguchi and Yakushiji ([1988](#)) investigated non-dietary sources of exposure to chlordane and levels of total chlordane (cis-chlordane, trans-chlordane, oxychlordane, cis-nonachlor and trans-nonachlor) in breast milk. Chlordane concentrations in human breast milk were significantly higher for mothers who had home termite treatments than for mothers whose homes had not been treated for termites. The

average concentration of total chlordane in breast milk was 58 µg/kg lipid (ppb) for the 7 non-exposed lactating women, compared with 162 µg/kg lipid (ppb) for the 15 lactating women whose homes had recently been treated for termites (i.e., within an average of 1.8 years). The authors concluded that the likely chlordane exposure routes in the group whose homes had been treated were inhalation of contaminated air, dermal absorption from contaminated air, or ingestion of foods that were contaminated through storage in the home ([Taguchi and Yakushiji, 1988](#)).

---

### **B.1.2.2. Is Chlordane Sequestered in Breast Milk or Other Human Tissues?**

#### ***Chlordane in Human Breast Milk***

Twenty-nine studies were identified that measured chlordane or oxychlordane<sup>5</sup> concentrations in human milk. Analytical techniques and reporting methods varied among these studies. Studies measured and reported some combination of both cis- and trans- congeners, oxychlordane, and mixtures of these and other metabolites. Oxychlordane concentrations were typically reported more often than cis- or trans-chlordane or chlordane mixtures. Concentrations of chlordane in either whole breast milk or milk fat (lipids) were reported in a variety of units (e.g., ppm, ppb, mg/kg, or ng/g). All identified studies were performed between 1977 and 1999. A summary of data from these studies is provided in [Table B-1](#). Specific results from each study are summarized in more detail in [Attachment B.1-1](#) in [Table Att. B.1-1](#) (whole breast milk) and [Table Att. B.1-2](#) (milk fat).

The chlordane concentrations in human breast milk vary widely, as summarized in [Attachment B.1-1](#). One reason for the variation may be that the reported breast milk concentrations represent averages across study populations ranging in size from less than 10 women ([Al-Omar et al., 1986](#); [Barnett et al., 1979](#)) to 1,436 women ([Savage et al., 1981](#)). Studies were conducted in a variety of international locations. Typically, studies conducted in Japan and Canada provided values that fell into the “majority” of average concentrations (see [Table B-1](#)). Studies also vary by the size of the geographical areas from which study participants were solicited—some studies examined a subpopulation chosen based on country-wide population distribution data ([Mes et al., 1993](#); [Mes et al., 1986](#)) whereas others recruited participants from a single rural community ([Strassman and Kutz, 1977](#)) or single city ([Al-Omar et al., 1985](#)). Many studies noted that the contaminant of concern was not detected in all samples and provided the detection frequency (%). Other studies did not provide any information on detection frequency and simply provided a mean or median concentration and a range that included a lower bound concentration of “not detected” or “detected at trace levels.” It is unclear how the detection frequency of chlordane and its metabolites affects the comparability of average concentrations among studies. Two studies reported average concentrations for the full study populations as well as the average concentrations of positive samples only. In these studies, concentrations averaged to include all samples were much lower than those averaged to include only positive samples both in whole milk ([Strassman and Kutz, 1977](#)) and in milk fat ([Mussalo-Rauhamaa et al., 1988](#)). However, in some studies it

---

<sup>5</sup> Oxychlordane is a biological metabolite of chlordane that persists in body fat as a predominant chlordane residue ([ATSDR, 1994](#)).

is unclear whether the average concentration reported includes all samples or only positive-detection samples.

**Table B-1. Summary of Breast Milk Concentration Studies**

Compound	Number of reported averages	Range of reported averages (ppb)	Range of reported detection frequencies	Majority values	Countries
<b>Measured in whole milk</b>					
Cis-chlordane	8	0.05–154	14%–80%	5/8 reported averages ≤1 ppb	Canada, Iraq, Japan, Jordan
Trans-chlordane	8	trace–125	7%–73%	5/8 reported averages ≤0.16 ppb	Canada, Iraq, Japan, Jordan
Oxy-chlordane	10	0.39–12	45.6%–100% (4/6 reported >90%)	6/10 reported averages ≤1 ppb	Canada, Japan, United States
Unspecified chlordane	1	0.3	17.8%	N/A	Spain
<b>Measured in milk fat</b>					
Cis-chlordane	13	0.21–460	5.2%–56%	9/13 reported averages ≤12 ppb	Canada, Finland, Japan, Jordan, Russia
Trans-chlordane	11	trace–590	2%–73%	8/11 reported averages ≤10 ppb	Canada, Finland, Japan, Jordan
Oxy-chlordane	19 (1 median)	7.9–400 (median)	3.9%–100% (7/11 reported >90%)	14/19 reported averages ≤61 ppb	Australia, Canada, Finland, Japan, Mexico, Russia, United States
Unspecified chlordane	4 (1 median)	<10 (median)–162	~17% (2 reported)	3/4 reported averages ≤58 ppb	Australia, Japan, Spain

Note: See [Attachment B.1-1](#), [Table Att. B.1-1](#), and [Table Att. B.1-2](#) for more detailed information and sources.

### Chlordane in Other Human Tissues

Mes et al. (1984) measured oxychlordane in breast milk and maternal blood. Researchers collected blood samples and breast milk samples at the same time, on multiple occasions, post-partum. The ratio of oxychlordane concentrations in breast milk to those in blood averaged 9:1, but the ratio varied greatly over time due to the variable concentrations of oxychlordane in blood and breast milk (as shown in Table B-2). The authors did not find a consistent correlation between milk and blood oxychlordane residue levels, and concluded that it is not possible to predict residue levels in breast milk based on residue levels in maternal blood (Mes et al., 1984). Several other studies were identified that measured chlordane concentrations in tissues other than breast milk, as shown in Table B-3.

In a study in Baghdad, Iraq, researchers presented data on the concentration of chlordane in whole breast milk and the placenta for four women (Al-Omar et al., 1986). Concentrations of chlordane in whole breast milk were 25 ( $\pm$  29 SD), 51 ( $\pm$  69 SD), 125 ( $\pm$  225 SD), and 118 ( $\pm$  310 SD) ppb and concentrations of chlordane in the placenta of the same women were 5, 7, 18, and 137 ppb, respectively (Al-Omar et al., 1986). As previously mentioned, the authors considered these measured values in context with the residue levels of chlordane measured in meats that were reported in other studies conducted in the region during the same 2-year time period. They also noted that chlordane was used for termite control in the region where the women resided, but did not investigate this specific source or other potential sources of chlordane exposure for the individuals in the study (Al-Omar et al., 1986).

**Table B-2. Concentration of Oxychlordane in Breast Milk and Maternal Blood (Mes et al., 1984)**

Day Post-Partum	Concentration in breast milk (ng/g whole milk)	% fat in whole milk	Concentration in maternal blood (ng/g whole blood)	% fat in whole blood	Ratio of Oxychlordane Concentration (whole milk / whole blood)
7	1.2	2.65	0.2	0.17	5:1
14	1.5	3.69	0.1	0.16	20:1
28	1.6	3.70	0.2	0.15	10:1
42	1.2	3.07	trace	0.15	10:1
56	1.2	3.44	0.5	0.13	2:1
70	1.1	3.06	0.1	0.14	10:1
84	1.1	2.89	trace	0.12	--
98	1.3	3.75	ND	0.13	--

Trace: detection limit < 0.05 ng/g; ND = Not detected/below detection limit

Source: Mes et al. (1984).

Frank et al. (1988) measured organochlorine residues in tissues of residents of Ontario, Canada over the course of several years. Chlordane residues were measured in adipose tissues of 50–88 adult females

each year for 4 years. Average concentrations ranged from 0.011 ( $\pm$  0.016 SD) to 0.07 ( $\pm$  0.05 SD) mg/kg. While the study did not measure chlordane concentrations in breast milk of those same participants or other study participants residing in the area, they did measure chlordane concentrations in adipose tissue from 13 children aged 1 day to 5 years old (9 of the 13 children were under 18 months old). Chlordane concentrations ranged from less than 0.005 mg/kg up to 0.02 mg/kg (Frank et al., 1988). The study authors did not identify the source of chlordane exposure.

**Table B-3. Concentrations of Chlordane in Human Tissues Other than Breast Milk**

Location	Year	Participant/ Samples	Compound	Concentration	Source
<b>Adipose tissue (mg/kg)</b>					
South West Ontario	1978 – 1979	50 females	chlordane	0.07 $\pm$ 0.05 SD detection frequency <sup>a</sup> : 92.7%	Frank et al. (1988)
South West Ontario	1976 – 1977	54 females	chlordane	0.03 $\pm$ 0.03 SD detection frequency <sup>a</sup> : 76%	Frank et al. (1988)
Western Australia	October 1990 – March 1991	31 women, samples taken at time of Caesarean section	chlordane	avg: 0.02 range: ND-0.04 detection frequency: 84%	Stevens et al. (1993)
South West Ontario	1980 – 1981	53 females	chlordane	0.02 $\pm$ 0.02 SD detection frequency <sup>a</sup> : 76.8%	Frank et al. (1988)
South West Ontario	1983 – 1984	88 females	chlordane	0.011 $\pm$ 0.016 SD detection frequency <sup>a</sup> : 24.4%	Frank et al. (1988)
South West Ontario	1976 – 1984	13 infants, age 1 day to 5 yr.	chlordane	range: < 0.005-0.02	Frank et al. (1988)
<b>Maternal blood (ng/g)</b>					
Western Australia	October 1990 – March 1991	31 women, samples taken at time of hospital admittance for delivery	chlordane	Not detected	Stevens et al. (1993)
Washington, D.C. area	NR <sup>c</sup> , before 1984	16 women, 7-98 days post-partum	oxychlordane	avg. range ND-0.5 depending on week	Mes et al. (1984)
<b>Placenta (mg/kg)</b>					
Baghdad, Iraq	NR <sup>c</sup> , before 1986	4 post-partum women	chlordane	0.005, 0.007, 0.018, 0.137	Al Omar et al. (1986)
<b>Cord blood (ng/g)</b>					
Western Australia	October 1990 – March 1991	31 women, samples taken at time of Caesarean delivery	chlordane	Not detected	Stevens et al. (1993)

<sup>a</sup> In both male and female adipose tissue samples, the percentage of samples that had detectable levels of chlordane.

<sup>b</sup> Measurement is for oxychlordane, the metabolite of chlordane.

<sup>c</sup> ND = Not detected/below detection limit; NR=not reported, assumed to be before the year the study was published.

In addition to measuring concentrations of chlordane in breast milk, Stevens et al. (1993) measured chlordane concentrations in adipose tissue taken from the incision site of 31 western Australian mothers who gave birth via a Caesarean section. The researchers also recorded concentrations in maternal blood at the time of admission to the hospital prior to the Caesarean section and concentrations in cord blood at time of delivery. Concentrations of chlordane in adipose tissue ranged from undetected to 0.04 mg/kg of the total extractable fat; 84% of samples had chlordane concentrations above the minimum quantifiable level. The median chlordane concentration in adipose tissue was 0.02 mg/kg extractable fat. Chlordane was not detected in maternal blood or cord blood samples (Stevens et al., 1993). While the study authors did not determine the source of exposure for the study population, they noted that chlordane was registered for use as a termite control agent in buildings under construction in the study region.

---

#### **B.1.2.3. How Much Chlordane Passes from Mother to Infant via Breast Milk?**

One study was identified that directly assessed the relationship between maternal chlordane body burden and infant lactational dose in humans, results of which were reported in two different publications (Quinsey et al., 1996; Quinsey et al., 1995). Up to three breast milk samples were taken from 23 Australian mothers at approximately 6 weeks, 10 weeks, and 14 weeks after birth. Daily milk intake by each infant was determined based on infant weight before and after each feeding in a 24-hour time period (Quinsey et al., 1996). Milk samples were analyzed for organochlorine content (including chlordane, DDT, HCB, and PCBs) and milk fat content. Infant weight during the study period averaged 7.08 kg (range: 3.85–10.45 kg). Daily milk intake averaged 722 mL (range: 355–1,110 mL) and fat content of milk averaged 3.73% (range: 0.97–9.63%) (Quinsey et al., 1996).

In the first publication, researchers estimated that approximately 77% of infants received a measurable dose of oxychlordane via breast milk, averaging 0.386 ( $\pm$  0.324)  $\mu$ g/kg-day (range: 0.016–1.09  $\mu$ g/kg-day) (Quinsey et al., 1995). Trans-nonachlor was detected in approximately 39% of samples, and the infant dose of trans-nonachlor calculated based on those samples averaged 0.239 ( $\pm$  0.209)  $\mu$ g/kg-day (range: 0.036–0.71  $\mu$ g/kg-day) (Quinsey et al., 1995). In both the first and second publication, the total chlordane concentration (represented trans-nonachlor and oxychlordane combined) in milk samples was not reported, but the mean daily intake of chlordane for infants based on 44 milk samples and the averages for infant weight, milk consumption, and milk fat content was calculated as 0.46 ( $\pm$  0.32)  $\mu$ g/kg-day. Total chlordane intake calculated for each infant in the study ranged from 0.02 to 1.09  $\mu$ g/kg-day. The metabolites  $\alpha$ -chlordane and  $\beta$ -chlordane were not detected in any of the samples (Quinsey et al., 1995).

In a study conducted by Stevens et al. (1993), the average concentration of chlordane in the breast milk of 128 mothers in Western Australia was determined to be less than 0.01 mg/kg lipid (range: not detected to 0.07 mg/kg lipid; see [Table Att. B.1-2](#)).

---

### B.1.3. Maternal Dose vs. Nursing Offspring Dose in Animals

No studies were available that quantified the maternal levels and/or nursing offspring dose of chlordane or its metabolites in the milk experimental animals.

---

### B.1.4. Developmental Effects of Chlordane

---

#### B.1.4.1. Human Studies

No studies were available that discussed the developmental effects of chlordane in humans.

---

#### B.1.4.2. Animal Studies

The animal toxicology literature on controlled exposures to chlordane was reviewed for studies presenting data specifically related to exposure occurring during gestation and/or lactation. To provide a comprehensive understanding of the hazard resulting from these specific types of exposures, all relevant studies that could be retrieved were reviewed and are summarized in [Table B-4](#). The endpoints examined and effects observed are noted, as are potential points of departure (PODs) for each study. For endpoints in the low-dose range, benchmark dose modeling using U.S. EPA's Benchmark Dose Software (BMDS) was considered. However, appropriate litter-specific data were not available for critical effects in the low-dose range, making BMD modeling inappropriate for this application. PODs are arrayed in [Figure B-1](#) and [Figure B-2](#) to facilitate comparisons across studies. [Table B-4](#) serves as a reference key for [Figure B-1](#) and [Figure B-2](#).

Studies were classified according to the timing of exposure for the pups. Gestational exposure studies were those in which maternal animals were exposed during gestation and (1) pups were sacrificed prenatally or at parturition, or (2) pups were nursed by unexposed dams. Combined lactational and gestational exposure studies were those in which maternal animals were exposed during gestation or during gestation and lactation and pups were nursed prior to sacrifice. Finally, lactational exposure studies were those in which dams were not dosed until lactation began or pups unexposed during gestation were cross-fostered to exposed mothers.

Chlordane induced a number of developmental effects in rats and mice including immune effects, endocrine effects, and neurotoxicity. A number of studies have investigated the effects of gestational chlordane exposure on the immune system in the mouse ([Blaylock et al., 1995](#); [Theus et al., 1992](#); [Barnett et al., 1990b](#); [Barnett et al., 1990a](#); [Blaylock et al., 1990](#); [Barnett et al., 1985a](#); [Barnett et al., 1985b](#); [Menna et al., 1985](#); [Spyker-Cranmer et al., 1982](#)). Several studies established that gestational exposure to mice resulted in depressed delayed typed hypersensitivity (DTH) responses in offspring ([Blaylock et al., 1995](#); [Barnett et al., 1985a](#); [Barnett et al., 1985b](#); [Spyker-Cranmer et al., 1982](#)). Barnett et al. ([1985b](#)) and Spyker-Cranmer et al. ([1982](#)) showed that even at 100 days of age, with no exposure



to chlordane since birth, the DTH response did not recover. Consistent with effects on DTH (in which macrophages play an important role), significant reductions in myeloid progenitor cells in female offspring ([Blyler et al., 1994](#); [Barnett et al., 1990b](#); [Barnett et al., 1990a](#)) and decreased macrophage tumoricidal activity were observed in exposed offspring ([Theus et al., 1992](#)). Thus, cells in the macrophage lineage may be the target for chlordane. Investigations of response to influenza infection generally showed increased survival but no effect on virus titers in the lung. Influenza is associated with pathology that results from a DTH response. The increased influenza survival observed with chlordane exposure is thought to be due to attenuation of this pathology due to depression of the DTH response ([Barnett et al., 1985a](#); [Menna et al., 1985](#)).

Chlordane is thought to affect the endocrine system ([NLM, 2011](#)). Two of the studies described in [Table B-4](#) report endocrine effects following chlordane exposure ([ATSDR, 1994](#); [Cranmer et al., 1984](#)). Plasma corticosterone was increased in prenatally exposed male mice at 28 and 100 days (low dose only) and at 400 days of age although animals seem to have recovered by 800 days ([Cranmer et al., 1984](#)). Female mice were similarly affected when examined at 400 days, at the low dose only. Cassidy et al. ([1994](#)) noted a significant depression in testosterone levels measured at 85 days of age in female rat offspring only.

Three studies demonstrated neurotoxicity in offspring exposed to very low levels of chlordane during gestation ([Al-Hachim and Al-Baker, 1973](#)) or during gestation followed by lactation ([Cassidy et al., 1994](#); [Ingle, 1952](#)). At a dose of 1 mg/kg-day during gestation, Al-Hachim and Al-Baker ([1973](#)) described neurological effects in mice aged 30–49 days. Ingle ([1952](#)) conducted a study in which the pups of rats exposed to an estimated chlordane dose as low as 1.5 mg/kg-day during gestation and lactation showed tremors and hyperactivity while higher doses resulted in pup mortality (see [Table B-4](#)). Although some of the studies are difficult to evaluate due to incomplete descriptions of the experimental design, the results are generally supported by the findings of effects on spatial ability and auditory startle by Cassidy et al. ([1994](#)) at an even lower dose (0.1 mg/kg-day) in rats exposed during gestation and lactation. While there is a dose-response pattern observed for these effects at the low and middle doses tested, the high dose animals appeared less affected than those at the lower doses. The authors suggest that the potential mechanism for the observed neurological effects is decreased chloride uptake through the GABA<sub>A</sub>-mediated receptor complex in the brains of exposed rats. The authors also identified several chlordane components and metabolites in the dams' milk as well as in the plasma of the offspring.

Cassidy et al. ([1994](#)) provided data indicating behavioral effects in offspring following exposure of maternal animals to 0.10 mg/kg-day chlordane via the diet during gestation and lactation. This LOAEL of 0.10 mg/kg-day is supported by critical changes in the GABA<sub>A</sub>-mediated receptor complex reported by Cassidy et al. ([1994](#)) as well by observations of neurotoxicity in other independent studies that occurred at similar exposures ([Al-Hachim and Al-Baker, 1973](#); [Ingle, 1952](#)). The LOAEL of 0.10 mg/kg-day may be protective of other effects reported in the immune system and endocrine system at slightly higher doses (4-8 mg/kg-day and 0.16-1 mg/kg-day, respectively). While the study by Cassidy et al. ([1994](#)) is considered to be of good quality, the data reported for this endpoint are not sufficient to support BMD modeling. Therefore, the LOAEL of 0.10 mg/kg-day is used as an example POD to assess the impact that

accumulated exposure to a mother may have on lactational exposure and postnatal development of her offspring. The POD is used in [Section B.1.6](#), along with results from PBPK models, to estimate a HED. The choice of the POD and HED presented in this packet are intended only as examples to stimulate discussion for the workshop participants.

### B.1.4.3. Developmental Effects Summary Tables

**Table B-4. Developmental Effects from Gestational and/or Lactational Exposure to Chlordane**

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
<b>Gestational Exposure</b>								
Mouse, BALB/c, 5 dams dosed GD 1-18, diet, 2-11 fetuses/ female, fetuses examined at GD 18	0, 8	<b>Measured:</b> fetal livers for cellularity, granulocyte/macrophage CFU and spleen-CFU; only examined female conceptus  <b>Observed:</b> depressed granulocyte-macrophage CFU at 8 mg/kg-d; decreased spleen-CFU at 8 mg/kg-d, no change in liver cellularity	Develop: NA	Develop: 8	DU	DU	Barnett et al. (1990b)  (Authors concluded that damage to stem cells in adult offspring reported by Barnett et al. (1990a) is a result of fetal exposure.)	Develop: [C1]
Rat, Osborne-Mendel, diet, 1 female/group dosed 24 weeks or 48 weeks-PND 1; pups cross-fostered to untreated dam	0, ≤ 0.5, 0.5-1, 1.5-3, 10-14, 18-30	<b>Measured:</b> pup body weight, growth and development  <b>Observed:</b> no effects in offspring from gestation only exposure	Develop: 18-30	Develop: NA	DU	DU	Ingle (1952)  (Doses are estimated from a figure showing doses prenatally; small sample size. Adverse effects were noted in offspring exposed after gestation. Described later in this table.)	Uncertainty in the doses precludes inclusion in the exposure response array

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
<b>Gestational and Lactational Exposure</b>								
Mouse, CD-1, 50 dams dosed GD 8–12, gavage, pups sacrificed PND 3	0, 50	<b>Measured:</b> maternal mortality, conception, maternal weight gain, pup body weight, litter size, litter viability  <b>Observed:</b> no effects	Maternal: NA  Develop: 50	Maternal: NA  Develop: NA	DU	DU	Chernoff and Kavlock ( <a href="#">1982</a> )  (Due to day of sacrifice lactational exposure cannot be excluded from consideration)	Develop: [A1]
Mouse, BALB/c, unreported number dams dosed GD 1–18, diet, pups sacrificed at PND 42-49	0, 8	<b>Measured:</b> bone marrow cells for cellularity, granulocyte/macrophage CFU and spleen-CFU examined in response to exogenous recombinant forms of cytokines granulocyte/macrophage-colony stimulating factor (CSF), macrophage-CSF, and interleukin 3  <b>Observed:</b> significant reduction in myeloid progenitor cells; females only	Develop: NA	Develop: 8	DU	DU	Blyler et al. ( <a href="#">1994</a> )	Develop: [B1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Mouse, BALB/c, unreported number dams dosed GD 1-18, diet, pups sacrificed at PND 100 or 200	0, 4, 8	<p><b>Measured:</b> granulocyte-macrophage CFU in bone marrow and spleen-CFU, in offspring; peripheral blood leukocyte count, clinical observations for overt toxicity</p> <p><b>Observed:</b> dose-related depressed granulocyte-macrophage colony-forming cells at PND 100 and 200 at <math>\geq 4</math> mg/kg-d, decreased spleen colony-forming cells at PND 100 and 200 (females only tested) at <math>\geq 4</math> mg/kg-d, no change in bone marrow cellularity</p>	Develop: NA	Develop: 4	DU	DU	<p>Barnett et al. (1990a)</p> <p>(There was an insufficient number of male offspring in the 8 mg/kg-d group to assay at 200 days.)</p>	Develop: [D1]
Mouse, BALB/c, 18 dams/group dosed GD 1-19, diet, 10-12 pups from two litters/group inoculated with FLU-A at PND 38, sacrificed PND 66	0, 0.16, 2.0, 4.0, 8	<p><b>Measured:</b> survival and influenza A antibodies in surviving mice at 21 and (following boost) at 28 days post infection</p> <p><b>Observed:</b> improved survival to influenza type A virus, increased antiviral antibody in females</p>	Develop: NA	Develop: NA	DU	DU	Menna et al. (1985)	This study is not included in the exposure response array. Authors note that the effect is “not defined on a mechanistic level.”

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Mouse, BALB/c, dams dosed GD 1-19, diet, 5-17 pups/group sensitized and challenged with oxazolone, examined at PND 30 or 100	0, 4, 16	<p><b>Measured:</b> oxazolone induced delayed hypersensitivity (DTH) response at PND 100 and 30; mixed lymphocyte reactivity (MLR), mitogen-induced T and B-cell blastogenesis in spleen cells PND 30</p> <p><b>Observed:</b> decreased DTH response at PND 30(variable) and 100 (pronounced) at <math>\geq 4</math> mg/kg-d, MLR at PND 30 (males only) at <math>\geq 4</math> mg/kg-d, enhanced Con A( T cell) induced blastogenesis at PND 30 at <math>\geq 4</math> mg/kg-d , but not significant in males at 16 mg/kg-d; no effects on B cell mitogenesis</p>	Develop: NA	Develop: 4	DU	DU	<p>Barnett et al. (<a href="#">1985b</a>)</p> <p>(Effects more severe in older animals)</p>	Develop [F1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Mouse, BALB/c, dams dosed GD 1-19, diet, 2–11 pups/group inoculated on PND 30 and examined 1, 3, 5, 7, and 11 days post-inoculation, 3–5 pups/group assayed for DTH	0, 4, 8, 16	<p><b>Measured:</b> offspring influenza A virus-specific DTH response, virus-specific T-cell blastogenesis, virus titers in lung</p> <p><b>Observed: decreased DTH response in offspring at 6-48 hrs post inoculation at ≥8 mg/kg-d</b> (not measured at 4 mg/kg-d); no effect on virus titers in lung at either dose; no effect on ability of T cells from thioglycolate induced peritoneal exudates cells to respond influenza antigen 60 days after infection</p>	Develop: NA	Develop: 8	DU	DU	<p>Barnett et al. (<a href="#">1985a</a>)</p> <p>(Authors concluded that increased survival was due to decreased specific DTH response, which is associated with pathology following influenza infection.)</p>	Develop: [G1]
Mouse, BALB/c, dams dosed GD 1-19, diet, 45 pups/group sensitized and challenged with oxazolone or inoculated to sheep red blood cells, examined at PND 101	0, 0.16, 8	<p><b>Measured:</b> offspring oxazolone DTH response and antibody-mediated response to sheep red blood cell inoculation</p> <p><b>Observed: decreased DTH response at 8 mg/kg-d;</b> no effect on T-cell dependent antibody response</p>	Develop: 0.16	Develop: 8	DU	DU	Spyker-Cranmer et al. ( <a href="#">1982</a> )	Develop: [H1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Mouse, BALB/c, dams dosed GD 1-18, diet, 5 pups/group sensitized and challenged with oxazolone, examined at PND 30	0, 8	<p><b>Measured:</b> offspring oxazolone DTH response compared to topical application of chlordane</p> <p><b>Observed:</b> decreased DTH response at 8 mg/kg-d</p>	Develop: NA	Develop: 8	DU	DU	<p>Blaylock et al. (1995)</p> <p>(Author topical chlordane treatment to be more effective in decreasing DTH response than prenatal exposure to chlordane, but it is really impossible to compare dermal vs oral prenatal exposure.)</p>	Develop: [I1]
Mouse, BALB/c, dams dosed GD 1-18, diet, 5 pups/group sacrificed at PND 100 or 200	0, 4, 8	<p><b>Measured:</b> fetal cytotoxic T-lymphocyte (CTL) and natural killer (NK) cell activities</p> <p><b>Observed:</b> decreased male NK response at PND 200 at 8 mg/kg-d; no effect on CTL</p>	Develop: ND	Develop: 8	DU	DU	<p>Blaylock et al. (1990)</p> <p>(Effects not considered adverse.)</p>	Develop: [J1]
Mouse, BALB/c, unreported number dams dosed GD 1-18, diet, macrophage cells collected from pups between PND 100 and 125	0, 8	<p><b>Measured:</b> tumoricidal induction activity in thioglycolate induced peritoneal macrophages of spring, overt signs of toxicity</p> <p><b>Observed:</b> delayed tumoricidal induction activity associated with delayed release of H<sub>2</sub>O<sub>2</sub></p>	Develop: NA	Develop: 8	DU	DU	<p>Theus et al. (1992)</p> <p>(Authors noted that total chlordane exposure to pups was 3.5 mg/kg; not clear how they arrived at that number.)</p>	Develop: [K1]



Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Mouse, F <sub>2</sub> dihybrid, 18 dams dosed GD 1-19, diet, 15 pups/group sacrificed PND 101, 400, and 800	0, 0.16, 8	<p><b>Measured:</b> plasma corticosterone levels at PND 101, 400, 800, pup weight gain, pup viability</p> <p><b>Observed:</b> increased plasma corticosterone in males at PND 101 at 0.16 mg/kg-d only, and at PND 400 at ≥0.16 mg/kg-d, increased plasma corticosterone in females at PND 400 at 0.16 mg/kg-d, 55% mortality of offspring at 8 mg/kg-d in the first week (FEL)</p>	Develop: NA	Develop: 0.16; 8 (FEL)	DU	DU	Cranmer et al. (1984)	Develop: [L1]
Mouse, F <sub>2</sub> dihybrid, 6 dams/group dosed GD 1-22, diet, pups culled to 4/sex/group and sacrificed PND 28	0, 0.16, 8	<p><b>Measured:</b> fetal adrenal production and liver reduction capacity for corticosterone, plasma corticosterone levels, adrenal and liver weights, pup weight gain, pup viability</p> <p><b>Observed:</b> increased plasma corticosterone in males at 0.16 mg/kg-d only, 55% mortality of offspring at 8 mg/kg-d in the first week (FEL)</p>	Develop: NA	Develop: 0.16; 8 (FEL)	DU	DU	<p>Cranmer et al. (1978)</p> <p>(Authors postulate that the lack of dose response is a result of non-monotonic metabolic response.)</p>	Develop: [E1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Mouse, albino, 6 dams/group dosed 5-7 times during 7 consecutive days of the third trimester, oral, 10 pups/group examined PND 30-49	0, 1, 2.5	<p><b>Measured:</b> pup conditioned avoidance response, electroshock seizure threshold, open-field performance</p> <p><b>Observed:</b> depressed acquisition of avoidance response, increased seizure threshold, and increased exploratory activity in open field at <math>\geq 1</math> mg/kg-d</p>	NA	Develop: 1	0.63 (field exploration)	2.28 (field exploration)	Al-Hachim and Al-Baker (1973)  (Exact days of dosing during gestation were not provided. Pups were randomly selected for each dose group regardless of sex.)	Develop: [M1]
Rat, F344, 18-23 dams/group, dosed GD 6-19, gavage, pups sacrificed PND 6	0, 21, 28	<p><b>Measured:</b> maternal mortality, conception, maternal weight gain, pup weight, litter size, litter viability, clinical signs of toxicity</p> <p><b>Observed:</b> decreased maternal weight gain at <math>\geq 21</math> mg/kg-d, postnatal mortality at <math>\geq 21</math> mg/kg-d</p>	<p>Maternal: NA</p> <p>Develop: NA</p>	<p>Maternal: 21</p> <p>Develop: 21 (FEL)</p>	DU	DU	Narotsky and Kavlock (1995)  (Due to day of sacrifice lactational exposure cannot be excluded from consideration)	Develop: [N1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Rat, Sprague-Dawley, diet, 5 dams/group dosed GD 4-PND 21; pups culled to 5/sex/group and dosed PND 22-80, sacrificed PND 85	0, 0.1, 0.5, 5	<p><b>Measured:</b> maternal body weight, plasma, and milk metabolite levels; pup active testosterone levels, body weights, mating behaviors (male only), Cincinnati water maze, open field activity, auditory startle, neurological chloride-36 uptake (male 5 mg/kg-d only)</p> <p><b>Observed:</b> decreased testosterone levels in females at <math>\geq 0.5</math> mg/kg-d, <b>increased spatial ability and auditory startle at <math>\geq 0.1</math> mg/kg-d</b>, increased body weight at 0.5 mg/kg-d in females, increased mating behavior at 0.1 and 0.5 mg/kg-d with no difference at 5 mg/kg-d, decreased chloride-36 uptake through GABA<sub>A</sub>-mediated receptor complex at 5 mg/kg-d in males, in females reduced errors (<math>\geq 0.1</math> mg/kg-d) and time to escape in Cincinnati maze at 0.1 and 0.5 mg/kg-d</p>	Develop: NA	Develop: 0.1	DU	DU	<p>Cassidy et al. (1994)</p> <p>(Plasma and milk levels of metabolites measured in dams; plasma levels reported in offspring. Concentration of cyclodienes was gender and dose related.)</p>	Develop: [O1]

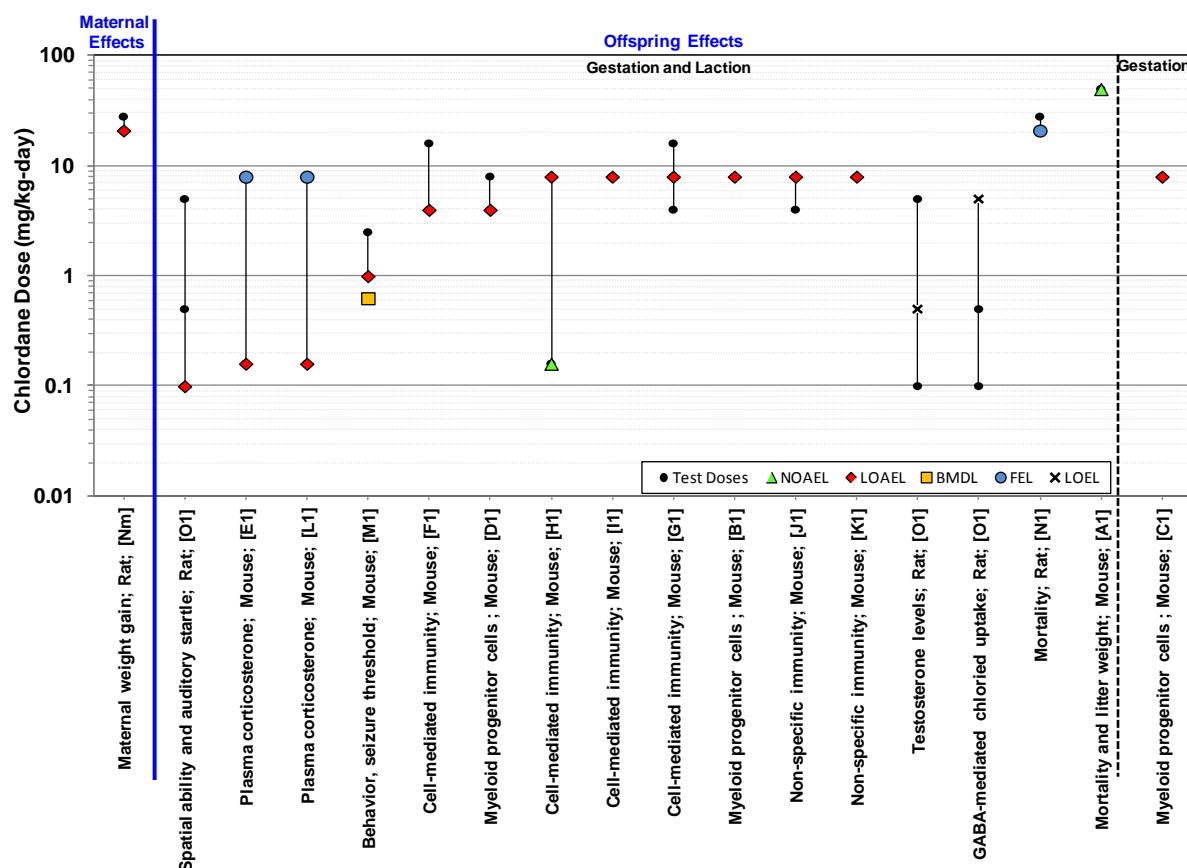
Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key <sup>b</sup>
Rat, Osborne-Mendel, diet, 1 female/group dosed 24 weeks or 48 weeks-lactation, litters culled to 3 pups/group	0, ≤ 0.5, 0.5-1, 1.5-3, 10-14, 18-30	<p><b>Measured:</b> pup body weight, growth and development</p> <p><b>Observed in dams exposed for 24 weeks pre-mating:</b> in pups, hyperexcitability and mild tremors at 10-14 mg/kg-d, marked tremors and mortality at 18-30 mg.kg-d</p> <p><b>Observed in dams exposed for 48 weeks pre-mating:</b> mortality in pups ≥ 10-14 mg/kg-d</p>	Develop: 1.5-3	Develop: 10-14	DU	DU	Ingle ( <a href="#">1952</a> )  (Doses are estimated from a figure showing doses pre-mating; small sample size.)	Uncertainty in the doses precludes inclusion in the exposure response array
<b>Lactational Exposure</b>								
Rat, Osborne-Mendel, diet, 1 female/group dosed lactation, litters culled to 3 pups/group	0, ≤ 0.5, 0.5-1, 1.5-3, 10-14, 18-30	<p><b>Measured:</b> pup body weight, growth and development</p> <p><b>Observed</b> pups from dams exposed for 24 or 48 weeks before mating showed: mortality ≥ 10-14 mg/kg-d</p>	Develop: 1.5-3	Develop: 10-14	DU	DU	Ingle ( <a href="#">1952</a> )  (Doses estimated from figure showing doses pre-mating. Specific endpoints observed not provided. Small sample size.)	Uncertainty in the doses precludes inclusion in the exposure response array

<sup>a</sup> Doses estimated using default body weight and food intake values ([U.S. EPA, 1988](#))

<sup>b</sup> The E-R Array Reference Key can be used to locate this study's element in [Figure B-1](#) and/or [Figure B-2](#).

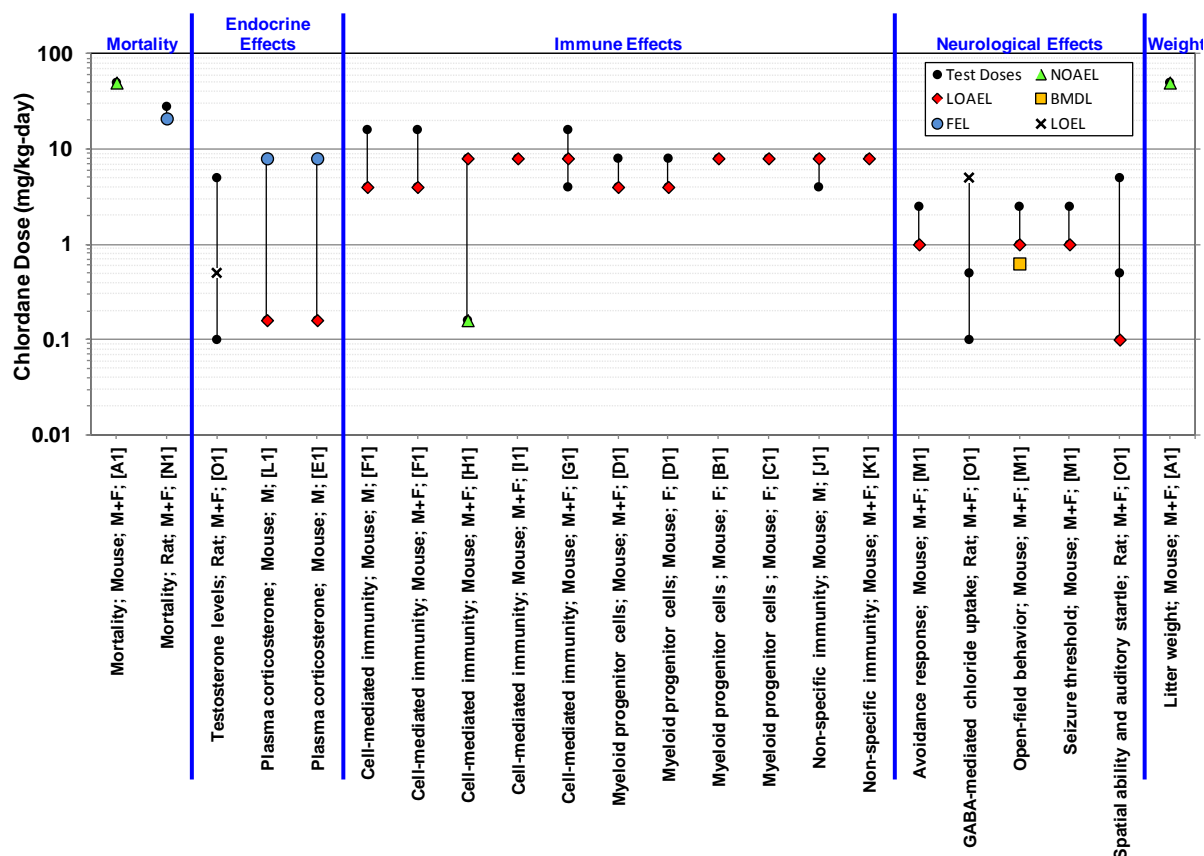
CFU = colony-forming unit; BMD = benchmark dose; BMDL = the 95% lower bound confidence limit on the BMD; DU = data unsuitable; GD = Gestational day; LOAEL = lowest observed adverse effect level; NA = not applicable; ND = no data; NOAEL = no observed adverse effect level; PND = Postnatal day

#### B.1.4.4. Exposure-Response Arrays for Developmental Effects



Note: Each array element represents the dose-response data for a unique study design. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. The changes in testosterone levels (indicative of endocrine effects) and GABA-mediated chloride uptake (indicative of neurological effects) were included on the array as LOELs to denote their significance despite the fact that they were observed at doses higher than the LOAEL. The endpoint “Behavior” includes depressed acquisition of avoidance response and increased exploratory activity in an open-field test. The endpoint “Cell-mediated immunity” includes one or more of the following endpoints: decreased delayed-type hypersensitivity response, mixed lymphocyte reactivity, and/or enhanced T-cell induced blastogenesis. The endpoint “Non-specific immunity” includes one or more of the following endpoints: decreased NK cell response and/or delayed tumoricidal induction activity associated with release of hydrogen peroxide. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. [Figure B-2](#) details the endocrine, immune, and neurological effects observed. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-4](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; BMDL = the 95% lower bound confidence limit on the BMD; FEL = frank effect level; LOEL = lowest observed effect level

**Figure B-1. Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to Chlordane**



Note: Each array element represents the dose-response data for a unique study design and endpoint. Elements are labeled with the adverse effect on which NOAEL and LOAEL decisions were based. The changes in testosterone levels (indicative of endocrine effects) and GABA-mediated chloride uptake (indicative of neurological effects) were included on the array as LOELs to denote their significance despite the fact that they were observed at doses higher than the LOAEL. The endpoint “Cell-mediated immunity” includes one or more of the following endpoints: decreased delayed-type hypersensitivity response, mixed lymphocyte reactivity, and/or enhanced T-cell induced blastogenesis. The endpoint “Non-specific immunity” includes one or more of the following endpoints: decreased NK cell response and/or delayed tumoricidal induction activity associated with release of hydrogen peroxide. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. Element labels also include the sex and test species in which the effect was observed and a Reference Key, which can be used to find detailed study information in [Table B-4](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; BMDL = the 95% lower bound confidence limit on the BMD; FEL = frank effect level; LOEL = lowest observed effect level

**Figure B-2. Exposure-Response Array Showing Developmental Effects in Animals after Gestational or Gestational and Lactational Exposure to Chlordane**

### B.1.5. Human Equivalent Dose (HED) Estimation

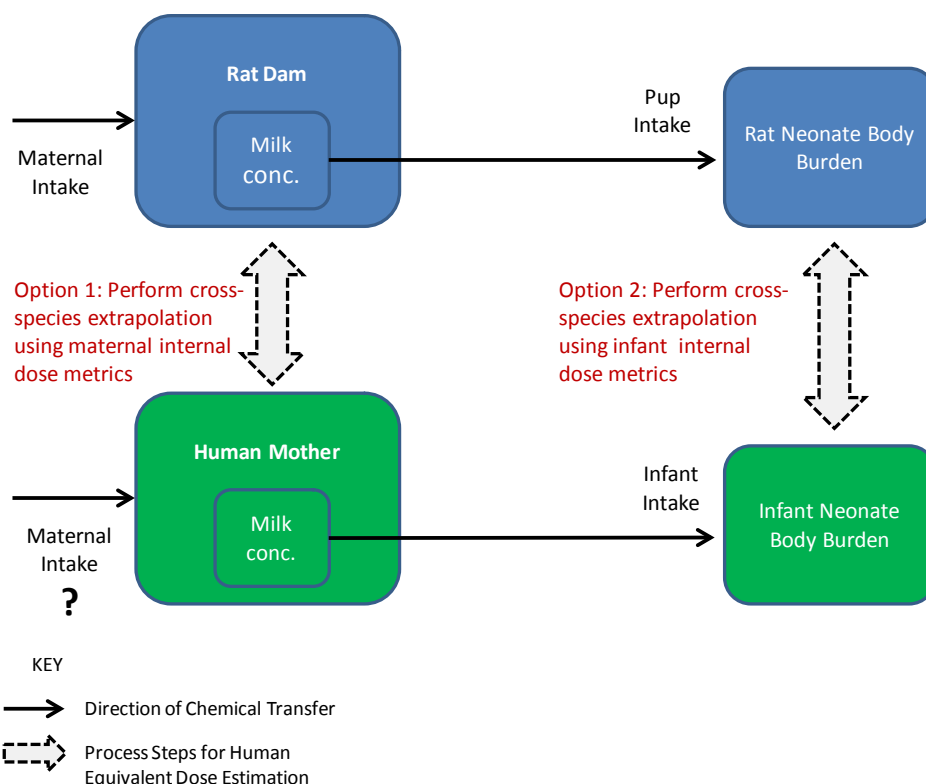
Once the POD has been selected, the next step in the reference value determination is estimation of the HED. This HED is intended to quantify the maternal continuous daily exposure in humans that may result

in an adverse developmental effect similar to that observed in animal offspring as described in [Section B.1.4](#).

The information provided in this section is designed to promote discussion amongst the workshop attendees as to how best to perform the HED estimation. In order to provide a concrete example to facilitate discussion, one method is chosen and carried through in this document to demonstrate an example calculation. This example is for illustrative purposes only and may not represent the conclusions of the workshop panel after discussion.

The HED can be estimated in a number of different ways. Generally speaking, an internal dose metric is selected for the human and the animal, and then these two metrics are assumed to be equal in magnitude. The decision must be made as to which internal dose metric is appropriate to serve as an indicator of the associated adverse effect.

For developmental effects resulting from lactational exposure, [Figure B-3](#) provides a diagram of the pathways that may be chosen to perform cross-species extrapolation to estimate the HED. The assumption is made that the maternal or infant animal intake is known, and the HED is the unknown human continuous daily exposure (depicted with a “?” in the figure). Because exposure occurs through the mother and then passes to the offspring by nursing, cross-species extrapolation may be performed by selecting either an internal maternal dose metric or an internal infant dose metric. Performing the extrapolation on internal maternal dose metrics will not account for species differences regarding infant feeding habits and infant kinetics, while performing the extrapolation on the infants requires more input data and more judgment about how to appropriately link very young members of both species. The overall choice will depend on judgment as to the reliability of the models available and the input data driving the models, balanced against the desire for increased biological accuracy.



**Figure B-3. Process diagram for performing cross-species extrapolation**

The main questions considered in derivation of an HED from an animal-derived POD are shown in the text box below ("Discussion Points for Chlordane HED Estimation"). This section discusses some of the chemical-specific properties that affect (1) the selection of the modeling method, (2) the kinetic information available for chlordane, and (3) the types of models available for assessing chlordane exposure in animals and humans. Summary statements for each of the discussion points are provided in the appropriate sections.

#### Discussion Points for Chlordane HED Estimation

- ? Chlordane is a mixture. How should the relationship between human and animal dose metrics for each constituent be effectively addressed?
- ? The constituents of chlordane are metabolized following exposure. How should differences in metabolism between animals and humans be effectively addressed?
- ? What pharmacokinetic data or relevant chemical-specific information are available for the chlordane constituents and metabolites in animals and humans to facilitate model implementation?
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ? What PBPK or other simpler biokinetic models are available for chlordane?
- ? **What is the best method for estimating the HED using the proposed POD?**



### B.1.5.1. Chemical-Specific Properties Affecting HED Estimation

Technical chlordane is a mixture composed of over 20 chemicals including trans-chlordane (25%), cis-chlordane (20%), heptachlor (10%), trans-nonachlor (7%), and cis-nonachlor (<5%) ([Asakawa et al., 1996](#)); however, not all experiments with technical chlordane contain this same ratio of components. In the proposed point of departure study ([Cassidy et al., 1994](#)), the administered chlordane is a defined mixture of components, although the study authors do not report the percentage of each component. In order to properly characterize the internal dose metrics in a mother or infant associated with a given adverse effect, the relative toxicity of the various components would require evaluation. In addition, it would have to be determined whether the kinetic properties of the different constituents are different between species to properly model the relationship between intake and internal dose metric. It has been shown that constituents of technical chlordane such as cis- and trans-chlordane can bind irreversibly with cellular macromolecules ([ATSDR, 1994](#)), and the rate and fraction of binding may affect downstream metabolism. Thus, because chlordane is a mixture, there is a greater burden on toxicity and pharmacokinetic data if a more sophisticated (e.g., pup-to-infant) cross-species extrapolation method is selected.

The constituents of chlordane may be metabolized to epoxides including heptachlor epoxide and oxychlordane. Because chlordane is metabolized and toxicity is related to the metabolites, modeling techniques applied to chlordane should account for metabolic rates and for excretion of the metabolites if possible. Sexual dimorphism in metabolic enzymes responsible for the metabolism of chlordane further complicates issues with potential toxicity in offspring ([Cassidy et al., 1994](#)). Additionally, metabolic enzymes in infants and pups may not be fully functional during early development, and infants and pups may express different metabolic enzymes than adult humans or animals, which may result in different metabolic rates and different metabolic products. Furthermore,

#### Chlordane is a Mixture

- ? How should the relationship between human and animal dose metrics for each constituent be effectively addressed?
- ✓ **If the cross-species extrapolation is performed on the infants, there is a greater burden on understanding the relative toxicity and kinetic properties of each constituent if more sophisticated HED methods are chosen.**

#### Chlordane Metabolism

- ? The constituents of chlordane are metabolized following exposure. How should differences in metabolism between animals and humans be effectively addressed?
- ✓ **If the cross-species extrapolation is performed on the infants, there is a greater burden on understanding the metabolism, excretion, and toxicity of each metabolite.**

human infants may differ from animal offspring in metabolic profile and activity. This puts a greater burden on toxicity and pharmacokinetic data if a more sophisticated (e.g., pup-to-infant) cross-species extrapolation method is selected.

#### **B.1.5.2. Available Pharmacokinetic Data for Chlordane**

Before discussing the potential modeling techniques for chlordane, the available database of pharmacokinetic data is summarized. This database should help to determine which modeling techniques may be most appropriate and subject to the least uncertainty.

Potential parameters that may be useful in estimating HED by a variety of methods are presented in [Table B-5](#). A discussion of the parameters and their sources follows the table. The various applications and uses of these parameters are discussed in the context of different modeling techniques in [Section B.1.5.4](#).

##### **Pharmacokinetic Data**

- ? What pharmacokinetic data or relevant chemical-specific information are available for the chlordane constituents and metabolites in animals and humans to facilitate model implementation?
- ✓ **Octanol-water partition coefficient,  $K_{ow}$**
- ✓ **Human and rat half-lives in plasma**
- ✓ **Absorption fractions and fraction of chemical stored in fat (assumptions can be made based on other PBT chemicals)**
- × **No available partition coefficients in humans**
- × **No available metabolism rates**

**Table B-5. Available Pharmacokinetic Parameters for Chlordane in Humans and Rats**

Parameter (units)	Variable	Value	Note/source
Chemical-Specific Only			
Octanol-water partition coefficient	log(K <sub>ow</sub> )	5.54 (reported log(K <sub>ow</sub> ) values for chlordane constituents ranged from 5.44–6.35)	ATSDR ( <a href="#">1994</a> ); Simpson et al. ( <a href="#">1995</a> )
Human Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	U.S. EPA ( <a href="#">1998</a> ) (default)
Biological elimination constant for chlordane (day <sup>-1</sup> )	k <sub>elim</sub>	0.0079	Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans for chlordane (days)	t <sub>1/2</sub>	88	Aldrich and Holmes ( <a href="#">1969</a> )
Partition coefficients- in different human body compartments for the chlordane constituents	None identified in the literature		
Metabolic rates for chlordane constituents	None identified in the literature		
Animal Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the rat pup (dimensionless)	f <sub>ai</sub>	0.8	Assumed
Fraction of ingested contaminant that is absorbed by the rat dam (dimensionless)	f <sub>am</sub>	0.8	Ohno et al. ( <a href="#">1986</a> ); value for gamma-chlordane (not technical chlordane)
Fraction of contaminant that is stored in rat dam/maternal fat (dimensionless)	f <sub>f</sub>	0.2	Calculated from data in Ohno et al. ( <a href="#">1986</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.013	Estimated as ln(2) / t <sub>1/2</sub>
Half-life of contaminant in rats (days)	t <sub>1/2</sub>	5.92–54.1 (range of half-lives of chlordane metabolites in fat)	Dearth and Hites ( <a href="#">1991b</a> )
Partition coefficients in different rat compartments for the chlordane constituents	Can be estimated from K <sub>ow</sub>		
Metabolic rates for chlordane constituents	None identified in the literature		

ATSDR ([1994](#)) used EPA's Graphical Exposure Modeling System (GEMS) computer program to estimate the octanol-water partition coefficient ( $K_{ow}$ ) of technical chlordane based on the chemical structure and no empirical data ( $\log K_{ow} = 5.54$ ). Simpson et al. ([1995](#)) used the slow-stirring method by De Bruijn et al. ([1989](#)) to determine  $\log K_{ow}$  for 13 major components of technical chlordane ( $\log K_{ow} = 5.44 - 6.35$ ).

In the absence of specific information on chlordane in humans, the input values presented here were patterned after those in the EPA's Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions ([U.S. EPA, 1998](#)). Chapter 9 of this methodology (hereafter "Combustor Assessment") presents a framework for evaluating exposure to persistent lipophilic contaminants in breast milk. Thus, the assumptions made in that document are applied to chlordane when values specific to chlordane could not be located. The **maternal absorption fraction** for chlordane was not available in the reviewed literature. An absorption fraction value of 1 is used in the current calculations to serve as the most conservative estimate, assuming complete absorption of the chemical ([U.S. EPA, 1998](#)). In the absence of information about infant absorption, an absorption fraction of 1 was applied to infants as well. Quantitative information about the **fraction of chlordane in the body that is stored in fat** also could not be found in the literature. The reviewed literature generally described chlordane as highly lipophilic. The Combustor Assessment states that for highly lipophilic compounds, >90% of the compound may be stored in the fat ([U.S. EPA, 1998](#)). In the absence of more specific information, this parameter was set to 0.9 in the example calculations.

Chlordane is a mixture, which complicates the estimation of **half-life**. Different components of the mixture are eliminated at different rates. In humans, the elimination of chlordane was determined to be biphasic, with constituents being eliminated at two distinct rates. The human half-life in plasma has been reported for the second phase as 88 days ([Aldrich and Holmes, 1969](#)), 21 days ([Curley and Garrettson, 1969](#)), and 34 days ([Olanoff et al., 1983](#)); the first phase was shorter and ranged from seven to 16 hours. No studies in the available literature quantified the half-life of chlordane in human fat or the whole body. Assuming that technical chlordane has a longer half-life in fat compared to plasma, using the highest reported half-life value of 88 days is the best available approximation of the full-body elimination rate. The half-life value is based on studies conducted after accidental exposure to an unknown amount of chlordane in a 4-year old child. The estimation of this parameter is made more complicated by the fact that chlordane is metabolized following exposure; thus, further data on half-life, especially in adipose tissue, would be helpful for conducting a chlordane assessment.

Animal parameters were determined from values found in the literature where available. The search focused on rats, since rats are the species studied in the candidate POD study ([Cassidy et al., 1994](#)). Studies on absorption of technical chlordane were not identified, but a study by Ohno et al. ([1986](#)) determined 80% absorption of gamma-chlordane in male Wistar rats following oral administration of either 0.05 mg/kg or 10 mg/kg gamma-chlordane. Therefore, 0.8 is used as the **maternal absorption fraction**. No absorption fractions could be found in the literature for rat pups. Thus, the value was set equal to the maternal absorption fraction.

To determine the **fraction of chlordane stored in fat**, calculations were performed based on data from the Ohno et al. (1986) study. Because low-level exposures are of interest, example calculations for the fraction of chlordane in fat will use the low-dose value of 0.05 mg/kg. In the experiment, animal weights ranged from 300 to 350 grams, and the concentration of chlordane in fat peaked at 4 days at a level of 93.5 ng/g. Assuming a fat volume fraction of 0.09 (Fisher et al., 1990) and an absorption fraction of 0.8 (Ohno et al., 1986), the fraction of chlordane stored in fat was then calculated as:

$$f_f = \frac{\text{mass of chlordane in fat compartment}}{\text{mass of chlordane in oral dose}} = \frac{C_{\text{FAT}} \times \text{BW} \times f_{\text{fm}}}{\text{DI} \times \text{BW} \times f_{\text{am}}}$$

Appendix Equation 1

Where:

- $f_f$  = the fraction of contaminant that is stored in maternal fat (dimensionless),
- $C_{\text{FAT}}$  = the measured concentration of contaminant in the fat (mg contaminant per kg fat),
- $\text{BW}$  = the animal body weight (kg),
- $f_{\text{fm}}$  = fraction of the mother's weight that is fat (kg fat/kg BW),
- $\text{DI}$  = the applied dose (mg contaminant/kg BW), and
- $f_{\text{am}}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless).

Using values reported above in Table B-5 and in Table B-8; this calculation for this study yields:

$$f_f = \frac{0.0935 \frac{\text{mg}}{\text{kg}} \times 0.325 \text{ kg} \times 0.09}{0.05 \frac{\text{mg}}{\text{kg}} \times 0.325 \text{ kg} \times 0.8} = 0.2$$

**Half-life** data for technical chlordane were not identified in the literature, but information is available on the half-lives of its metabolites. Dearth and Hites (1991b) examined the half-lives of 14 chlordane compounds in the fat of Holtzman rats fed technical chlordane (10 ppm) in diet for 28 days. Half-lives ranged from 5.92 days (alpha-chlordane) to 54.1 days (nonachloro III). The longest half-life available (54.1 days) is used as a surrogate to estimate half-life of technical chlordane.

**Partition coefficients** for use in PBPK models could not be found for rats or humans. However, a paper by Poulin and Krishnan (1995) describe an algorithm for predicting rat tissue:blood partition coefficients based on  $K_{ow}$ . The algorithm accounts for chemical partitioning including phospholipids in tissues and blood, with erythrocytes and plasma considered separately. However, the estimates for tissue:blood partition coefficients were validated using hydrophilic organic compounds and may not be an appropriate representation of lipophilic compounds. No similar algorithm is available for humans.

### B.1.5.3. Appropriate Dose Metric for Selected Point of Departure (POD)

Developmental effects may be associated with a critical exposure window during which exposure to chlordane will result in the adverse effect. Metrics can be selected to look at either peak or average maternal or infant exposure, depending on the effect in question and the dosing protocol in the animal study. For developmental effects, if a critical window can be determined, then the model solutions during the critical window can be used for the cross-species extrapolation; otherwise, peak or average concentrations from different exposure metrics (see below) can be used based on the judgment of the assessor as to what is most appropriate. For the proposed POD presented here, no critical window could be determined. Thus, possible dose metrics for specified lactation durations include:

- The average maternal body burden,
- The peak maternal body burden,
- The average maternal fat concentration,
- The peak maternal fat concentration,
- The average milk concentration,
- The peak milk concentration,
- The average infant body burden, and
- The peak infant body burden.

#### Dose Metric

- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ✓ **Peak or average concentrations from maternal or infant dose metrics**
- × **The critical window could not be defined**

### B.1.5.4. Available Models for Estimating Chlordane Internal Dose

The following section discusses potential models that can be used to estimate internal dose metrics based on maternal intake. The section starts with the most biologically-robust class of models, physiologically-based pharmacokinetic (PBPK) models. The section then discusses simpler first-order kinetic modeling techniques and a biotransfer method for estimating maternal exposure. Thus, the section generally progresses from the most biologically robust to the more simple models. Where possible, the parameters needed for each technique have been collected and presented. The final choice in technique depends on weighing both the biological sophistication of the method and the uncertainty in the model parameters, as discussed in [Section B.1.6](#).

#### *Multi-Compartment PBPK Models*

Generally speaking, the most biologically complete kinetic model for a given chemical is a multi-compartment, data-validated, PBPK model. However, no such model is available for chlordane in rats or

humans, either with or without a lactation component. Thus, this section discusses other models that could be applied to chlordane.

### Human Models

Byczkowski and Lipscomb (2001) developed a human PBPK model to simulate neonatal exposure to methyl mercury; Ayotte et al. (1996), Gentry et al. (2003), and Kreuzer et al. (1997) developed lactation models for 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD); Redding et al. (2008) constructed a lactational model for PCB-153 and validated the predictions against human biomonitoring data, and Byczkowski et al. (1994) developed a lactational model for tetrachloroethylene.

These models could potentially be adapted to chlordane using chlordane-specific kinetic parameters. However, as stated above, limited information is available on the kinetic properties of chlordane and its metabolites in humans. There is no information about the partition coefficients in different compartments, the permeability area cross products in the gastric and mammary tissues, or the elimination rates that would be needed for these models.

One group in Canada has developed a generic maternal-infant multi-compartment model for persistent organic pollutants (POPs) (Verner et al., 2009; Verner et al., 2008). The primary utility of this model for chlordane is that the chemical-specific parameters can be estimated using only the half-life and octanol-water partition coefficient ( $K_{ow}$ ) of the compound.

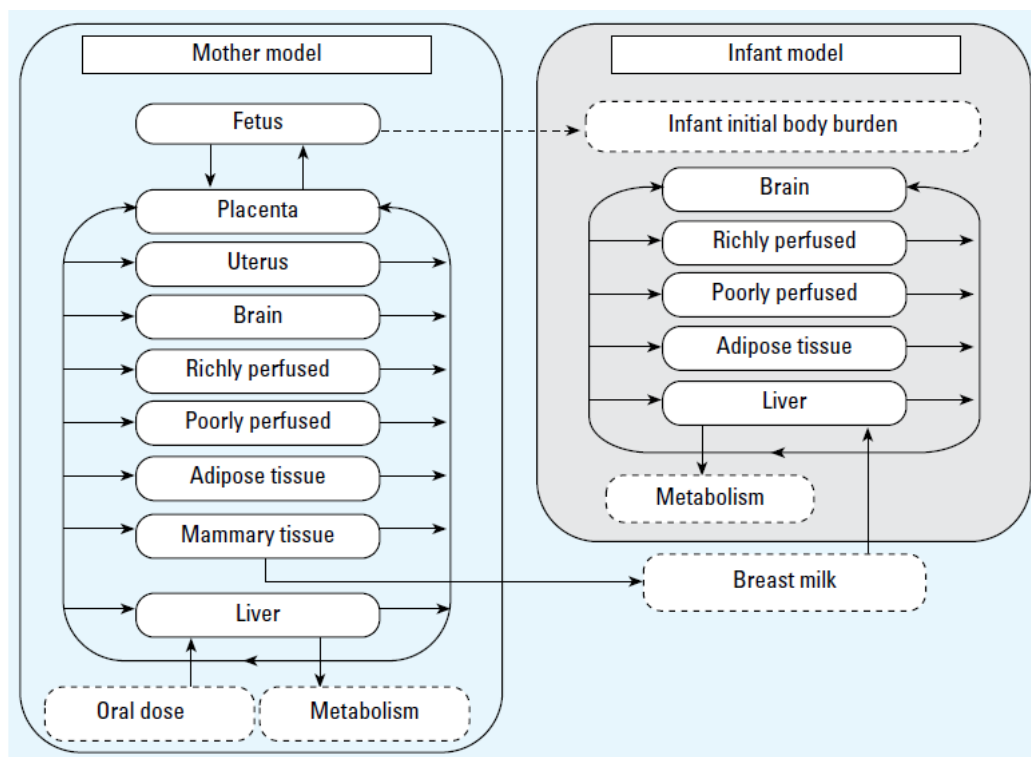
The full maternal and infant model (Verner et al., 2009) is intended to simulate the entire life-cycle of a woman up to age 55 years and includes nine maternal tissue compartments and five infant tissue compartments, as shown in Figure B-4.

The oral dose is modeled as being directly absorbed into the liver and assumed to be fully bioavailable. First-order hepatic metabolism is included and is intended to represent all excretion in the absence of breastfeeding or childbirth. The elimination rate constant is determined for the chemical from the adult whole-body half-life. The concentrations in each

compartment are determined using partition coefficients, and the chemical-specific partition coefficients are estimated using an equation based on  $K_{ow}$  (Poulin and Krishnan, 1996) and using the fraction of blood and tissue that are lipid and water (Price et al., 2003). Physiological parameters such as body weight and fat volume fraction are assumed to vary in time to capture changes over the life of the woman.

#### Available Models

- ? What PBPK or other simpler biokinetic models are available for chlordane?
- × **No chlordane-specific human or rat PBPK model available (existing models could be modified for chlordane if appropriate model parameters are identified)**
- ✓ **Simple first order models are available**
- ✓ **Simple biotransfer methods are available**



**Figure B-4. Maternal and Infant POP Model (Verner et al., 2009)**

Although chlordane was not included, the model has been validated against human cord blood, breast milk, and infant blood concentrations for seven POPs (*p,p'*-DDE, *p,p'*-DDT, HDB,  $\beta$ -HCH, PCB-138, PCB-153, and PCB-180). The computer code was not provided in the publication, although a number of details about the parameters and equations are included in the main papers and in supplemental information. Considerable time would be needed in order to further evaluate the potential for this model to be used for chlordane, including model implementation and manipulation in modeling software.

### Animal Models

The candidate POD study by Cassidy et al. (1994) investigates developmental effects in rats, so rat models are the focus of this section. Although no chlordane-specific lactation PBPK model currently exists, there are several lactation models for rats for exposure to other chemicals. Fisher et al. (1990) constructed a lactation model for trichloroethylene while Clewell et al. (2003) constructed a lactation model for iodide and perchlorate. The lactation components of these models could potentially serve as a guide for developing a chlordane animal model, although the chemicals modeled in these models are not lipophilic and the model might have to be adapted to account for fat storage or other chemical-specific differences. The necessary chlordane-specific parameters would include mammary partition coefficients and permeability cross products, the clearance rate from the mother to the infant, and



partition coefficients in the infant. It may be possible to determine partition coefficients in different compartments for rat models. A paper by Poulin and Krishnan ([1995](#)) describe an algorithm for predicting rat tissue:blood partition coefficients based on  $K_{ow}$ . The algorithm accounts for chemical partitioning including phospholipids in tissues and blood, with erythrocytes and plasma considered separately. However, the estimates for tissue:blood partition coefficients were validated using hydrophilic organic compounds and may not be an appropriate representation of lipophilic compounds.

### ***First-Order Steady-State Single-Compartment Model*** ([U.S. EPA, 1998](#))

A first-order single-compartment model is an intermediate step between a full, multi-compartment PBPK model and simpler dosimetric conversions or biotransfer models. The model presented here is patterned after the first-order models presented in the Combustor Assessment ([U.S. EPA, 1998](#)) and the dioxin reassessment ([U.S. EPA, 2012b](#)) and is adapted to fully incorporate time-dependence during lactation. The general model equations are presented first, and then the model is applied to both humans and rats in the next section. Rats are the focus here since that is the species in the candidate POD study. Because the equations are applied for rats and humans as an example in this briefing packet, they are presented in greater detail than the equations in the PBPK models discussed in [Section B.2.5.4](#). However, this is not meant to imply that the first-order models are superior for chlordane.

The single body compartment represented by the model is generally defined as “body burden,” or the total average concentration of contaminant in the body. In a first-order model, the elimination from the body is represented as a rate constant multiplied by the total body burden. This rate constant can be estimated from the whole-body half-life, which is often readily available in the literature. The input to the body is the dose, assumed to be normalized by the total body weight. Thus, the simple model can be represented by the differential equation<sup>6</sup>:

$$\frac{\partial BB(t)}{\partial t} = f_{am} DI_{mat}(t) - k_{elim} \times BB(t)$$

Appendix Equation 2

Where:

- $BB(t)$  = the time-dependent total body burden (ng/kg),
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{mat}(t)$  = the time-dependent maternal dose (ng/kg-day), and
- $f_{am}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless).

This equation can be converted to a difference equation and iterated in time:

<sup>6</sup> Strictly speaking, this equation is only correct if the maternal body weight is not changing in time. However, this approximation is sufficient given the overall uncertainty in using a first-order model.

$$BB_{t+\Delta t} = BB_t + \Delta t(f_{am} DI_{mat,t} - k_{elim} \times BB_t)$$

## Appendix Equation 3

Where:

$BB_{t+\Delta t}$	=	the total body burden at the next time step (ng/kg),
$BB_t$	=	the total body burden at the current time step (ng/kg),
$k_{elim}$	=	the first order elimination rate ( $\text{days}^{-1}$ ) = $\ln(2)/\text{half-life (days)}$ ,
$DI_{mat,t}$	=	the maternal dose at the current time step (ng/kg-day),
$f_{am}$	=	the fraction of ingested contaminant absorbed by the mother (dimensionless), and
$\Delta t$	=	the time step (days).

This equation will approximate the solution to the differential equation. To help eliminate errors introduced by converting the differential equation to an algebraic equation, the time step ( $\Delta t$ ) should be much smaller than the elimination time scale.

This equation allows approximation of the maternal body burden; however, additional assumptions can be made to estimate the time dependent infant intake from breast milk. First, the concentration in the maternal milk fat is assumed to be equal to the concentration in the maternal fat and can be estimated as:

$$C_{milk\ fat,t} = \frac{BB_t \times f_f}{f_{fm}}$$

## Appendix Equation 4

Where:

$C_{milkfat,t}$	=	the concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
$f_f$	=	the fraction of contaminant that is stored in maternal fat (dimensionless), and
$f_{fm}$	=	the fraction of the mother's weight that is fat (kg fat/kg BW).

Although nonpolar compounds partition to some degree into the aqueous compartment, the most commonly analyzed organic contaminants, including chlordane, are highly concentrated in the lipid phase; the concentration of chlordane in breast milk is not expected to be significantly underestimated by ignoring the aqueous phase.

To account for the fact that lactation acts as an additional removal mechanism from the mother, the elimination rate during lactation is increased to equal:

$$k_{elac} = k_{elim} + \frac{CR_{milk,t} \times f_f \times f_{mbm}}{f_{fm} \times BW_{Mat}}$$

Appendix Equation 5

Where:

- $k_{elac}$  = the maternal elimination rate during lactation (days<sup>-1</sup>),
- $k_{elim}$  = the first order elimination rate (days<sup>-1</sup>) = ln(2)/half-life (days),
- $CR_{milk,t}$  = the time-dependent infant ingestion rate of milk (kg/day),
- $f_f$  = the fraction of contaminant that is stored in maternal fat (dimensionless)
- $f_{mbm}$  = the fraction of fat in milk (dimensionless),
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW), and
- $BW_{Mat}$  = the maternal body weight (kg).

Then, the time-dependent infant intake can be estimated as:

$$DI_{INF,t} = \frac{(C_{milk\ fat,t} \times f_{mbm}) \times CR_{milk,t}}{BW_{INF,t}}$$

Appendix Equation 6

Where:

- $DI_{INF,t}$  = the time-dependent infant daily ingestion (ng/kg-day),
- $C_{milk\ fat,t}$  = the time-dependent concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_{mbm}$  = the fraction of fat in milk (dimensionless),
- $CR_{milk,t}$  = the time-dependent infant ingestion rate of milk (kg/day), and
- $BW_{INF,t}$  = the time-dependent infant's body weight (kg).

The time-dependent body weights and ingestion rates are incorporated in the models by using different point estimates over the course of the simulation (see the [Biotransfer Method](#) section). Finally, if the infant is modeled using a first-order model as well, the infant body burden can be estimated as:

$$BB_{INF,t+\Delta t} = BB_{INF,t} + \Delta t (f_{ai} DI_{INF,t} - k_{elim,INF} \times BB_{INF,t})$$

Appendix Equation 7

Where:

$BB_{INF,t+\Delta t}$	=	the infant total body burden at the next time step (ng/kg),
$BB_{INF,t}$	=	the infant total body burden at the current time step (ng/kg),
$k_{elim,INF}$	=	the first order elimination rate in the infant ( $\text{days}^{-1}$ ) = $\ln(2)/\text{half-life (days)}$ ,
$DI_{INF,t}$	=	the infant dose at the current time step (ng/kg-day),
$f_{ai}$	=	the fraction of ingested contaminant absorbed by the infant (dimensionless), and
$\Delta t$	=	the time step (days).

The above equations approximate the maternal body burden and exposure to a lactating infant. The model does not, however, include any fetal exposure *in utero*.

This model is generic and not species specific. For purposes of illustration, the model was implemented in Excel and time integrated through the life of the mother. Then, the time-dependent solution can be used to estimate the internal dose metrics in [Section B.1.6](#).

The above model was parameterized so that the maternal dose, the infant body weight, and the infant ingestion rate all vary with time. In the case of the maternal dose, this allows the model to follow dosing protocols from animal studies. In the case of the body weight and ingestion rate, this allows the model to more accurately simulate effects on infants during very early exposure when body weight is changing rapidly and when critical windows for developmental effects may occur.

### ***Input Parameters and Application to Chlordane in Humans***

For humans, the model was implemented using a time step of 1 week. This represents a balance between a time step short enough to capture changes in the infant during lactation and long enough so that data are not applied beyond their measurement range. Since the Exposure Factors Handbook ([U.S. EPA, 2011a](#)) provides infant estimates on a monthly basis (see below), values across weeks within a single month were kept equal.

The input parameters used for chlordane for humans are shown in [Table B-6](#). In addition to using mid-range (i.e., mean) values in the model, low and high end estimates were also used for some parameters, where available, to determine the approximate range of human variability. Values for the parameters that are not chemical-specific were retained from the Combustor Assessment ([U.S. EPA, 1998](#)) or taken from the Exposure Factors Handbook ([U.S. EPA, 2011a](#)). Chlordane-specific values are the same as those presented and discussed in [Section B.1.5.2](#).

**Table B-6. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model**

Parameter (units)	Variable	Low	Mid	High	Note/source
Human Parameters (not chemical-specific)					
Maternal age at pregnancy (years)	Age	18	25	40	Assumptions
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 1 (kg)	BW <sub>INF,t</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 2-3 (kg)		5.9			
Infant body weight, Month 4-6 (kg)		7.4			
Infant body weight, Month 7-12 (kg)		9.2			
Infant ingestion rate, Month 1 (kg milk/day)	CR <sub>milk,t</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, Month 2-3 (kg milk/day)		0.69			
Infant ingestion rate, Month 4-6 (kg milk/day)		0.77			
Infant ingestion rate, Month 7-12 (kg milk/day)		0.62			
Fraction of mother’s weight that is fat (kg maternal fat/kg BW) <sup>b</sup>	f <sub>fm</sub>	0.2	0.3	0.4	Timson and Coffman ( <a href="#">1984</a> ); U.S. EPA ( <a href="#">1998</a> )
Fraction of fat in breast milk (dimensionless) <sup>c</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Human Chemical-Specific Parameters					
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1			U.S. EPA ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	1			U.S. EPA ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9			U.S. EPA ( <a href="#">1998</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.0079			Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans (days)	t <sub>1/2</sub>	88			Aldrich and Holmes ( <a href="#">1969</a> )

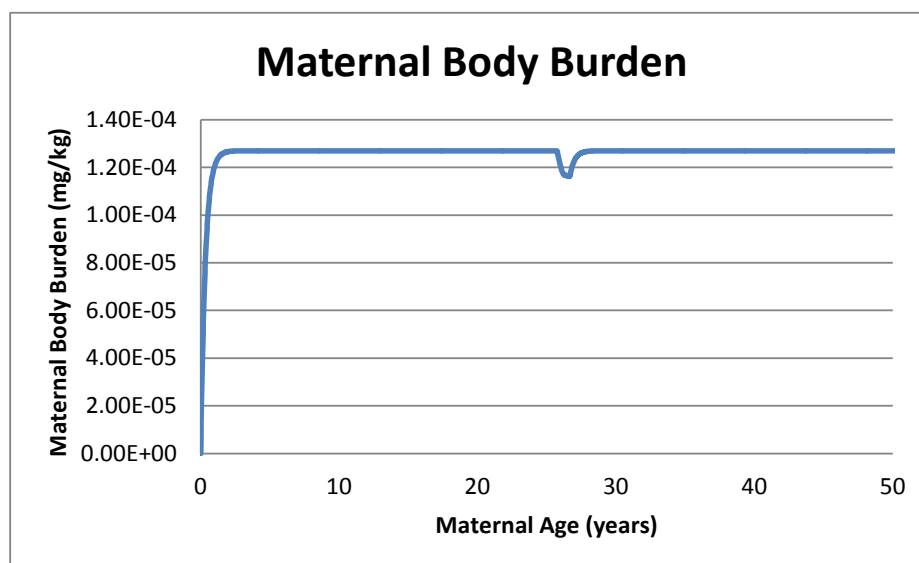
<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents mean ± standard deviation.

<sup>c</sup> Represents the typical range and midpoint of the range.

An example figure showing the maternal body burden assuming pregnancy at age 25 years and a daily intake of 1 ng/kg-day is shown in [Figure B-5](#). Because the model is linear with respect to dose, this intake level is chosen for illustrative purposes and the body burden would scale linearly with any change in intake. The dip at age 25 years and 9 months is due to the additional elimination during lactation.

One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, assuming the “mid” parameters and pregnancy at age 25 years, the average infant to maternal dose ratio was estimated to be 1.2 (1 month lactation duration) and 0.9 (12 month lactation duration). The overall range of the ratio, accounting for human variability and including the peak estimates, was from 0.5 to 2.6 (see [Table B-7](#)).



**Figure B-5. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day**

**Table B-7. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model**

Infant Nursing Duration	Pregnancy at 18 years; Low parameters	Pregnancy at 25 years; Mid parameters	Pregnancy at 40 years; High parameters
1 month, Peak Infant Intake	0.6	1.2	2.4
1 month, Average Infant Intake	0.6	1.2	2.4
12 months, Peak Infant Intake	0.7	1.3	2.6
12 months, Average Infant Intake	0.5	0.9	1.8

### *Input Parameters and Application to Chlordane in Rats*

For rats, the model was implemented using a time step of one half a day (one week was used for humans). This was deemed sufficient to resolve changes during the typical three-week lactation period. The input parameters used for chlordane are shown in [Table B-8](#). The age of the rats at the beginning of gestation was not reported in the candidate POD study, so the age at which rats become sexually mature (5 weeks) was assumed for this analysis. In addition, a gestation time of 3 weeks and lactation duration of 3 weeks were assumed for the analysis as representative typical values. A litter size

parameter was added so that the nursing rat fed 10 pups at a time, rather than the single infant assumed in the human model. This litter size increased the maternal lactational elimination rate and matches the average litter size in the POD study

The physiological parameters needed for the model were not presented in the candidate POD study. Knight et al. ([1984](#)) reported the suckling rate in rat litters was assumed to be equal to the milk production in the dam. Experimentally determined milk production values of 0.0009, 0.0016, 0.0018, and 0.0016 liters/hr were measured on lactation days 3, 10, 17, and 23, respectively ([Knight et al., 1984](#)). The values for day 3, 10, and 17 were used for weeks 1, 2, and 3, respectively, assuming a milk density of 1 kg/liter and dividing by the total number of pups to get a per pup ingestion rate. Knight et al. ([1984](#)) also reported average pup body weights corresponding to these suckling rates of 8.5, 21, and 31 grams for weeks 1, 2, and 3, respectively.

Knight et al. ([1984](#)) reported the weight of lactating Wistar dams at day 2, 7, 14, and 21. The average across these days for the control group, 273 g, was used in the model. Fisher et al. ([1990](#)) reported fat as a percentage of body weight to be 6.0 to 12.0% in lactating F344 dams. A midpoint of 9.0% was used for this application. The fraction of fat in milk was reported to be 15.0% in Fisher et al. ([1990](#)), compared to a value of 4% in humans ([Welch and Findlay, 1981](#)).

The same chemical-specific values as presented in [Section B.1.5.2](#) were used: maternal absorption fraction, the fraction of contaminant stored in the fat relative to the total body burden, and the half-life.

**Table B-8. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat**

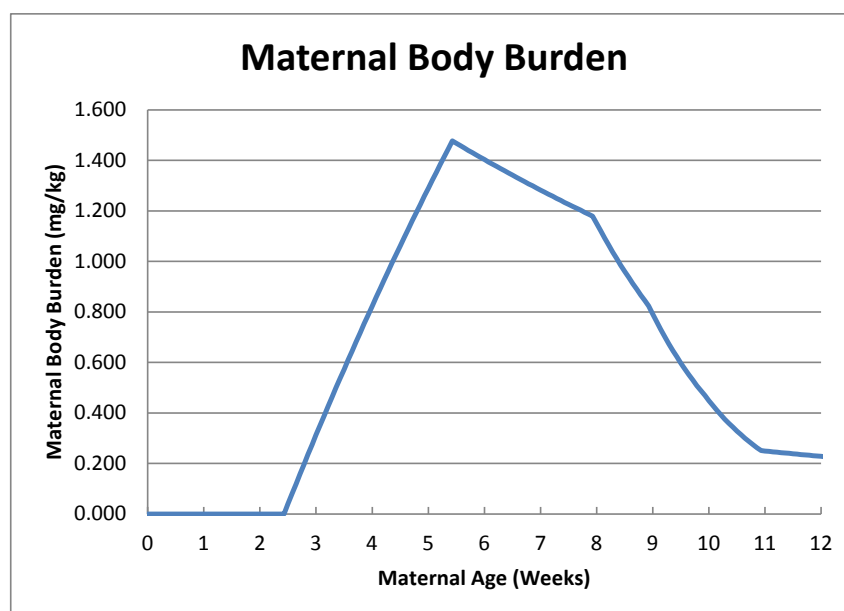
Parameter (units)	Variable	Estimated Value	Note/source
<b>Rat Parameters (not chemical-specific)</b>			
Dam age at pregnancy (weeks)	Age	5	Assumption
Dam weight during lactation (kg)	BW <sub>Mat</sub>	0.273	Knight et al. (1984)
Litter size (number of pups)	Litter	10	Fisher et al. (1990)
Pup body weight, Week 1 (kg)	BW <sub>INF,t</sub>	0.0085	Knight et al. (1984),
Pup body weight, Week 2 (kg)		0.021	
Pup body weight, Week 3 (kg)		0.031	
Pup ingestion rate, Week 1 (kg milk/day)	CR <sub>milk,t</sub>	0.0031	Knight et al. (1984), adjusted for litter size
Pup ingestion rate, Week 2 (kg milk/day)		0.0054	
Pup ingestion rate, Week 3 (kg milk/day)		0.0061	
Fraction of dam's weight that is fat (kg maternal fat/kg BW)	f <sub>fm</sub>	0.09	Fisher et al. (1990)
Fraction of fat in maternal milk (dimensionless)	f <sub>mbm</sub>	0.15	Welch and Findlay (1981)
<b>Rat Chemical-Specific Parameters</b>			
Fraction of ingested contaminant that is absorbed by the pup (dimensionless)	f <sub>ai</sub>	0.8	Assumed
Fraction of ingested contaminant that is absorbed by the dam (dimensionless)	f <sub>am</sub>	0.8	Ohno et al. (1986); value for gamma-chlordane (not technical chlordane)
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.2	Calculated from data in Ohno et al. (1986)
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.013	Estimated as ln(2) / t <sub>1/2</sub>
Half-life in rats (days)	t <sub>1/2</sub>	5.92–54.1 (range of half-lives of chlordane metabolites in fat)	Dearth and Hites (1991b)

The dosing protocol in the POD study (Cassidy et al., 1994) was applied to this rat model. In the study, the dams were dosed daily from gestation day 4 until lactation day 21. To implement this in the current model with the weekly time step, dosing was assumed to occur daily throughout gestation and lactation (gestation day 1 to lactation day 21). The model is able to capture transfer to the nursing pup during lactation, but it does not model any infant body burden during gestation.

An example figure showing the maternal body burden using the candidate POD dose (0.1 mg/kg-day) is shown in Figure B-6. One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, the average infant to maternal



dose ratio was estimated to be 1.2 (1 week lactation duration) and 0.6 (3 week lactation duration, see [Table B-9](#)).



**Figure B-6. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study ([Cassidy et al., 1994](#)) and an Administered Dose of 0.1 mg/kg-day (POD)**

**Table B-9. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model**

Pup Nursing Duration	Ratio
1 week, Peak Pup Intake	1.4
1 week, Average Pup Intake	1.2
3 weeks, Peak Pup Intake	1.4
3 weeks, Average Pup Intake	0.6

### ***Biotransfer Method***

The simplest technique for linking maternal and infant intake involves estimating the transfer of chlordane to milk using a biotransfer factor. This method is discussed in the Combustor Assessment ([U.S. EPA, 1998](#)). The technique is based on a study by Travis and Arms ([1988](#)) and assumes that the milk fat contaminant concentration is proportional to maternal contaminant intake, with the proportionality constant represented by a biotransfer factor:

$$C_{\text{milk fat}} = DI_{\text{MAT}} \times BTF_m \times BW_{\text{Mat}}$$

Appendix Equation 8

Where:

- $C_{\text{milk fat}}$  = the concentration of contaminant in the maternal milk fat (ng/kg milk fat),  
 $DI_{\text{MAT}}$  = daily maternal intake of contaminant (ng/kg BW-day),  
 $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and  
 $BW_{\text{Mat}}$  = the maternal body weight (kg).

The biotransfer factor is then estimated using a regression equation based on the octanol-water partition coefficient ( $K_{ow}$ ) using the expression:

$$BTF_m = 0.00062 \times K_{ow}$$

Appendix Equation 9

Where:

- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and  
 $K_{ow}$  = the octanol-water partition coefficient (unitless).

The regression equation was estimated using six highly lipophilic compounds with  $\log(K_{ow})$  in the range of 5.16 to 6.5. According to the Combustor Assessment ([U.S. EPA, 1998](#)), the model tends to over-predict concentrations. For example, for dioxin, the equation predicts milk fat concentrations that are ten times higher than those actually measured in the United States when assuming background maternal intake values. Thus, the Combustor Assessment suggests the equation should only be used if parameters for more sophisticated kinetic models cannot be found in the literature and if the  $K_{ow}$  of the chemical in question is within the range of the  $K_{ow}$  used to fit the model. Because the pharmacokinetic data for chlordane are generally sparse and the half-lives are relatively uncertain, this method is included for consideration.

The daily infant contaminant dose can then be estimated for the biotransfer method using the equation:

$$DI_{\text{INF}} = \frac{(C_{\text{milk fat}} \times f_{\text{mbm}}) \times CR_{\text{milk}} \times ED_{\text{INF}}}{BW_{\text{INF}} \times AT}$$

Appendix Equation 10

Where:

$DI_{INF}$	=	the daily infant contaminant dose (ng/kg-day),
$C_{milkfat}$	=	the concentration of contaminant in the maternal milk fat (ng/kg milk fat),
$f_{mbm}$	=	the fraction of fat in milk (dimensionless),
$CR_{milk}$	=	the infant ingestion rate of milk (kg/day),
$ED_{INF}$	=	the infant exposure duration (year),
$BW_{INF}$	=	the infant's body weight (kg), and
$AT$	=	the averaging time (year).

Using the above equations, the ratio between the infant and maternal intake can be estimated as:

$$\frac{DI_{INF}}{DI_{MAT}} = \frac{0.00062 \times K_{OW} \times f_{mbm} \times CR_{milk} \times ED_{INF} \times BW_{Mat}}{BW_{INF} \times AT}$$

Appendix Equation 11

### ***Input Parameters and Application to Chlordane in Humans***

The input parameters used for chlordane are shown in [Table B-10](#). In addition to using mid-range (e.g., mean) values in the model, low and high end estimates were also used for some parameters where available to determine the approximate range of human variability. The  $ED_{INF}$  and  $AT$  values are equal to each other (either 1 month or 12 months) and cancel from the equation.

**Table B-10. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age**

Parameter (units)	Variable	Low	Mid	High	Note/source
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, 1 month (kg)	BW <sub>INF, 1</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, average over 12 months (kg)	BW <sub>INF, 12</sub>	7.8			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, 1 month (kg milk/day)	CR <sub>milk,1</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, average over 12 months (kg milk/day)	CR <sub>milk,12</sub>	0.66			U.S. EPA ( <a href="#">2011a</a> )
Fraction of fat in breast milk (dimensionless) <sup>b</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Octanol-water partition coefficient	log(K <sub>ow</sub> )	5.54 (reported log(K <sub>ow</sub> ) values for chlordane constituents ranged from 5.44–6.35)			ATSDR ( <a href="#">1994</a> ); Simpson et al. ( <a href="#">1995</a> )

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents the typical range and midpoint of the range.

Based on this K<sub>ow</sub> value and using [Appendix Equation 9](#), the biotransfer factor for chlordane is 215. Using this BTF<sub>m</sub>, the average infant to maternal dose ratio was estimated for infants at 1 and 12 months of age and is presented in [Table B-11](#). For the model parameterization presented here, these ratios tend to be one to two orders of magnitude higher than the estimates from the simple one-compartment kinetic model.

**Table B-11. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method**



Age	Low	Mid	High
1 month	34.3	51.4	68.5
12 months	27.3	40.9	54.6

### B.1.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation

The previous sections discuss the tools and input data available to estimate internal dose metrics for chlordane in rats and humans. The next step is to select a method for performing cross-species extrapolation by weighing biological sophistication against methodology and data uncertainty.

[Table B-12](#) presents potential dose metrics and methods for performing the cross-species extrapolation. This list may not be exhaustive but is intended to foster discussion at the workshop. For illustrative purposes in this briefing packet, one method for HED calculation was selected (shown in bold type), and sample calculations were performed.

**Table B-12. Potential Dose Metrics and Methods for HED Estimation**

Should the Cross-Species Extrapolation Be Performed on Maternal or Offspring Dose Metric?	What Technique is Used to Estimate the Dose Metrics?	What Dose Metric Should Be Used for Cross-Species Extrapolation?
<b>Maternal Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• <b>First order kinetic maternal model in rats and humans</b></li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Maternal average or peak body burden during lactation</li> <li>• <b>Maternal average or peak fat concentration during lactation</b></li> <li>• Maternal average or peak milk concentration during lactation</li> </ul>
<b>Offspring Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic model with infant/pup body burden</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Infant average or peak body burden during lactation</li> </ul>
<b>Other?</b>	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• First order kinetic model with infant/pup body burden</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Other?</li> </ul>

In selecting the dose metric to use for cross-species extrapolation and the technique to use to estimate the dose metrics, the discussion points listed in the text boxes throughout this section should be considered. This information is synthesized in the following text box.

As an example, the points were considered, and an example was provided to foster discussion. The chemical-specific information available should be considered to help guide the decision on whether a maternal or offspring dose metric should be utilized. Chlordane is a mixture with multiple metabolites

that are linked to toxicity. The mother will be exposed to the full mixture, but the metabolism within her body and the way the metabolites will be passed to the offspring are difficult to characterize in the simple models available. Thus, in the example provided here, the decision was made to perform the cross-species extrapolation on the maternal concentrations because the mothers receive the initial dose. This method will not account for differences in metabolism between the two species or differences in offspring feeding patterns or offspring kinetics.

Next, a model was selected to characterize the dose metrics. No full PBPK model was available, and the pharmacokinetic data were not deemed sufficient to build or adapt a model. Half-life information is not available in the whole-body, but the upper end of the range can be used to approximate the half-life. For this reason, the maternal portion of the first-order model was judged to be sufficiently parameterized to justify its use instead of the simpler biotransfer method. Thus, the first-order, time-dependent model in MS Excel was used to estimate the human and rat maternal average concentrations. Again, the dynamic metabolic elimination and the effects of chlordane's chemical composition as a mixture are not captured in this simple model.

Finally, the maternal dose metric was selected. No critical window could be determined for the developmental effect in the proposed POD. However, it is expected that developmental effects may have such a critical window. For this reason, the peak maternal concentration was selected instead of the average concentration during lactation. This choice reflects the possibility that short-lived high concentrations may affect the developing offspring during lactation. For the model compartment, the maternal milkfat concentration was selected. In the first-order model, the fat and milk fat concentrations (assumed equal to each other) are directly proportional to the body burden, although this proportionality may be different between the species. Because the half-life data for chlordane tend to be plasma-based (rather than whole body-based), the differences in preferential fat storage between rats and humans may not be reflected in the half-lives alone. Thus, the peak fat/milk fat concentrations during lactation were selected for the dose-metric to use in the cross-species extrapolation to account for potential differences in fat storage potentials between the species.

The selections described in the preceding paragraphs were applied to the candidate POD study. The dose was administered between gestation day 4 and lactation day 21. The peak fat concentration in the maternal rat during lactation at the point of departure dose (0.1 mg/kg-day) was predicted to be 2.6 mg/kg. The human first-order model was used to find the maternal continuous daily exposure that resulted in the same peak fat concentration, assuming "mid" parameters for the human model (e.g., pregnancy at age 25). Continuous daily exposure was used because the reference dose should reflect a chronic maternal intake. In this example, the resulting HED is 0.0069 mg/kg-day.

### Discussion Points for Chlordane HED Estimation

- ? Chlordane is a mixture. How should the relationship between human and animal dose metrics for each constituent be effectively addressed?
  - ✓ If the cross-species extrapolation is performed on the infants, there is a greater burden on understanding the relative toxicity and kinetic properties of each constituent if more sophisticated HED methods are chosen.
- ? Constituents of chlordane are metabolized. How should differences in metabolism between animals and humans be effectively addressed?
  - ✓ If the cross-species extrapolation is performed on the infants, there is a greater burden on understanding the metabolism, excretion, and toxicity of each metabolite.
- ? What pharmacokinetic data or relevant chemical-specific information are available for the chlordane constituents and metabolites for both animals and humans to facilitate model implementation?
  - ✓  $K_{ow}$
  - ✓ Human and rat half lives in plasma
  - ✓ Absorption fractions and fraction of chemical stored in fat (assumptions can be made based on other PBTs)
  - ✗ No available partition coefficients in humans
  - ✗ No available metabolism rates
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
  - ✓ Peak or average concentrations from maternal or infant dose metrics
  - ✗ The critical window could not be defined
- ? What PBPK or other simpler biokinetic models are available for chlordane?
  - ✗ No chlordane-specific human or rat PBPK model available (existing models could be modified for chlordane if appropriate model parameters are identified)
  - ✓ Simple first order models are available
  - ✓ Simple biotransfer methods are available
- ? What is the best method for estimating the human equivalent dose using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

## Attachment B.1-1. Concentrations of Chlordane in Human Milk

**Table Att. B.1-1. Concentrations of Chlordane in Human Milk (whole milk, ppb)**

Location	Year	Participants / Samples	cis-Chlordane	trans-Chlordane	Chlordane, unspecified <sup>a</sup>	oxy-chlordane	Source
Canada; regionally distributed	1982	210 women, 3-4 weeks post-partum	avg in positive samples: 1 detection frequency: 56% max: 4	avg in positive samples: trace detection frequency: 73% max: 2	NR	avg in positive samples: 1 detection frequency: 100% max: 2	Mes et al. ( <a href="#">1986</a> )
Canada	NR, before 1987	18 Inuit/Indian women	avg in positive samples: 0.2 detection frequency: 50%	avg in positive samples: 0.1 detection frequency: 17%	NR	avg in positive samples: 0.7 detection frequency: 100%	Davies and Mes ( <a href="#">1987</a> )
Tokyo, Japan	1979	12 women	concentration in pooled sample: 0.13	concentration in pooled sample: 0.16	NR	avg in positive samples: 0.52 max: 1.1	Miyazaki et al. ( <a href="#">1980</a> )
Tokushima City, Japan	NR, before 1986	29 women age 19-34 yr, 3 months post-partum	geometric mean in positive samples: 0.09 ± 2.27	geometric mean in positive samples: 0.04 ± 1.59	NR	geometric mean in positive samples: 0.39 ± 1.86	Tojo et al. ( <a href="#">1986</a> )
Baghdad, Iraq	NR, before 1986	4 women	avg in positive samples: 154 ± 18	avg in positive samples: 125 ± 14	NR	NR	Al Omar et al. ( <a href="#">1986</a> )
Canada; regionally distributed	1986	412 women	avg in all samples: 0.05 detection frequency: 38% max: 0.26	avg in all samples: trace detection frequency: 7% max: 0.59	NR	avg in all samples: 0.71 detection frequency: 93% max: 3.16	Mes et al. ( <a href="#">1993</a> )
Baghdad, Iraq	1983–1984	50 women	avg: 78 <sup>b</sup> detection frequency: 80% max: 536	avg: 34 <sup>b</sup> detection frequency: 68% max: 482	NR	NR	Al Omar et al. ( <a href="#">1985</a> )



Location	Year	Participants / Samples	cis-Chlordane	trans-Chlordane	Chlordane, unspecified <sup>a</sup>	oxy-chlordane	Source
Jordan; regionally distributed	NR, before 1998	411 women	avg in positive samples: $20 \pm 10$ detection frequency: 14%	avg in positive samples: $20 \pm 10$ detection frequency: 11%	NR	NR	Nasir et al. ( <a href="#">1998</a> )
Mississippi, high and low pesticide-use areas	1973–1975	high pesticide-use area: 34 women low pesticide-use area: 6 women	NR	NR	NR	high pesticide-use area avg: $5 \pm 1^b$ detection frequency: 100% max: 22 low pesticide-use area avg: $2 \pm 1^b$ detection frequency: 68% max: 4	Barnett et al. ( <a href="#">1979</a> )
Washington, D.C.	NR, before 1984	16 women, 7–98 days post-partum	NR	NR	NR	avg in all samples per week: 1.1–1.6	Mes et al. ( <a href="#">1984</a> )
Arkansas, Mississippi	1973–1974	57 women, avg 41 days post-partum	NR	NR	NR	avg in all samples: 0.5 avg in positive samples: 12 detection frequency: 45.6% max: 20	Strassman and Kutz ( <a href="#">1977</a> )
Spain	1979	45 women	NR	NR	avg: $0.3^b$ detection frequency: 17.8%	NR	Lora et al. ( <a href="#">1979</a> ) as cited by Jensen ( <a href="#">1983</a> )

<sup>a</sup>Authors referred to compound as only “chlordane,” may include a combination of isomers and metabolites, such as cis-, trans-, and oxy-chlordane and cis- and trans- nonachlor.

<sup>b</sup>Averaging methods not reported

Avg = Average; NR= Not reported

**Table Att. B.1-2. Concentrations of Chlordane in Human Milk (milk fat, ppb)**

Location	Year	Participants/Samples	cis-Chlordane	trans-Chlordane	Chlordane, unspecified <sup>a</sup>	Oxychlordane	Source
Osaka, Japan	1984–1985	non termite-treated homes: 7 women termite-treated homes avg 1.8 years ago: 15 women	non termite-treated homes avg: 2.1 <sup>b</sup> termite-treated homes avg: 5.9 <sup>b</sup>	non termite-treated homes avg: 1.5 <sup>b</sup> termite-treated homes avg: 7.9 <sup>b</sup>	non termite-treated homes avg: 58 <sup>b</sup> termite-treated homes avg: 162 <sup>b c</sup>	non termite-treated homes avg: 19.3 <sup>b</sup> termite-treated homes avg: 33.6 <sup>b</sup>	Taguchi and Yakushiji ( <a href="#">1988</a> )
Canada; regionally distributed	1982	210 women, 3–4 weeks post-partum	avg in positive samples: 27 detection frequency: 56% max: 84	avg in positive samples: 10 detection frequency: 73% max: 68	NR	avg in positive samples: 27 detection frequency: 100% max: 109	Mes et al. ( <a href="#">1986</a> )
Finland	1984–1985	155 women, 18–30 yrs, 1–3 weeks post-partum	avg in all samples: 20 ± 20 avg in positive samples: 100 ± 60 max: 230 detection frequency: 5.2%	avg in all samples: 20 ± 60 avg in positive samples: 200 ± 190 max: 580 detection frequency: 5.8%	NR	avg in all samples: 20 ± 60 avg in positive samples: 230 ± 121 max: 630 detection frequency: 3.9%	Mussalo-Rauhamaa et al. ( <a href="#">1988</a> )
Canada; regionally distributed	1986	412 women	avg in all samples: 1.6 detection frequency: 38% max: 12.1	avg in all samples: trace detection frequency: 61% max: 39.2	NR	avg in all samples: 23.3 detection frequency: 93% max: 197.4	Mes et al. ( <a href="#">1993</a> )
Canada; regionally distributed	1992	497 women	avg in all samples: 0.21 detection frequency: 11%	avg in all samples: 0.16 detection frequency: 2%	NR	avg in all samples: 13.4 detection frequency: 97%	Newsome et al. ( <a href="#">1995</a> )
Northern Canada	1996–1997	12 women, median age 25	avg in all samples: 1.27	avg in all samples: trace	NR	avg in all samples: 59.0	Newsome and Ryan ( <a href="#">1999</a> )

Location	Year	Participants/Samples	cis-Chlordane	trans-Chlordane	Chlordane, unspecified <sup>a</sup>	Oxychlordane	Source
Canada	NR, before 1987	18 Inuit/Indian women	avg in positive samples: 12 detection frequency: 50%	avg in positive samples: 3 <sup>b</sup> detection frequency: 17%	NR	avg in positive samples: 35 detection frequency: 100%	Davies and Mes ( <a href="#">1987</a> )
Tokushima City, Japan	NR, before 1986	29 women age 19-34 yr; 3 months post-partum	geometric mean in positive samples: 3.08 ± 2.5	geometric mean in positive samples: 1.2 ± 2.1	NR	geometric mean in positive samples: 11.5 ± 1.7	Tojo et al. ( <a href="#">1986</a> )
Jordan; regionally distributed	NR, before 1998	411 women	avg in positive samples: 460 ± 340 detection frequency: 14% <sup>b</sup>	avg in positive samples: 590 ± 630 detection frequency: 11% <sup>b</sup>	NR	NR	Nasir et al. ( <a href="#">1998</a> )
Murmansk and Monchegorsk, Russia	1993	Murmansk: 15 women, <2 weeks post-partum Monchegorsk: 14 women, <2 weeks post-partum	Murmansk avg in all samples: 6.9 ± 4.4 max: 19 Monchegorsk avg in all samples: 3.3 ± 2.2 max: 9.5 ug/kg	NR	NR	Murmansk avg in all samples: 8.1 ± 11 max: 38 Monchegorsk avg in all samples: 7.9 ± 18 max: 66	Polder et al. ( <a href="#">1998</a> )
United States, regionally distributed	NR, before 1981	1436 women	NR	NR	NR	avg in all samples: 95.8 ± 195.1 detection frequency: 73.9%	Savage et al. ( <a href="#">1981</a> )
Mexico	1976	620 women	NR	NR	NR	median <sup>b</sup> : 400	WHO/FAO ( <a href="#">1981</a> ) as cited by Jensen ( <a href="#">1983</a> )
Mississippi, high and low pesticide-use areas	1973–1975	high pesticide-use area: 34 women low pesticide-use area: 6 women	NR	NR	NR	high pesticide-use area avg: 130 ± 20 <sup>b</sup> detection frequency: 100% max: 700 low pesticide-use area avg: 50 ± 20 <sup>b</sup> detection frequency: 68% max: 120	Barnett et al. ( <a href="#">1979</a> )

Location	Year	Participants/Samples	cis-Chlordane	trans-Chlordane	Chlordane, unspecified <sup>a</sup>	Oxychlordane	Source
Victoria, Australia	1992	17 women age 22–36 yr, 6 wks–2 mo post-partum	NR	NR	NR	avg in positive samples: 130 ± 140 detection frequency: 80% max: 540	Quinsey et al. ( <a href="#">1995</a> )
Oahu, Hawaii and Neighbor Islands	1979–1980	Oahu sample: 38 women, median 16 days post-partum Neighbor Islands sample: 12 women, median 16 days post-partum	NR	NR	NR	Oahu avg in positive samples: 61 detection frequency: 100% max: 160 Neighbor Islands avg in positive samples: 53 detection frequency: 100% max: 110	Takahashi et al. ( <a href="#">1981</a> )
Spain	1979	45 women	NR	NR	avg: 26 <sup>b</sup> detection frequency: 17.8% max: 720	NR	Lora et al. ( <a href="#">1979</a> ) as cited by Jensen ( <a href="#">1983</a> )
Perth, Australia	1990–1991	128 women	NR	NR	median in all samples: <10 detection frequency: 17% max: 70	NR	Stevens et al. ( <a href="#">1993</a> )

<sup>a</sup> Authors referred to compound as only “chlordane”, may include a combination of isomers and metabolites, such as cis-, trans-, and oxy-chlordane and cis- and trans- nonachlor.

<sup>b</sup> Calculation methods not reported

<sup>c</sup> Author specified that “chlordane” included cis-, trans-, and oxy-chlordane and cis- and trans- nonachlor.

Avg = Average; ID= Indigenous population; NR= Not reported; NS= National survey

---

## B.1.7. References

- [Al-Hachim, GM; Al-Baker, A.](#) (1973). Effects of chlordane on conditioned avoidance response, brain seizure threshold and open-field performance of prenatally-treated mice. *Br J Pharmacol* 49: 311-315.
- [Al-Omar, M; Abdul-Jalil, FH; Al-Ogaily, NH; Tawfig, SJ; Al-Bassomy, MA.](#) (1986). A follow-up study of maternal milk contamination with organochlorine insecticide residues. *Environ Pollut Ecol Biol* 42: 79-92. [http://dx.doi.org/10.1016/0143-1471\(86\)90046-2](http://dx.doi.org/10.1016/0143-1471(86)90046-2)
- [Al-Omar, MA; Tawfig, SJ; Al-Ogaily, N.](#) (1985). Organochlorine residue levels in human milk from Baghdad. *Bull Environ Contam Toxicol* 35: 65-67. <http://dx.doi.org/10.1007/BF01636481>
- [Aldrich, FD; Holmes, JH.](#) (1969). Acute chlordane intoxication in a child: case report with toxicological data. *Arch Environ Occup Health* 19: 129-132.
- [Asakawa, F; Jitsunari, F; Shiraishi, H; Suna, S; Takeda, N; Kitamado, T.](#) (1996). Accumulation of chlordanes in adipose tissues of mice caused by long-term exposure of low level technical chlordane. *Bull Environ Contam Toxicol* 57: 909-916.
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (1994). Toxicological profile for chlordane. (RISKLIN/1995020003). Washington, DC: U.S. Department of Health and Human Services.
- [Ayotte, P; Carrier, G; Dewailly, E.](#) (1996). Health risk assessment for inuit newborns exposed to dioxin-like compounds through breast feeding. *Chemosphere* 32: 531-542.
- [Barnett, JB; Blaylock, BL; Gandy, J; Menna, JH; Denton, R; Soderberg, LS.](#) (1990a). Long-term alteration of adult bone marrow colony formation by prenatal chlordane exposure. *Fundam Appl Toxicol* 14: 688-695. [http://dx.doi.org/10.1016/0272-0590\(90\)90294-T](http://dx.doi.org/10.1016/0272-0590(90)90294-T)
- [Barnett, JB; Blaylock, BL; Gandy, J; Menna, JH; Denton, R; Soderberg, LSF.](#) (1990b). Alteration of fetal liver colony formation by prenatal chlordane exposure. *Fundam Appl Toxicol* 15: 820-822. [http://dx.doi.org/10.1016/0272-0590\(90\)90198-S](http://dx.doi.org/10.1016/0272-0590(90)90198-S)
- [Barnett, JB; Holcomb, D; Menna, JH; Soderberg, LSF.](#) (1985a). The effect of prenatal chlordane exposure on specific anti-influenza cell-mediated immunity. *Toxicol Lett* 25: 229-238. [http://dx.doi.org/10.1016/0378-4274\(85\)90202-4](http://dx.doi.org/10.1016/0378-4274(85)90202-4)
- [Barnett, JB; Soderberg, LSF; Menna, JH.](#) (1985b). The effect of prenatal chlordane exposure on the delayed hypersensitivity response of BALB/c mice. *Toxicol Lett* 25: 173-183. [http://dx.doi.org/10.1016/0378-4274\(85\)90079-7](http://dx.doi.org/10.1016/0378-4274(85)90079-7)
- [Barnett, RW; D'Ercole, AJ; Cain, JD; Arthur, RD.](#) (1979). Organochlorine pesticide residues in human milk samples from women living in Northwest and Northeast Mississippi, 1973-75. *Pestic Monit J* 13: 47-51.
- [Blaylock, BL; Newsom, KK; Holladay, SD; Shipp, BK; Bartow, TA; Mehendale, HM.](#) (1995). Topical exposure to chlordane reduces the contact hypersensitivity response to oxazolone in BALB/c mice. *Toxicol Lett* 81: 205-211. [http://dx.doi.org/10.1016/0378-4274\(95\)03436-6](http://dx.doi.org/10.1016/0378-4274(95)03436-6)
- [Blaylock, BL; Soderberg, LS; Gandy, J; Menna, JH; Denton, R; Barnett, JB.](#) (1990). Cytotoxic T-lymphocyte and NK responses in mice treated prenatally with chlordane. *Toxicol Lett* 51: 41-49.
- [Blyler, G; Landreth, KS; Barnett, JB.](#) (1994). Gender-specific effects of prenatal chlordane exposure on myeloid cell development. *Fundam Appl Toxicol* 23: 188-193. <http://dx.doi.org/10.1093/toxsci/23.2.188>

- [Byczkowski, JZ; Kinkead, ER; Leahy, HF; Randall, GM; Fisher, JW.](#) (1994). Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically based model. *Toxicol Appl Pharmacol* 125: 228-236. <http://dx.doi.org/10.1006/taap.1994.1068>
- [Byczkowski, JZ; Lipscomb, JC.](#) (2001). Physiologically based pharmacokinetic modeling of the lactational transfer of methylmercury. *Risk Anal* 21: 869-882.
- [Cassidy, RA; Vorhees, CV; Minnema, DJ; Hastings, L.](#) (1994). The effects of chlordane exposure during pre- and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. *Toxicol Appl Pharmacol* 126: 326-337. <http://dx.doi.org/10.1006/taap.1994.1123>
- [Chan, HM; Berti, PR; Receveur, O; Kuhnlein, HV.](#) (1997). Evaluation of the population distribution of dietary contaminant exposure in an Arctic population using Monte Carlo statistics. *Environ Health Perspect* 105: 316-321. <http://dx.doi.org/10.2307/3433269>
- [Chernoff, N; Kavlock, RJ.](#) (1982). An in vivo teratology screen utilizing pregnant mice. *J Toxicol Environ Health* 10: 541-550. <http://dx.doi.org/10.1080/15287398209530275>
- [Clewell, RA; Merrill, EA; Yu, KO; Mahle, DA; Sterner, TR; Fisher, JW; Gearhart, JM.](#) (2003). Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. *Toxicol Sci* 74: 416-436. <http://dx.doi.org/10.1093/toxsci/kfg147>
- [Cranmer, JM; Cranmer, MF; Goad, PT.](#) (1984). Prenatal chlordane exposure: Effects on plasma corticosterone concentrations over the lifespan of mice. *Environ Res* 35: 204-210. [http://dx.doi.org/10.1016/0013-9351\(84\)90128-2](http://dx.doi.org/10.1016/0013-9351(84)90128-2)
- [Cranmer, JS; Avery, DL; Grady, RR; Kitay, JL.](#) (1978). Postnatal endocrine dysfunction resulting from prenatal exposure to carbofuran, diazinon or chlordane. *J Environ Pathol Toxicol* 2: 357-369.
- [Curley, A; Garrettson, LK.](#) (1969). Acute chlordane poisoning: Clinical and chemical studies. *Arch Environ Occup Health* 18: 211-215.
- [Davies, D; Mes, J.](#) (1987). Comparison of the residue levels of some organochlorine compounds in breast milk of the general and indigenous Canadian populations. *Bull Environ Contam Toxicol* 39: 743-749.
- [De Bruijn, J; Busser, F; Seinen, W; Hermens, J.](#) (1989). Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the slow-stirring method. *Environ Toxicol Chem* 8: 499-512. <http://dx.doi.org/10.1002/etc.5620080607>
- [Dearth, MA; Hites, RA.](#) (1991b). Depuration rates of chlordane compounds from rat fat. *Environ Sci Technol* 25: 1125-1128.
- [Fisher, JW; Whittaker, TA; Taylor, DH; Clewell, HJ, III; Andersen, ME.](#) (1990). Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol* 102: 497-513.
- [Frank, R; Rasper, J; Smout, MS; Braun, HE.](#) (1988). Organochlorine residues in adipose tissues blood and milk from Ontario, Canada residents 1976-1985. *Can J Public Health* 79: 150-158.
- [Gentry, PR; Covington, TR; Clewell, HJ, III.](#) (2003). Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. *Regul Toxicol Pharmacol* 38: 1-16. [http://dx.doi.org/10.1016/S0273-2300\(03\)00047-3](http://dx.doi.org/10.1016/S0273-2300(03)00047-3)
- [Ingle, L.](#) (1952). Chronic oral toxicity of chlordane to rats. *Arch Environ Occup Health* 6(4): 357-367.
- [Jensen, AA.](#) (1983). Chemical contaminants in human milk [Review]. *Residue Rev* 89: 1-128.
- [Knight, CH; Docherty, AH; Peaker, M.](#) (1984). Milk yield in rats in relation to activity and size of the mammary secretory cell population. *J Dairy Res* 51: 29-35.

- [Kreuzer, PE; Csanády, GA; Baur, C; Kessler, W; Pápke, O; Greim, H; Filser, JG.](#) (1997). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and congeners in infants. A toxicokinetic model of human lifetime body burden by TCDD with special emphasis on its uptake by nutrition. *Arch Toxicol* 71: 383-400.
- [Menna, JH; Barnett, JB; Soderberg, LS.](#) (1985). Influenza type A virus infection of mice exposed in utero to chlordane; survival and antibody studies. *Toxicol Lett* 24: 45-52. [http://dx.doi.org/10.1016/0378-4274\(85\)90138-9](http://dx.doi.org/10.1016/0378-4274(85)90138-9)
- [Mes, J; Davies, DJ; Doucet, J; Weber, D; McMullen, E.](#) (1993). Levels of chlorinated hydrocarbon residues in Canadian human breast milk and their relationship to some characteristics of the donors. *Food Addit Contam* 10: 429-441. <http://dx.doi.org/10.1080/02652039309374166>
- [Mes, J; Davies, DJ; Turton, D; Sun, WF.](#) (1986). Levels and trends of chlorinated hydrocarbon contaminants in the breast milk of Canadian women. *Food Addit Contam* 3: 313-322. <http://dx.doi.org/10.1080/02652038609373598>
- [Mes, J; Doyle, JA; Adams, BR; Davies, DJ; Turton, D.](#) (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. *Arch Environ Contam Toxicol* 13: 217-223. <http://dx.doi.org/10.1007/BF01055879>
- [Miyazaki, T; Akiyama, K; Kaneko, S; Horii, S; Yamagishi, T.](#) (1980). Chlordane residues in human milk. *Bull Environ Contam Toxicol* 25: 518-523. <http://dx.doi.org/10.1007/BF01985565>
- [Mussalo-Rauhamaa, H; Pyysalo, H; Antervo, K.](#) (1988). Relation between the content of organochlorine compounds in Finnish human milk and characteristics of the mothers. *J Toxicol Environ Health* 25: 1-19. <http://dx.doi.org/10.1080/15287398809531185>
- [Narotsky, MG; Kavlock, RJ.](#) (1995). A multidisciplinary approach to toxicological screening: II. Developmental toxicity. *J Toxicol Environ Health* 45: 145-171. <http://dx.doi.org/10.1080/15287399509531987>
- [Nasir, K; Bilot, YY; Al-Shuraiki, Y.](#) (1998). Residues of chlorinated hydrocarbon insecticides in human milk of Jordanian women. *Environ Pollut* 99: 141-148. [http://dx.doi.org/10.1016/S0269-7491\(97\)00195-4](http://dx.doi.org/10.1016/S0269-7491(97)00195-4)
- [Newsome, WH; Davies, D; Doucet, J.](#) (1995). PCB and organochlorine pesticides in Canadian human milk-1992. *Chemosphere* 30: 2143-2153. [http://dx.doi.org/10.1016/0045-6535\(95\)00086-N](http://dx.doi.org/10.1016/0045-6535(95)00086-N)
- [Newsome, WH; Ryan, JJ.](#) (1999). Toxaphene and other chlorinated compounds in human milk from northern and southern Canada: A comparison. *Chemosphere* 39: 519-526. [http://dx.doi.org/10.1016/S0045-6535\(98\)00600-6](http://dx.doi.org/10.1016/S0045-6535(98)00600-6)
- [NLM](#) (National Institutes of Health, National Library of Medicine). (2011). Hazardous Substances Data Bank (HSDB). Bethesda, MD. <http://toxnet.nlm.nih.gov>
- [Ohno, Y; Kawanishi, T; Takahashi, A; Nakaura, S; Kawashima, K; Tanaka, S; Takanaka, A; Omori, Y; Sekita, H; Uchiyama, M.](#) (1986). Comparisons of the toxicokinetic parameters in rats determined for low and high dose of "gamma"-chlordane. *J Toxicol Sci* 11: 111-123.
- [Olanoff, LS; Bristow, WJ; Colcolough, J, Jr; Reigart, JR.](#) (1983). Acute chlordane intoxication. *Clin Toxicol* 20: 291-306.
- [Polder, A; Becher, G; Savinova, TN; Skaare, JU.](#) (1998). Dioxins, PCBs and some chlorinated pesticides in human milk from the Kola Peninsula, Russia. *Chemosphere* 37: 1795-1806.
- [Poulin, P; Krishnan, K.](#) (1995). An algorithm for predicting tissue: Blood partition coefficients of organic chemicals from n-octanol: Water partition coefficient data. *J Toxicol Environ Health* 46: 117-129. <http://dx.doi.org/10.1080/15287399509532021>
- [Poulin, P; Krishnan, K.](#) (1996). A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. *Toxicol Appl Pharmacol* 136: 126-130. <http://dx.doi.org/10.1006/taap.1996.0015>

- [Price, K; Haddad, S; Krishnan, K.](#) (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol Environ Health A* 66: 417-433.  
<http://dx.doi.org/10.1080/15287390306450>
- [Quinsey, PM; Donohue, DC; Ahokas, JT.](#) (1995). Persistence of organochlorines in breast milk of women in Victoria, Australia. *Food Chem Toxicol* 33: 49-56.
- [Quinsey, PM; Donohue, DC; Cumming, FJ; Ahokas, JT.](#) (1996). The importance of measured intake in assessing exposure of breast-fed infants to organochlorines. *Eur J Clin Nutr* 50: 438-442.
- [Redding, LE; Sohn, MD; Mckone, TE; Chen, JW; Wang, SL; Hsieh, DP; Yang, RS.](#) (2008). Population physiologically based pharmacokinetic modeling for the human lactational transfer of PCB-153 with consideration of worldwide human biomonitoring results. *Environ Health Perspect* 116: 1629-1635.  
<http://dx.doi.org/10.1289/ehp.11519>
- [Savage, EP; Keefe, TJ; Tessari, JD; Wheeler, HW; Applehans, FM; Goes, EA; Ford, SA.](#) (1981). National study of chlorinated hydrocarbon insecticide residues in human milk, USA: I. Geographic distribution of dieldrin, heptachlor, heptachlor epoxide, chlordane, oxychlordane, and mirex. *Am J Epidemiol* 113: 413-422.
- [Simpson, CD; Wilcock, RJ; Smith, TJ; Wilkins, AL; Langdon, AG.](#) (1995). Determination of octanol-water partition coefficients for the major components of technical chlordane. *Bull Environ Contam Toxicol* 55: 149-153.
- [Spyker-Cranmer, JM; Barnett, JB; Avery, DL; Cranmer, MF.](#) (1982). Immunotoxicology of chlordane: Cell-mediated and humoral immune responses in adult mice exposed in utero. *Toxicol Appl Pharmacol* 62: 402-408. [http://dx.doi.org/10.1016/0041-008X\(82\)90141-7](http://dx.doi.org/10.1016/0041-008X(82)90141-7)
- [Stevens, MF; Ebell, GF; Psaila-Savona, P.](#) (1993). Organochlorine pesticides in Western Australian nursing mothers. *Med J Aust* 158: 238-241.
- [Strassman, SC; Kutz, FW.](#) (1977). Insecticide residues in human milk from Arkansas and Mississippi, 1973-74. *Pestic Monit J* 10: 130-133.
- [Taguchi, S; Yakushiji, T.](#) (1988). Influence of termite treatment in the home on the chlordane concentration in human milk. *Arch Environ Contam Toxicol* 17: 65-71.
- [Takahashi, W; Saidin, D; Takei, G; Wong, L.](#) (1981). Organochloride pesticide residues in human milk in Hawaii, 1979-80. *Bull Environ Contam Toxicol* 27: 506-511. <http://dx.doi.org/10.1007/bf01611056>
- [Theus, SA; Tabor, DR; Soderberg, LS; Barnett, JB.](#) (1992). Macrophage tumoricidal mechanisms are selectively altered by prenatal chlordane exposure. *Agents and Actions* 37: 140-146.
- [Timson, BF; Coffman, JL.](#) (1984). Body composition by hydrostatic weighing at total lung capacity and residual volume. *Med Sci Sports Exerc* 16: 411-414.
- [Tojo, Y; Wariishi, M; Suzuki, Y; Nishiyama, K.](#) (1986). Quantitation of chlordane residues in mothers' milk. *Arch Environ Contam Toxicol* 15: 327-332.
- [Travis, CC; Arms, AD.](#) (1988). Bioconcentration of organics in beef, milk, and vegetation. *Environ Sci Technol* 22: 271-274. <http://dx.doi.org/10.1021/es00168a005>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1988). Recommendations for and documentation of biological values for use in risk assessment [EPA Report]. (EPA/600/6-87/008). Cincinnati, OH.  
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1998). Methodology for assessing health risks associated with multiple pathways of exposure to combustor emissions [EPA Report]. (EPA 600/R-98/137). Washington, DC. <http://cfpub.epa.gov/ncea/CFM/recordisplay.cfm?deid=55525>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2011a). Exposure factors handbook 2011 edition (final) [EPA Report]. (EPA/600/R-09/052F). <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252>



U.S. EPA. (2012b). EPA's reanalysis of key issues related to dioxin toxicity and response to NAS comments (CAS No. 1746-01-6) [EPA Report]. (EPA/600/R-10/038F). Washington, DC.

Verner, MA; Ayotte, P; Muckle, G; Charbonneau, M; Haddad, S. (2009). A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. *Environ Health Perspect* 117: 481-487.

Verner, MA; Charbonneau, M; López-Carrillo, L; Haddad, S. (2008). Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: A new tool in breast cancer epidemiologic studies. *Environ Health Perspect* 116: 886-892. <http://dx.doi.org/10.1289/ehp.10917>

Welch, RM; Findlay, JW. (1981). Excretion of drugs in human breast milk [Review]. *Drug Metab Rev* 12: 261-277. <http://dx.doi.org/10.3109/03602538108994032>

## B.2. DDT Briefing Packet



# Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk

**Workshop Briefing Packet:  
DDT, DDD, and DDE**

### **Prepared for**

National Center for Environmental Assessment,  
U.S. EPA  
109 T.W. Alexander Drive  
Durham, NC 27711

### **Prepared by**

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713

### **Notice**

Although the research described in this briefing packet has been supported by the U. S. Environmental Protection Agency through EP-C-09-009 to ICF International, it does not necessarily reflect the views of the Agency and no official endorsement should be inferred.



## Contents B.2

<b>Appendix B. Original Briefing Packets, as Distributed to Workshop Attendees .....</b>	<b>B-1</b>
B.2. DDT Briefing Packet .....	B-61
B.2.1. Introduction.....	B-65
B.2.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-66
B.2.3. Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-79
B.2.4. Developmental Effects of DDT .....	B-82
B.2.5. Human Equivalent Dose (HED) Estimation .....	B-105
B.2.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-130
B.2.7. References.....	B-134

## List of Figures

Figure B-7. Total human milk levels of DDT (left) and p,p'-DDE (right), as µg/g milk fat, from 144 studies. ....	B-70
Figure B-8. Dietary Intake versus DDT Level in Blood.....	B-79
Figure B-9. Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to DDT Compounds.....	B-103
Figure B-10. Exposure-Response Array Showing All Developmental Effects in Male Pups after Gestational or Gestational and Lactational Exposure to DDT Compounds.....	B-104
Figure B-11. Process diagram for performing cross-species extrapolation .....	B-106
Figure B-12. Maternal and Infant POP Model (Verner et al., 2009) .....	B-114
Figure B-13. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day .....	B-122
Figure B-14. Mouse Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Palanza et al., 1999) and an Administered Dose of 0.02 mg/kg-day (POD) .....	B-126

## List of Tables

Table B-13. DDT in Breast Milk in Japan .....	B-71
Table B-14. DDT in Breast Milk in Canada .....	B-72
Table B-15. DDT in Breast Milk in Eastern Europe.....	B-73
Table B-16. DDT in Breast Milk in India .....	B-74
Table B-17. DDT in Breast Milk in China .....	B-76
Table B-18. Concentration of ΣDDT in Children's Adipose Tissue Related to Breast Milk Consumption Patterns.....	B-77
Table B-19. Concentration of DDE in Various Human Tissues.....	B-78
Table B-20. Overview of Developmental Effects of DDT and/or DDE in Humans Reported in Epidemiological Literature .....	B-85
Table B-21. Developmental Effects from Gestational and/or Lactational Exposure to DDT Compounds <sup>a</sup> .....	B-94
Table B-22. Available Pharmacokinetic Parameters for DDT in Humans and Mice.....	B-111
Table B-23. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model.....	B-121
Table B-24. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-122

Table B-25. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Mouse.....	B-125
Table B-26. Estimated Mouse Pup-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-126
Table B-27. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-129
Table B-28. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method .....	B-130
Table B-29. Potential Dose Metrics and Methods for HED Estimation .....	B-131

### B.2.1. Introduction

Technical-grade DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) may vary in composition, but is generally composed of three main DDT isomers: p,p'-DDT (63-85%), o,p'-DDT (8-21%), and o,o'-DDT (trace amount) ([WHO, 2011](#); [ATSDR, 2002a](#)). Technical-grade DDT may also contain varying amounts of DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) (trace to 4%) and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) (trace) as contaminants ([WHO, 2011](#); [ATSDR, 2002a](#)). While DDD was historically manufactured and used as a pesticide and a medical treatment, DDD and DDE are also breakdown products of DDT ([WHO, 2011](#); [ATSDR, 2002a](#)).

DDT was first synthesized in 1874, but its insecticidal properties were not discovered until 1935 ([WHO, 2005](#)). In the following decades, DDT was used as a general agricultural pesticide and as a disease control measure—to control lice during World War II and to control mosquitoes during international malaria control efforts in the 1950s and 1960s ([WHO, 2005](#)). DDT use was banned in the US and many other countries in 1972, except for public health purposes ([ATSDR, 2002a](#)). In 2001, global production and use of DDT was restricted to public health purposes only under the Stockholm Convention for Persistent Organic Pollutants, and such uses must be monitored and reported ([WHO, 2011](#)).

Following the convention set forth by ATSDR ([2002a](#)), the terms DDT, DDD, and DDE will refer to all isomers of that compound (i.e., o,p'-, p,p'- and o,o'-); and, when information is available, individual isomers will be identified. The term “DDT” will also be used henceforth in discussion to refer to the collection of all DDT, DDD, and DDE isomers (e.g., p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE) that are present in technical-grade DDT or exist as a breakdown product from technical-grade DDT. The distinction between “DDT” referring to just 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane isomers versus the collection of all DDT, DDD, and DDE isomers should be clear based on the context, but in cases where further clarification is needed, the term  $\Sigma$ DDT (read: sum of DDTs) will be used. Many references that discuss health effects from DDT report levels of individual isomers of DDT, in biological and environmental media, but most commonly focus on p,p'-DDT, o,p'-DDT, and DDE. When those studies are summarized, “Total DDT” will be referred to unless otherwise noted. For the purposes of this briefing packet, Total DDT represents the sum of p,p'-DDT, o,p'-DDT, and DDE.

In this briefing packet, the available literature on lactational and gestational exposure to DDT and its metabolites was reviewed for key findings and data to help estimate the maternal dose and breastfeeding infant dose of DDT in humans. Selected references are summarized and evaluated in this briefing packet in five sections: maternal dose and breastfeeding infant dose of DDT in humans; maternal dose and nursing offspring dose in animals; developmental effects of DDT in humans and animals; human and animal kinetic models; and a final section, which discusses the application of kinetic models to derive an example human equivalent dose (HED).

These briefing packets are intended to stimulate ideas and provide material for discussion regarding innovative approaches to the assessment of risk to breastfeeding infants from DDT and other PBT chemicals in breast milk. The information in this and other briefing packets is provided to help workshop

participants discuss and formulate answers to the workshop charge questions and does not reflect Agency opinion or policy regarding DDT. Areas where there exists uncertainty, data gaps, or where there is a lack of understanding in the current literature are highlighted to foster further discussion.

---

## **B.2.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans**

This section discusses how women of child-bearing age may be exposed to DDT; how their bodies sequester DDT in various tissues, and the extent and manner that DDT is eliminated through breast milk, leading to infant exposure. DDT exposure levels vary geographically and are at least partially dependent on social factors (e.g., dietary choices) and environmental factors (e.g., the amount of residual DDT in the local environment). Concentrations of DDT have been reported in various human tissues, including breast milk, blood plasma, and adipose tissue.

In this briefing packet, measured concentrations of DDT in the diet or environmental media and their relationship with measured body burdens in mothers are presented. Studies are also described that inform the characterization of the relationship between the body burden of DDT in mothers and intake by breastfeeding infants. However, the relationships between maternal exposure to DDT (i.e., intake from environmental and dietary sources), maternal body burden, and infant dose in humans are not well described in the literature. Attempts have been made to draw connections between studies showing intake and breast milk concentrations in similar geographic areas during similar time periods to illustrate relationships between exposure and body burden of DDT.

---

### **B.2.2.1. Mothers' Exposure to DDT**

Environmental residues of DDT and DDE exist globally due to (1) releases that occur during production and manufacturing of DDT products, (2) their use in vector control, and (3) environmental contamination that remains from past use ([WHO, 2011](#)). The persistence of DDT in the environment also leads to long-range transport of residues throughout global food webs and carbon cycles ([ATSDR, 2002a](#)).

Environmentally released DDT is hydrophobic and has a tendency to bioaccumulate within food webs, so ingestion of DDT through the diet, specifically fatty foods, is considered to be a significant route of exposure for the general population ([Galassi et al., 2008](#)). DDT is metabolized in the environment and assimilated by organisms before uptake by humans; therefore, the main residue found in human tissues is *p,p'*-DDE ([Galassi et al., 2008](#)). This is especially true in countries where DDT was historically used for agricultural purposes but is no longer used as DDT has a long biological half-life ([WHO, 2011](#)). In countries where DDT is still used for disease control purposes, more direct exposures to the technical-grade chemical may occur through skin contact with contaminated surfaces, ingestion of food contaminated in the home, and hand-to-mouth activity ([WHO, 2011](#)).

Several studies have measured levels of DDT in foods consumed by humans. However, only a few studies to date have concurrently measured DDT in foods as well as in blood or other human tissues to draw connections between dietary intake and body burdens ([WHO, 2011](#)). In a study conducted in 2003 in Germany (where DDT had been banned about 10 years prior) and Poland (where DDT was banned 20+ years prior), concentrations of DDT residues measured in blood, placenta, and various food and beverage samples showed that p,p'-DDE concentration in human tissues is related to p,p'-DDE dietary intake ([Galassi et al., 2008](#)). Estimated average daily p,p'-DDE intake from consumption of various foods was 83.38 µg for the Polish study population compared to 17.93 µg for the German study population (no body weight data provided). With the single outlier datapoint removed for each population (107.35 ng/g for German and 10.41 ng/g for the Polish), the average placenta concentration of p,p'-DDE was 1.88 ng/g dry weight for the Polish population and 2.92 ng/g dry weight for the German population ([Galassi et al., 2008](#)). Mean levels of p,p'-DDE in the blood were higher for the Polish population as compared to the German population, but they were not statistically significantly different, due to high data variability ([Galassi et al., 2008](#)). The ratio of placenta to blood p,p'-DDE concentrations for both the German and Polish samples was estimated at 5, and the lipid concentration was about six times higher in placental tissue than in blood ([Galassi et al., 2008](#)).

Kashyap et al. ([1994](#)) found a correlation between food intake and blood DDT levels in a duplicate diet study of 20 adult vegetarians in a metropolitan city in India (14 men and 6 women, 25-45 years old, no body weight data provided). The average intake of total DDT via the diet was 19.24 µg/day, but no range was provided. Residues of DDT were found in each food type analyzed (non-fatty foods, vegetables, milk, water, and other beverages). The concentration of DDT in fatty foods was higher than in non-fatty foods, so even a slight increase in consumption of fatty foods was predicted to increase total DDT intake. The study authors found that blood DDT levels were positively correlated with DDT intake from the duplicate diet analysis, which illustrates that DDT levels in blood can be related to dietary intake ([Kashyap et al., 1994](#)).

Using similar methods, Frank et al. ([1993](#)) concluded that 96% of human DDE body burden was a result of dietary intake. The authors measured DDE residues in diet and blood of Ontario, Canada residents from 1986-1987. Residues of DDE in whole foods ranged from 0.05 to 0.77 µg/kg, and residues in extractable fats from animal products were higher, at 0.5 to 8.6 µg/kg. Concentrations of DDE in whole blood from 750 Ontario residents averaged 3.7 µg/kg, with a maximum of 51 µg/kg. The authors concluded that 96% of blood DDE levels result from dietary intake and less than 5% comes from occupational or accidental exposure, assuming whole blood levels of DDE derived from diet fall between 0 and 10 µg/kg ([Frank et al., 1993](#)).

Several market basket/total diet studies were identified that analyzed DDT concentrations in foods and determined an average dietary intake of DDT for the study population. In a study by Matsumoto et al. ([1987](#)), a range of foods were collected from local supermarkets in Japan and analyzed for organochlorine pesticide and PCB content on seven occasions between 1977 and 1985. Estimated daily dietary intakes of total DDT ranged from 1.10 to 4.8 µg/day, with an average of 3.06 µg/day. A similar study was performed by Rawn et al. ([2004](#)) in Whitehorse, Yukon, as part of the Canadian Total Diet



study. Residues of DDT and its metabolites were found in a wide variety of food categories and total DDT was found in over 25% of all food composites analyzed. Study authors determined an estimated daily intake level of the contaminant using standard food consumption data for 11 different age-sex groups. The estimated daily intake of total DDT for females aged 20-39 years (average body weight of 57.2 kg) was 0.05 µg/kg body weight ([Rawn et al., 2004](#)). Chen and Gao ([1993](#)) also reported dietary intake of DDT as estimated from a market basket study and standard food consumption data relevant to their study population in mainland China in 1990. The study authors analyzed twelve food group composites based on average-consumed foods from each of 4 regional markets. The authors determined that average DDT intake in the current study ranged from 8.8 µg/person/day to 46.1 µg/person/day (mean 20.5 µg/person/day; assumed standard body weight of 60 kg), a decrease from the previously estimated mean intake of 41.4 µg/person/day in 1973-1978 ([Chen and Gao, 1993](#)).

While several other studies were identified that report levels of DDT in foods typical for a specific population [e.g., Galván-Portillo et al. ([2002](#)), Subramanian et al. ([2007](#))], these study authors did not determine estimates of daily or total dietary intake of DDT.

---

#### **B.2.2.2. Is DDT Sequestered in Breast Milk or Other Human Tissues?**

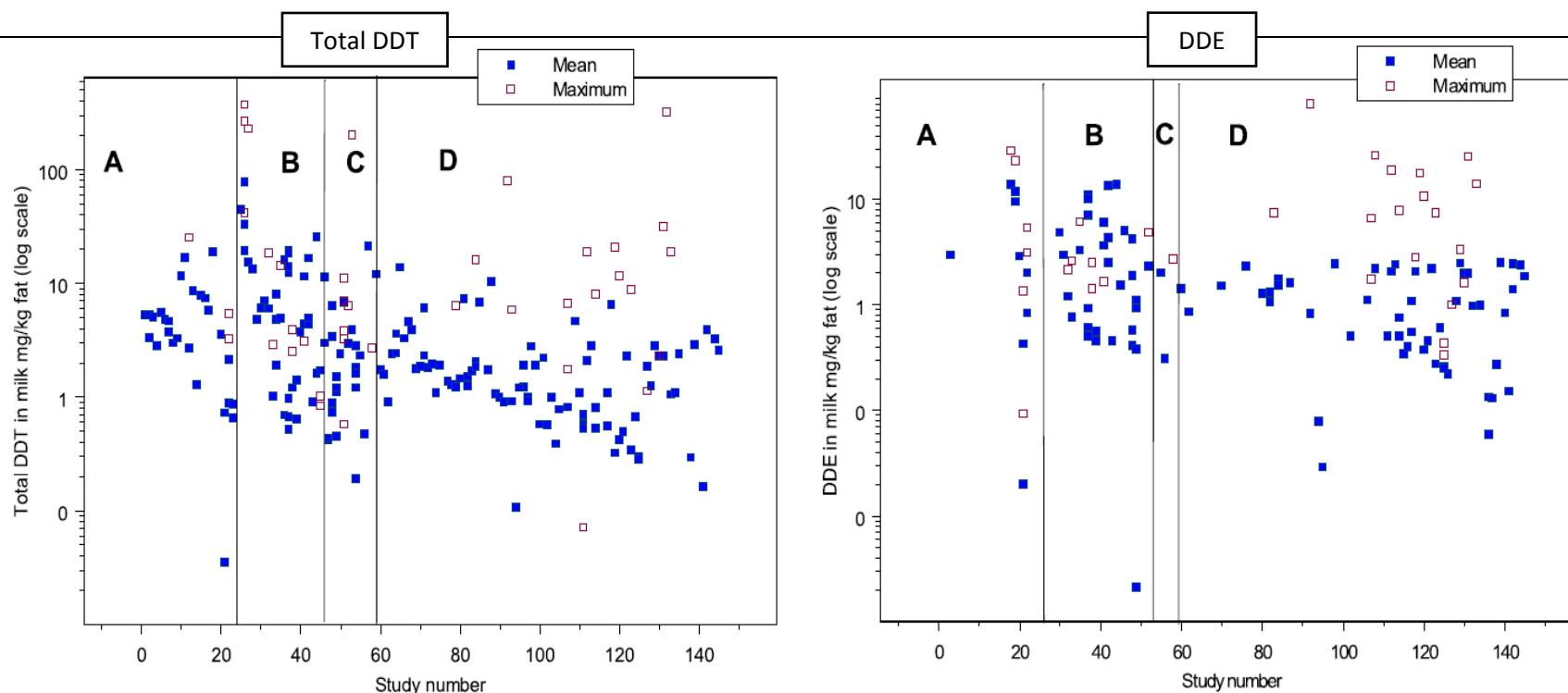
The available literature was reviewed for studies that reported maternal intake levels of DDT and breast milk levels of DDT. A number of studies were found worldwide that reported intake of DDT as discussed in the previous section. In addition, hundreds of studies were found that reported levels of DDT in breast milk. No studies were found that reported both maternal intake of DDT and levels of DDT in breast milk. To summarize the results of intake studies and illustrate the relationship between maternal intake and breast milk levels of DDT, the results of intake studies were grouped with studies reporting levels of DDT in breast milk from similar geographic areas. Studies from Japan, Canada, Eastern Europe, India, and China are summarized and reported in [Table B-13](#), [Table B-14](#), [Table B-15](#), [Table B-16](#), and [Table B-17](#) below.

#### ***DDT in Human Breast Milk***

No studies were found to derive a quantitative relationship between DDT exposure and concentrations of DDT, or its metabolites, in breast milk. The World Health Organization recently reviewed data on human health issues surrounding DDT use for malaria control in an Environmental Health Criteria Report ([WHO, 2011](#)). The report included an exposure assessment focused primarily on direct exposure from Indoor Residual Spraying (IRS), but framed within the context of understanding exposures to DDT from previous agricultural and public health uses of the insecticide. One hundred forty-four studies on DDT and DDE levels in breast milk were reviewed, including both studies where DDT was used for malaria control at the time of sampling and studies where DDT had not been used in the study location for at least 3 years. [Figure B-7](#) is a reproduction of plots from that document illustrating the values measured across studies for Total DDT and DDE in milk fat (left and right panels, respectively). The studies are grouped according to date and DDT use status where the sampling occurred ([WHO, 2011](#)). The studies in sector B in this left panel represent chronic, low level exposure resulting from the use of DDT for IRS at

the time of sampling. The mean concentration of total DDT in milk fat was 7,930 µg/kg (range of reported means: 190 – 76,800 µg/kg) ([WHO, 2011](#)).

The WHO report states that there is a downward trend in total DDT measured in breast milk over time, potentially reflecting the global reduction in DDT use ([WHO, 2011](#)). However, many uncertainties exist that complicate the comparison of studies across locations and time periods. For example, many studies report highly skewed data in which measured DDT or DDE residues are much higher in a subset of the population than in the rest of the population. Also, exposure varied widely across studies due to different DDT use and the time of sampling in relation to DDT application in each location ([WHO, 2011](#)).



Notes:

- A: Countries using DDT; studies arranged in date order of sampling from left to right, 1950-2006.
- B: Countries with endemic malaria using DDT for indoor residual spraying (IRS) studies arranged in date order of sampling from left to right (1970-2000).
- C: Countries where samples were taken immediately following cessation of use of DDT; dates of sampling between 1970 and 2000.
- D: Countries not using DDT for either agriculture or IRS; studies arranged in date order of sampling from left to right; dates are between 1973 and 2004.

Source: Reprinted with permission from WHO ([2011](#)). References for all studies plotted on these graphs can be found in the original source document.

**Figure B-7. Total human milk levels of DDT (left) and p,p'-DDE (right), as  $\mu\text{g/g}$  milk fat, from 144 studies.**

Concentrations of DDT and DDT metabolites in breast milk have decreased over time, as seen in studies of women in Japan ([Konishi et al., 2001](#)), Canada ([Craan and Haines, 1998](#)), and Eastern Europe ([Krauthacker et al., 2009](#)) (see [Table B-13](#), [Table B-14](#), and [Table B-15](#)). For example, Konishi et al. (2001) measured DDT in breast milk from primiparous women (between 9 and 60 participants) in Osaka, Japan between 1972-1998. Measurements were taken yearly, with the exception of 1983 through 1985 (when no measurements were taken). In 1972, the average DDT concentration was  $0.538 \pm 0.319$  µg/g lipid basis (range 0.0130–1.380 µg/g lipid basis) and the average DDE concentration was  $1.686 \pm 0.663$  (range 0.640–2.630 µg/g). Over the 2 decades of measurements, the concentration of DDT steadily decreased while DDE concentration appeared to increase and decrease sporadically until 1982 before following a downward trend from 1986 through 1998 ([Konishi et al., 2001](#)). Study authors estimated that the average daily intake of DDT by infants (assumed body weight of 4.5 kg) as a result of consumption of breast milk decreased from a high of 67.68 µg/kg-day to a low of 0.234 µg/kg-day during the study period.

**Table B-13. DDT in Breast Milk in Japan**

Intake of Total DDT* (µg/day)	Reference	Reported Concentration Range of Total DDT* in Breast Milk (µg/g lipid basis)	Notes	Reference
<b>Japan</b>				
<b>Average:</b> 3.06 (±1.58) <b>Range:</b> 1.10-4.8 <b>Years:</b> 1977-1985	<a href="#">(Matsumoto et al., 1987)</a>	0.052 – 15.04	Concentrations recorded 1972-1998. Represents sum of DDE and DDT concentrations. High concentration in 1976, low concentration in 1996 with a general downward trend over time.	<a href="#">(Konishi et al., 2001)</a>
		0.023 – 0.970	Concentrations from three cities in Japan (Sendai, Kyoto, and Takayama), from a total of 60 participants, 2007-2008. Average level: 0.170 µg/g lipid.	<a href="#">(Haraguchi et al., 2009)</a>
		0.088 – 0.145	Review of six studies from Japan, data collected 2007-2009. Geometric mean level: 0.119 µg/g lipid.	<a href="#">(Fujii et al., 2011)</a>

\* For the purposes of this briefing packet, total DDT includes: p,p'-DDT, o,p'-DDT, and DDE.

**Table B-14. DDT in Breast Milk in Canada**

Intake of Total DDT* (µg/kg bodyweight-day)	Reference	Reported Concentrations of Total DDT* in Breast Milk (µg/g)	Notes	Reference
<b>Canada</b>				
<b>Average:</b> 0.006 <b>Range:</b> 0.003 (in females ≥ 65 years old) – 0.017 (in males and females 1-4 years old) <b>Years:</b> 1992-1996	(Rawns et al., 2004)	Average: 0.246 lipid basis Median: 0.188 lipid basis <sup>a</sup>	Concentrations from 497 participants throughout all provinces of Canada in 1992.	(Newsome et al., 1995)
		0.00751 – 0.134 whole milk	Concentrations recorded in six Canadian surveys from 1967 to 1992. High concentration in 1967, low concentration in 1992.	(Craan and Haines, 1998) <sup>b</sup>
		Average: 0.385 lipid basis Median: 0.2682 lipid basis <sup>a</sup>	Concentrations from all provinces of Canada, from 412 samples in 1986.	(Mes et al., 1993)
		16.9 – 32.7 <sup>c</sup> whole milk, 0.507 – 0.981 milk fat	Concentrations recorded in six surveys of Ontario, Canada from 1975 to 1985. High concentration reported in the western region of Ontario in 1978. Low concentration reported for all of Ontario in 1983-1984.	(Frank et al., 1988)

\* For the purposes of this briefing packet, total DDT includes: p,p'-DDT, o,p'-DDT, and DDE.

<sup>a</sup> Concentrations are the sum of DDE, p,p' DDT, and o,p' DDT concentrations reported in the study.

<sup>b</sup> Review includes the Newsome (1995) study, which is also individually cited.

<sup>c</sup> Concentrations are composed of 81.6-100% DDE and 0-12.4% DDT depending on the year. See Frank et al. (1988) for exact DDE and DDT contributions.

<sup>d</sup> Calculation from whole milk values based on 3% fat in breast milk, from U.S. EPA (1998)

**Table B-15. DDT in Breast Milk in Eastern Europe**

Intake of Total DDE (µg/day)	Reference	Reported Concentrations of Total DDT* in Breast Milk (µg/g lipid basis)	Notes	Reference
<b>Eastern Europe</b>				
<b>Average:</b> 83.38 in Poland and 17.93 in Germany <b>Year:</b> 2003	<a href="#">(Galassi et al., 2008)</a>	Total DDT: Primiparae: Mean: 1.195, Median: 1.123 Secundiparae: Mean: 1.126, Median: 0.822 <sup>a</sup>  DDE: Primiparae: Mean: 1.195, Median: 1.051; Secundiparae: Mean: 0.822, Median: 1.126	Concentrations from 14 primiparae and 13 secundiparae donors in the Wielkopolska region of Poland in 2003.	Szyrwińska and Lulek <a href="#">(2007)</a>
		Mean: 2.5301 <sup>b</sup>	Concentrations from 28 samples from women living in Warsaw, Poland and surrounding areas from 2002 to 2005.	Hernik et al. <a href="#">(2011)</a>
		Mean: 0.1688 Median: 0.113	Concentrations recorded from 1999 to 2006 from 4,314 participants living in Lower Saxony, Germany.	Zietz et al. <a href="#">(2008)</a>
		Total DDT: 0.256 – 2.08 <sup>b</sup> DDE: 0.247 – 1.900	Concentrations recorded in eight surveys in Zagreb, Croatia from 1981 to 2000. High concentration in 1981-1982, low concentration in 1994-1995.	Krauthacker, et al. <a href="#">(2009)</a>

\* For the purposes of this briefing packet, total DDT includes: p,p'-DDT, o,p'-DDT, and DDE.

<sup>a</sup> Concentrations are ΣDDT concentrations reported in the study.

<sup>b</sup> Concentrations are the sum of DDE and p,p' DDT concentrations reported in the study.

**Table B-16. DDT in Breast Milk in India**

Intake of Total DDT* (µg/day)	Reference	Reported Concentrations of Total DDT* in Breast Milk (µg/g lipid basis)	Notes	Reference
India				
<b>Average:</b> 19.24 <b>Range:</b> N/A <b>Year:</b> Not reported. Study submitted for publication in 1992 Authors reported a correlation between blood DDT levels and DDT intake (R=0.685)  Note: Study group consisted of all vegetarians.	(Kashyap et al., 1994)	New Delhi mean: 1.5 Mumbai mean: 0.45 Kolkata mean: 1.1 <sup>a</sup>	Concentrations recorded 2005-2006 in New Delhi (n=21) and Mumbai (n=26), 2004-2005 in Kolkata (n=17), and 2002-2004 in Chennai (n=12).  Note: No mention of dietary choices in study group.	(Devanathan et al., 2009)
		Means from Chennai (city) Primipara: 1.2 Multipara: 1.1 Chennai-Perungudi (suburb-dumping site) Primipara: 0.62 Multipara: 0.31 Parangipettai (fishing village) Primipara: 0.41 Multipara: 0.19 Chidambaram (farm village) Primipara: 0.27 Multipara: 0.077 <sup>a</sup>	Concentrations recorded 2002-2003 from women living in four areas of Chennai, India. Total samples collected: n=43; city: n=12; suburb-dumping site: n= 20; fishing village: n=6; farm village: n=8. Note: No mention of dietary choices in study group.	(Subramanian et al., 2007)
		Means from Bakhoti: 0.179 Chiraigaon: 0.170 Ghodhakhas: 0.174 Minahas: 0.179 <sup>ab</sup>	Concentrations from four rural villages in the Agra region of India (n=36). Note: Study group consisted of all vegetarians. Study year not reported.	(Kumar et al., 2006)

\* For the purposes of this briefing packet, total DDT includes: p,p'-DDT, o,p'-DDT, and DDE.

<sup>a</sup> Concentrations are ΣDDT concentrations reported in the study.

<sup>b</sup> Study authors did not specify whether value was determined in whole milk or in milk fat.

Studies in India (Table B-16) corroborate the concept that concentrations of DDE measured in breast milk are usually higher than concentrations of DDT. However, in one study conducted in the rural Agra region, reported levels of DDT and DDE in breast milk were both about 0.056 µg/g across the areas studied (Kumar et al., 2006). In another study, Devanathan et al. (2009) measured concentrations of

p,p'-DDT and p,p'-DDE in breast milk in 3 metropolitan regions in India (n=12 to 26 per location) in 2005-2006 and determined that the concentration of DDE in breast milk was 4.6 to 5.7 times higher than the concentration of DDT on a lipid basis. The same research group had previously reported breast milk DDT and DDE concentrations measured in 2002-2003 in women living in four areas within 250 km of one another near the southeastern Bay of Bengal coast of India ([Subramanian et al., 2007](#)). The four areas varied from urban to rural: Chennai is the fourth largest metropolitan city in India with a population of about 7.45 million; Perungudi is one of Chennai city's two open solid waste dumpsites; Chidambaram is an agricultural town with a population of about 60,000; and Parangipettai is a fishing village with population of less than 5,000. Overall, total DDT concentrations in India ranged from 0.077 µg/g in multiparous mothers in the agricultural town of Chidambaram ([Subramanian et al., 2007](#)) to 1.5 µg/g in mothers from New Delhi ([Devanathan et al., 2009](#)). While the ratios of DDE:DDT were still greater than 1, they varied considerably among areas and in comparison to those reported by Devanathan et al. ([2009](#)). The concentration of DDE ranged from 13.2 times higher than DDT on a lipid basis for multiparous mother in the city of Chennai to 1.1 times higher for multiparous mothers in the agricultural town, Chidambaram ([Subramanian et al., 2007](#)). The Subramanian et al. ([2007](#)) study provides an additional analysis: average concentration of DDE and DDT were presented separately for primiparous versus multiparous mothers in each location. In all locations, breast milk from primiparous mothers contained higher concentrations of both DDT and DDE than breast milk from multiparous mothers. Furthermore, the ratio of DDE:DDT was lower for multiparous mothers than for primiparous mothers in 3 of the 4 locations (DDE:DDT in multiparous mothers was 112%, 52%, 74%, and 26% that of primiparous mothers across locations). This finding indicates a preferential elimination of DDE compared to DDT in breast milk, which may have implications for exposure via breast milk.

A study in China in 1993 ([Chen and Gao, 1993](#)) ([Table B-17](#)) showed the average intake of DDT to be 20.5 µg/day, with a range of 4.2 to 46.1 µg/day. Mean levels of Total DDT detected in breast milk, on a lipid basis, ranged from 0.58 to 1.5 µg/g lipid. Studies monitoring milk levels of DDT were conducted between December 2001 and November 2007. The results of the studies are reported in [Table B-17](#). For all of the studies, p,p'-DDE contributed the majority of the residue levels reported in breast milk, ranging from 92.0% ([Hui et al., 2008](#)) to 94.6% ([Leng et al., 2009](#)) of the total DDT measured.

### ***DDT in Infants' and Children's Tissues***

In a study by Bouwman et al. ([1992](#)) as cited in ([WHO, 2011](#)), a relationship between the percent DDT in maternal breast milk and percent DDT in infant blood was illustrated in mothers and their infants (0–2 years old). The mothers and their infants were exposed to DDT through its annual use for malaria control in their community. While total DDT levels measured in infant blood increased with infant age, multiple regression analysis also showed a correlation between DDT levels in breast milk and in infant blood. In a study by Niessen et al. ([1984](#)), ΣDDT was measured in adipose tissue from 48 children at a single hospital in Germany between the spring of 1982 and spring of 1983. It is important to note that the study authors did not directly measure the amount of ΣDDT in samples of the mothers' breast milk, however they state that written records provided "exact information on mothers' milk intake and length of the nursing period" ([Niessen et al., 1984](#)). As seen in [Table B-18](#), breast milk consumption correlated



significantly with the level of  $\Sigma$ DDT in children's adipose tissue. The average  $\Sigma$ DDT for the low milk consumption groups was significantly lower than for the high milk consumption groups when measured within 10 weeks post-weaning (0.61 ppm compared to 1.44 ppm,  $p < 0.002$ ) as well as when measured > 10 weeks post-weaning (0.25 ppm compared to 0.86 ppm,  $p < 0.01$ ). When the intake of breast milk stopped and growth dilution occurred over time, the concentration in adipose tissue decreased ([Niessen et al., 1984](#)).

**Table B-17. DDT in Breast Milk in China**

Intake of Total DDT* ( $\mu\text{g/day}$ )	Reference	Reported Concentrations of Total DDT* in Breast Milk ( $\mu\text{g/g}$ lipid basis)	Notes	Reference
<b>China</b>				
<b>Average:</b> 20.5 <b>Range:</b> 4.2 – 46.1 <b>Year:</b> 1993	<a href="#">(Chen and Gao, 1993)</a>	Lower bound mean: 0.5828 Upper bound mean: 0.5843 <sup>a</sup>	Concentrations recorded from August to November 2007 in twelve provinces of China (Heilongjiang, Liaoning, Hebei, Henan, Shanxi, Ningxia, Jiangxi, Fujian, Shanghai, Hubei, Sichuan and Guangxi). 1,237 individual samples were collected and pooled by urban and rural area for each province, resulting in 24 pooled samples.	<a href="#">(Zhou et al., 2011)</a>
		Average: 1.50 <sup>a</sup>	Concentrations recorded from mothers who gave birth in Hong Kong from December 2001 to September 2002. 238 individual milk samples were collected and pooled into 10 groups based on mothers' residential background, diet, or smoking status.	<a href="#">(Hui et al., 2008)</a>
		Tianjin Median: 0.5804 Yantai Median: 0.7568 <sup>b</sup>	Concentrations recorded between November 2006 and April 2007 from 60 donors in Tianjin and 48 donors in Yantai, China. All donors were primipara.	<a href="#">(Leng et al., 2009)</a>

\* For the purposes of this briefing packet, total DDT includes: p,p'-DDT, o,p'-DDT, and DDE.

<sup>a</sup> Concentrations are  $\Sigma$ DDT concentrations reported in the study.

<sup>b</sup> Concentrations are the sum of p,p'-DDE

**Table B-18. Concentration of  $\Sigma$ DDT in Children's Adipose Tissue Related to Breast Milk Consumption Patterns**

Average Interval (weeks) <sup>a</sup>	Average Total Breast Milk Intake <sup>b</sup> (kg)	$\Sigma$ DDT Average (ppm)	$\Sigma$ DDT Range (ppm)	$\Sigma$ DDT Median (ppm)	P-value compared to low intake group
<b>Low Intake, Short Interval (n=10)</b>					
4.95 $\pm$ 2.3	2.9 $\pm$ 3.3	0.61 $\pm$ 0.20	0.33-0.86	0.58	NA
<b>Low Intake, Long Interval (n=12)</b>					
44.9 $\pm$ 30.4	3.6 $\pm$ 3.1	0.25 $\pm$ 0.11	0.11-0.46	0.23	NA
<b>High Intake, Short Interval (n=11)</b>					
1.2 $\pm$ 2.4	30.5 $\pm$ 21.9	1.44 $\pm$ 0.77	0.65- 2.92	1.28	<0.002
<b>High Intake, Long Interval (n=8)</b>					
47.5 $\pm$ 26.7	38.7 $\pm$ 17.9	0.86 $\pm$ 0.58	0.19-1.85	0.67	<0.01

<sup>a</sup>Interval refers to the time between weaning and collection of the adipose sample.

<sup>b</sup>Total breast milk intake refers to total amount of breast milk consumed by the child from birth through weaning.

NA = Not applicable

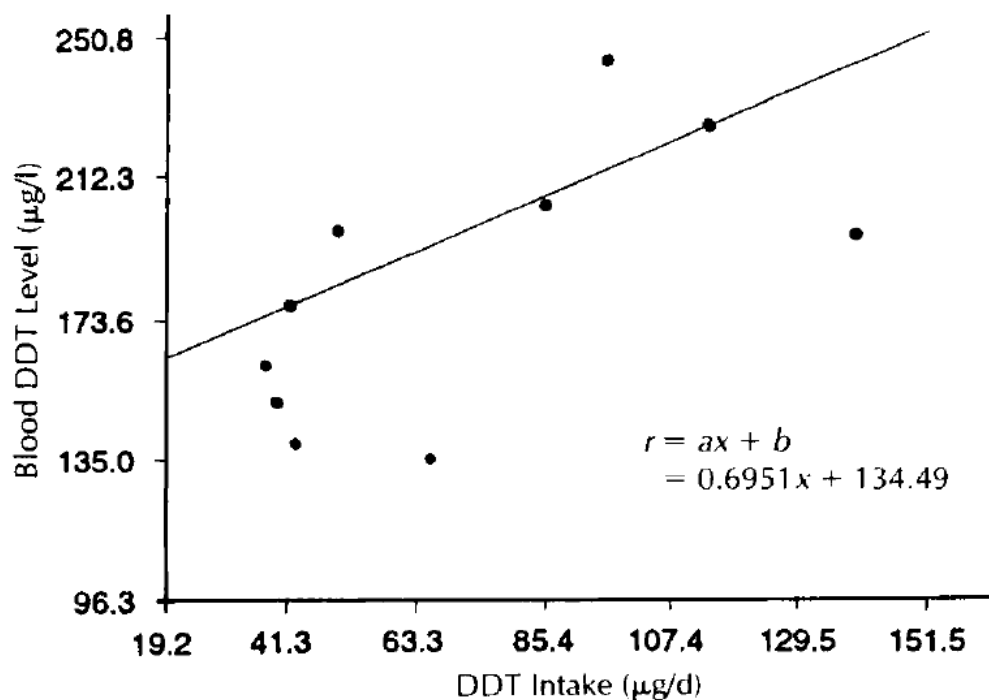
Source: Niessen ([1984](#))

### *DDT in Other Human Tissues*

As mentioned in [Section B.2.2.1](#), few studies have concurrently measured DDT in both foods and blood or other human tissues, making it difficult to draw connections between dietary intake and body burdens of DDT. Galassi et al. ([2008](#)) found that average placenta and blood plasma concentrations of p,p'-DDE were higher in the Polish population than the German population surveyed as shown in [Table B-19](#). Frank et al. ([1993](#)) measured DDE residues in the blood of 750 Ontario, Canada residents from 1986-1987. Whole blood concentrations of DDE averaged  $3.7 \pm 3.9$   $\mu\text{g}/\text{kg}$  (maximum: 51  $\mu\text{g}/\text{kg}$ ) ([Frank et al., 1993](#)). Kashyap et al. ([1994](#)) evaluated the relationship between blood DDT levels and DDT intake among 12 study participants. Blood levels of DDT ranged from 135.0 to 250.8  $\mu\text{g}/\text{l}$ , and DDT intake was estimated from the duplicate diet analysis (see [Figure B-8](#)). The investigators calculated a correlation coefficient of 0.685, illustrating that DDT levels in blood are related in a quantifiable manner to dietary intake ([Kashyap et al., 1994](#)).

**Table B-19. Concentration of DDE in Various Human Tissues**

Location; Date	Participants/Samples	p,p'-DDE	Source
Karlsruhe and Boone, Germany; 2003	15 placenta samples; mean age of mothers $31 \pm 5.6$ years	(without max outlier): mean $1.88 \pm 2.73$ ng/g median: 0.89 ng/g <sup>a</sup>	Galassi et al. (2008)
Gdansk, northern Poland; 2003	15 placenta samples; mean age of mothers $28.4 \pm 5.7$ years	(without max outlier): mean $2.92 \pm 1.56$ ng/g median: 2.69 ng/g	Galassi et al. (2008)
Karlsruhe and Boone, Germany; 2003	15 blood plasma samples; 33.3% females; mean age $39.5 \pm 15.2$ years	mean: $0.16 \pm 0.40$ ng/g median 0.17 ng/g	Galassi et al. (2008)
Gdansk, northern Poland; 2003	15 blood plasma samples; 33.3% females; mean age $38.0 \pm 13.9$ years	mean: $0.47 \pm 0.72$ ng/g median 0.20 ng/g	Galassi et al. (2008)
Large urban centers, Ontario Canada; 1986-1987	581 whole blood samples; patients suspecting oral, dermal, or inhalation exposure to PCBs	mean: $3.8 \pm 4.0$ µg/kg max: 51.0 µg/kg	Frank et al. (1993)
Medium/small urban centers, Ontario Canada; 1986-1987	169 whole blood samples; patients suspecting oral, dermal, or inhalation exposure to PCBs	mean: $3.2 \pm 3.2$ µg/kg max: 16.0 µg/kg	Frank et al. (1993)
All sizes urban centers, Ontario Canada; 1986-1987	750 whole blood samples; patients suspecting oral, dermal, or inhalation exposure to PCBs	mean: $3.7 \pm 3.9$ µg/kg max: 51.0 µg/kg	Frank et al. (1993)



<sup>a</sup>1 ng/g DDE = 0.001 μg/kg DDE

Note: Reprinted with permission of Taylor & Francis; Kashyap et al. (1994).

**Figure B-8. Dietary Intake versus DDT Level in Blood**

### B.2.3. Maternal Dose vs. Nursing Offspring Dose in Animals

#### B.2.3.1. Maternal Exposure and Elimination in Milk

Several studies have examined the fate of DDT following administration to maternal animals such as rats and ruminants.

##### *Rats*

Analyses from rat intake and milk level studies show that lactation is a significant elimination route for DDT in rat dams and that DDT is transferred from dams to offspring via milk (You et al., 1999; Ando and Wakisaka, 1978; Fang et al., 1977; Telford and Guthrie, 1945).

Using a PBPK model, You et al. (1999) showed that transfer by milk, rather than placental transfer, is the predominant route of transfer of DDE from dams to offspring in rats using a study design that dosed some mothers during gestation periods only, some during gestation and lactation, and some during

lactation only. This study is discussed in more detail in [Section B.2.5](#). Ando and Wakisaka ([1978](#)) found that 21.5% of an intraperitoneal dose of DDT was transferred from the rat dams to neonates via milk ([Ando, 1978](#)). The authors do not provide an elimination rate for DDT from the nursing dams.

A study by Hayes ([1976](#)) found that rat dams fed 32.4 mg/kg-day DDT produced milk with a DDT concentration between 50.73 and 102.82 µg/ml of milk. The calculated percentage of the total administered DDT dose excreted in milk by PND 14 ranged from 17.2% to 30.0%.

Fang et al. ([1977](#)) found that the maternal transfer of DDT to infants via milk resulted in lower DDT levels in dams than in their offspring, indicating a decreased body burden in mothers following nursing. In this study, female rats were given a single oral dose of 0.9 mg of <sup>14</sup>C-ring labeled p,p'-DDT immediately after parturition. Newborn rats from three litters of the dosed group were sacrificed on postnatal day (PND) 1, 2, 3, 4, 7, 11, 14, 21, or 28, and their stomach contents were analyzed to measure DDT concentration in milk. On the first day after dosing, the mean DDT concentration in rat milk was 15.26 µg/g dry weight, equivalent to 2.3 µg/g whole weight (assuming that rat milk contains 85% water). The radioactive concentration of DDT in milk decreased gradually over the course of the experiment (rate constant - 0.096), indicating a first-order excretion process for DDT in milk and a half-life of 7.2 days. The study authors estimated that the maximum initial concentration of DDT was 3,600 µg/kg, assuming even distribution of DDT throughout the whole body. When tissues from nursing dams sacrificed 14 to 28 days after dosing were compared to offspring sacrificed at corresponding times, dams had much lower radioactivity. Radioactivity was predominately in dams' fatty tissue, with about five times less present in their livers. The highest radioactivity in offspring occurred in liver, intestine, and carcass ([Fang et al., 1977](#)).

### *Ruminants*

Studies on DDT intake and milk levels in cows and buffalo may contribute to our understanding of the behavior of DDT in mammals and may have important implications for human dietary exposure. However, these studies are less useful for understanding how DDT intake affects human milk levels due to the likely differences in metabolism between ruminants (e.g., cows and buffalo) and humans. In addition, ruminants such as dairy cows would likely be lactating during a greater proportion of their exposure duration than would humans, which could potentially decrease their body burden and the resulting levels of DDT in milk. In fact, levels of DDT in cow's milk are typically lower than in human breast milk ([ATSDR, 2002a](#)). Studies reviewed in this briefing packet investigated differences in cows' and buffalo's milk levels based on concentration and duration of DDT intake ([Kalra et al., 1986](#); [Fries and Marrow, 1974](#); [Witt et al., 1966](#)), route of exposure ([Kalra et al., 1986](#); [Witt et al., 1966](#)), and analog of DDT (p,p'-DDT, p,p'-DDD, or p,p'-DDE) ([Kapoor and Kalra, 1993](#); [Fried et al., 1969](#)).

As shown by the results of several studies in ruminants, increased concentration and duration of DDT intake results in increased contaminant concentrations in milk ([Kalra et al., 1986](#); [Fries and Marrow, 1974](#); [Witt et al., 1966](#)). Fries and Marrow ([1974](#)) measured DDT levels every 5 days in milk from groups of lactating cows dosed with 5 or 25 mg of DDE per day, for 60 days. At 60 days, the concentration of

DDE in milk fat in the low dose group was 2.3 ppm compared to 11.1 ppm in the the high dose group. The authors determined that the half-life for DDE in milk ranged from 29 to 64 days and was inversely related to the level of milk fat production.

There are also studies that show differences in the metabolite profiles of DDT in cow and buffalo milk based on route of exposure ([Kalra et al., 1986](#); [Witt et al., 1966](#)). Witt et al. (1966) showed that doses of DDT that passed through the rumen are metabolized (to DDE and DDD) to a much greater extent than DDT doses that bypassed the rumen. Kalra et al. (1986) found that milk residues from buffalo exposed dermally to DDT contained mainly p,p'-DDT while milk residues from buffalo exposed orally to DDT contained greater concentrations of DDT metabolites.

Other studies have compared milk levels resulting from exposure to different analogs of DDT ([Kapoor and Kalra, 1993](#); [Fried et al., 1969](#)). Fried et al. (1969) orally exposed cows to 25 mg/day of p,p'-DDT, p,p'-DDD, or p,p'-DDE for 60 days and analyzed milk samples throughout the exposure period and 60 days after termination of exposure. The study authors found that milk from cows dosed with p,p'-DDE and p,p'-DDD contained only the compounds administered to the animals; no metabolites were observed. However, cows dosed with p,p'-DDT excreted the metabolite p,p'-DDD in addition to p,p'-DDT. During days 40 through 60 of the dosing period, 25.8% of the initial p,p'-DDE, 7.6% of the initial p,p'-DDD, and 5.1% of the initial p,p'-DDT (as 3.0% p,p'-DDD and 2.1% p,p'-DDT) was excreted in milk, almost at equilibrium. Pesticide decline in milk was also modeled, indicating that p,p'-DDT and p,p'-DDE levels decline at about the same rate, but p,p'-DDD levels decline twice as fast ([Fried et al., 1969](#)).

Kapoor and Kalra (1993) dosed 4 groups of Indian buffalo with 25 mg of p,p'-DDT, p,p'-DDD, p,p'-DDE, or o,p'-DDT daily for 100 days. Pesticide residues were measured in milk throughout the dosing period and for 100 days afterward. When pesticide levels in milk reached the 'plateau level,' the average milk fat concentrations of total DDT were 5.0, 7.2, 6.7. and 1.1 mg/kg for buffalo dosed with p,p'-DDT, p,p'-DDD, p,p'-DDE, and o,p'-DDT, respectively. Buffalo that were fed p,p'-DDT excreted p,p'-DDD (73%) and p,p'-DDT (25%) in their milk. Buffalo fed p,p'-DDD or p,p'-DDE excreted unaltered residues of the compounds they were fed. Milk residues from buffalo fed o,p'-DDT contained o,p'-DDD (60%) and p,p'-DDT (40%) ([Kapoor and Kalra, 1993](#)). Kapoor and Kalra (1993) noticed one rapid phase of decline for buffalo fed o,p'-DDT (half- life was 2.8 days) and two phases of decline for buffalo fed p,p'-DDT, p,p'-DDD, and p,p'-DDE. For the latter compounds, the first phase of decline was rapid and resulted in half-lives of 3.8, 2.8, and 10.0 days for p,p'-DDT, p,p'-DDD, and p,p'-DDE, respectively. The second "slow-decline" phase resulted in half-lives of 12.4, 11.8, and 36.8 days for the three compounds, respectively. These phases may be explained by the excretion of compounds from the circulatory system in the first phase and then from the fatty tissues in the second phase ([Kapoor and Kalra, 1993](#)).

---

## B.2.4. Developmental Effects of DDT

---

### B.2.4.1. Human Studies

Developmental effects of concern examined in human epidemiological studies included: cryptorchidism and hypospadias in males; wheezing, asthma, and atopy; immunological impairments; pubertal development; neurological effects; and neurodevelopmental effects. Two studies identified associations between DDE infant blood levels and effects examined postnatally ([Sunyer et al., 2006](#); [Dewailly et al., 2000](#)). In one study, diagnosed asthma and persistent wheezing at 6.5 years of age were associated with elevated infant serum DDE levels at birth but not with serum DDE levels at 4 years of age, regardless of breastfeeding status of the child ([Sunyer et al., 2006](#)). Breastfeeding was found to be protective against the diagnosis of asthma or wheezing in children at age 6, regardless of DDE levels at birth. Study authors concluded that postnatal exposure of DDE via breastfeeding is less relevant to asthma status than prenatal exposure ([Sunyer et al., 2006](#)). In the second study, authors found no clinically relevant differences in immunological parameters in breastfed and formula-fed infants within the first year of life and no association of infant DDE serum levels with immunological parameters ([Dewailly et al., 2000](#)). The relative risk of ear infections in infants (ages 4 to 7 months) with the highest prenatal p,p'-DDE exposure compared to infants with the lowest prenatal p,p'-DDE exposure (as measured by breast milk DDE concentration 3 days postpartum) was 1.87 (95% confidence interval 1.10–2.03). The relative risk of ear infections over 12 months was also increased with increased prenatal DDE exposure (1.52; 95% confidence interval 1.05–2.22) ([Dewailly et al., 2000](#)).

In reviewing epidemiological studies to evaluate the developmental effects from exposure to DDT, it can be difficult to separate effects attributable to lactational exposure from those that may be a result of gestational exposure. This is mainly due to the nature of human exposures to DDT and the design of most epidemiological studies. Damgaard et al. ([2006](#)) and Bhatia et al. ([2005](#)) examined effects such as cryptorchidism and hypospadias in infants at birth and 3 months or 2 years after birth, respectively. Although lactational or environmental postnatal exposure to DDT is possible, the adverse reproductive effects examined in these two studies are present at birth and can be attributed to gestational exposure ([Damgaard et al., 2006](#); [Bhatia et al., 2005](#)).

Six studies evaluated the neurodevelopmental impact of exposure to DDT using various developmental scales ([Torres-Sánchez et al., 2009](#); [Morales et al., 2008](#); [Fenster et al., 2007](#); [Torres-Sanchez et al., 2007](#); [Eskenazi et al., 2006](#); [Ribas-Fito et al., 2003](#)). In many cases, DDT levels were associated with lower performance by children at ages ranging from 1 month to 4 years. Some researchers observed sex differences in the effect of DDT on performance on neurodevelopmental tests; others observed gene-dependent differences in effects.

Eskenazi et al. ([2006](#)) studied the neurodevelopmental effects of prenatal exposures to p,p'-DDT, o,p'-DDT, and p,p'-DDE in a cohort of Mexican farm workers' children living in California. Children were evaluated at 6, 12, and 24 months of age using two neurodevelopmental (Bayley) scores, the Psychomotor Development Index (PDI) and Mental Developmental Index (MDI). A 10-fold increase in

maternal p,p'-DDT and p,p'-DDE serum levels was associated with 5.9- and 6.5-point reductions in PDI scores across the range of exposures at 6 months (both were significant at  $p > 0.05$ ). At 12 months, a 7.9-point decrease in PDI scores was associated with increased p,p'-DDT levels ( $p < 0.05$ ), but there was no association with p,p'-DDE levels. Increased levels of o,p'-DDT in boys had a significant negative association with PDI scores at 12 months ( $P = 0.10$ ); however, there was no association for girls. At 24 months, there was no association between DDT/DDE levels and PDI scores in either gender. There was no association between DDT/DDE serum levels and MDI scores at 6 months. At 12 months, a 10-fold increase in levels of p,p'-DDT and o,p'-DDT was associated with a 1.71- ( $p < 0.05$ ) and 2.56-point ( $p < 0.01$ ) decrease in MDI scores, respectively. However, there was no association for p,p'-DDE levels and MDI scores. At 24 months, decreased MDI scores were associated with increased levels of p,p'-DDT ( $p < 0.05$ ), o,p'-DDT ( $p < 0.01$ ) and p,p'-DDE ( $p < 0.10$ ). MDI point reductions were 2.12, 3.06, and 2.44 points, respectively. Although maternal DDT/DDE serum levels were associated with decreased Bayley scores in infants, breastfeeding was positively associated with increases of about 0.20 points in MDI scores at 12 and 24 months ( $p < 0.05$ ) and positively, but not significantly, associated with increased MDI and PDI scores at 6 months.

In the same cohort studied by Eskenazi et al. ([2006](#)), Fenster et al. ([2007](#)) measured neurodevelopmental effects of prenatal exposures to p,p'-DDT, o,p'-DDT and p,p'-DDE based on the Brazelton neonatal behavioral assessment scale (BNBAS). Infants were assessed using the seven BNBAS clusters: habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and reflex. Maternal DDT/DDE serum levels were not significantly associated with BNBAS performance.

Ribas-Fitó et al. ([2003](#)) studied exposures to p,p'-DDE in a cohort of 92 mother-infant pairs living in the vicinity of an electrochemical factory in Spain. Mental and psychomotor development were assessed in infants at around 13 months of age ( $\pm 6$  weeks) using the Bayley and Griffiths Scales of Infant Development. Researchers found a negative dose-response relationship between the Bayley scores (MDI and PDI) and maternal serum levels of p,p'-DDE. For each two-fold increase in p,p'-DDE levels, there was a 3.50-point decrease in MDI and 4.01-point decrease in PDI score. Scores were significantly decreased at the highest level of exposure for both MDI ( $p = 0.02$ ) and PDI ( $p = 0.01$ ). Increased maternal serum levels of p,p'-DDE were also associated with decreased scores on the Locomotor, Personal-Social, and Performance areas of the Griffiths Scales. Bayley scores were positively associated with duration of breastfeeding ( $p = 0.04$ ).

The authors also compared Bayley scores for infants based on breastfeeding duration (none, 2-16 weeks, or  $> 16$  weeks) and maternal p,p'-DDE serum levels ( $\leq$  the median,  $0.85 \mu\text{g/L}$ , or  $> 0.85 \mu\text{g/L}$ ) ([Ribas-Fito et al., 2003](#)). Infants breastfed for more than 16 weeks who were exposed to less than the median serum level ( $0.85 \mu\text{g/L}$ ) of p,p'-DDE had significantly higher MDI ( $p < 0.05$ ) and PDI ( $p < 0.05$ ) scores than infants breastfed for 2 to 16 weeks who were exposed to greater than the median serum level of p,p'-DDE. There were no other significant differences within these groups.



A cohort study in Morelos, Mexico, a city where malaria was endemic, assessed effects of prenatal p,p'-DDE exposure on the neurodevelopment of 244 children ([Torres-Sanchez et al., 2007](#)). PDI and MDI scores were measured in children at 1, 3, 6 and 12 months of age. PDI scores were negatively associated with maternal serum p,p'-DDE measured during the first trimester: the PDI score was reduced 0.52 points for every 2-fold increase in p,p'-DDE. There were no associations between PDI scores and exposure during the second or third trimesters or between MDI scores and p,p'-DDE throughout the pregnancy. Children who were breastfed tended to have higher PDI and MDI scores, but the trend was only significant for MDI scores. There was no significant correlation between breastfeeding and maternal serum DDE during the first trimester ( $p=0.20$ ). Torres-Sanchez et al. ([2009](#)) measured the PDI and MDI scores in children from the same cohort at 12-30 months of age. Although there was a negative association between PDI scores and prenatal DDE exposure during the first 12 months ([Torres-Sanchez et al., 2007](#)), it did not persist for the full duration of the study. There was no association between prenatal DDE exposure and MDI scores.

Morales et al. ([2008](#)) studied the cognitive effects of early-life exposure to p,p'-DDT in children of different genotypes at 4 years of age. Prenatal exposure to p,p'-DDT was indicated by cord blood serum concentrations, and cognitive skills were assessed using the McCarthy Scales of Children's Abilities. Genotyping was conducted for glutathione S-transferase (GST) genes (GSTP1, GSTM1, and GSTT1). Researchers found that measures of cognitive function were inversely associated with p,p'-DDT cord serum levels in children with the GSTP1 Val-105 allele. The presence of GSTP1 polymorphisms and prenatal exposure to p,p'-DDT significantly reduced general cognitive skills ( $p=0.05$ ), quantitative skills ( $p=0.02$ ), executive function ( $p=0.01$ ), and working memory ( $p=0.02$ ), but not motor skills ( $p=0.47$ ). Children with the GSTM1 and GSTT1 polymorphisms did not show significant associations between impaired cognitive function and p,p'-DDT exposure.

**Table B-20. Overview of Developmental Effects of DDT and/or DDE in Humans Reported in Epidemiological Literature**

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 360 Mexican farm workers' children examined at 6, 12, and 24 months; California; October 1999–October 2000	Neurodevelopmental effects; Bayley scores at 6, 12, and 24 months	Geometric mean maternal serum lipid levels: p,p'-DDT: 0.022 o,p'-DDT: 0.0018 p,p'-DDE: 1.4369	NR	NR	NR	Maternal prenatal DDT/DDE serum levels were variably associated with decreased neurodevelopmental scores at 6, 12, and 24 months, yet breastfeeding was positively associated with increases of about 0.20 points in MDI scores at 12 and 24 months (p<0.05) and positively but not significantly associated with increased MDI and PDI scores at 6 months	Eskenazi et al. ( <a href="#">2006</a> )
Cohort; 303 Mexican farm workers' children examined at 6, 12, and 24 months; California; October 1999–October 2000	Neurodevelopmental effects; BNBAS	Geometric mean maternal serum level of p,p'-DDT was 0.0232, o,p'-DDT was 0.0018, and p,p'-DDE was 1.4642 µg/g	NR	NR	NR	DDT/DDE maternal serum levels were not significantly associated with infants' BNBAS performance, which included markers of habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and reflex.	Fenster et al. ( <a href="#">2007</a> )

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 92 participants <sup>b</sup> ; infants with varied breastfeeding durations (none, 2-16 weeks, or >16 weeks) examined at 13 months $\pm$ 6 weeks; near an electrochemical factory, Spain; 1997–1999	Mental and psychomotor development; Bayley and Griffiths Scales	Median p,p'-DDE serum level = 0.85 $\mu$ g/L	NR	NR	NR	Negative dose-response relationship between Bayley scores and serum levels of p,p'-DDE. For each two-fold increase in p,p'-DDE levels, there was a 3.50-point decrease in MDI and 4.01-point decrease in PDI score; scores were positively associated with duration of breastfeeding ( $p=0.04$ ). Increased serum levels of p,p'-DDE were also associated with decreased scores on the Locomotor, Personal-Social, and Performance areas of the Griffiths Scales.	Ribas-Fitó et al. ( <a href="#">2003</a> )

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 244 participants <sup>b</sup> ; infants examined at 1, 3, 6, 12 months and 12-30 months; Morelos, Mexico; January 2001–June 2005	Neurodevelopmental effects; Bayley Scales	Geometric mean p,p'-DDE level 6.4, 6.8, and 7.8 µg/L (ppb) for the first, second, and third trimesters, respectively	NR	NR	NR	PDI scores were negatively associated with p,p'-DDE during the first trimester; the PDI score was reduced 0.52 points for every 2-fold increase in p,p'-DDE measured in the first trimester. There were no associations between PDI scores and exposure during the second or third trimesters or between MDI scores and p,p'-DDE throughout the pregnancy. Children who were breastfed tended to have higher PDI and MDI scores, although the trend was only significant for MDI scores. The correlation between breastfeeding and DDE exposure during the first trimester was not significant (p=0.20). Although there was a negative association between PDI scores and prenatal DDE exposure during the first 12 months but did not persist for the full duration of the study.	Torres-Sanchez et al. ( <a href="#">2009</a> ; <a href="#">2007</a> )

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 326 children examined at 4 years of age; Menorca, Spain; enrolled prenatally over 1 year starting in mid1997	Cognitive function; McCarthy Scales of Children's Abilities; Genotyping for glutathione S-transferase genes (GSTP1, GSTM1, and GSTT1)	NR	NR	cord serum mean concentration p,p'-DDT = $0.17 \pm 0.23$ ppb; p,p'-DDE = $1.63 \pm 1.93$ ppb	NR	levels of some cognitive functions (including general cognitive, memory, quantitative, and verbal skills as well as executive function and working memory) were inversely associated with p,p'-DDT cord serum levels for children with the GSTP1 Val-105 allele. The presence of GSTP1 polymorphisms and prenatal exposure to p,p'-DDT significantly reduced general cognitive skills ( $p=0.05$ ), quantitative skills ( $p=0.02$ ), executive function ( $p=0.01$ ), and working memory ( $p=0.02$ ), but not motor skills ( $p=0.47$ ). Children with the GSTM1 and GSTT1 polymorphisms did not show significant associations between impaired cognitive function and p,p'-DDT exposure.	Morales et al. ( <a href="#">2008</a> )
Case-control; 62 cases, 68 controls; male infants examined at birth and at 3 months; breast milk sampled 1–3 months postpartum; Denmark and Finland; 1997–2001	Cryptorchidism in males at 3 months of age	NR	Controls: 0.12 Cases: 0.14 (ppm-lipid, median)	NR	NR	No significant differences in maternal breast-milk concentrations of total DDT between cryptorchid and healthy male infants	Damgaard et al. ( <a href="#">2006</a> )

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Case-control; 75 cases of cryptorchidism, 66 cases of hypospadias, and 283 controls; maternal serum sampled at birth; San Francisco, CA, USA; 1959–1967	Cryptorchidism and hypospadias in males at 2-years of age	Controls: 6.6 Cryptorchid cases: 6.7 Hypospadias cases: 5.8 (ppm-lipid, median)	NR	NR	NR	No significant association between maternal serum concentrations of DDE or DDT and birth outcomes	Bhatia et al. (2005)  (Authors used prenatal serum samples for 86 women for whom birth samples were not available. Authors reported median concentrations for DDE and DDT separately; these concentrations were added here to reflect total DDT concentrations.)
Cohort; 402 participants <sup>b</sup> ; children examined at 1, 2, 3, 4, and 6 years of age; cord serum collected at birth, serum of children collected at 4 years of age, Menorca, Spain; 1997–1998	Wheezing, asthma, and atopy	0.001 (ppm, median)	NR	0.0009 (ppm, median)	NR	No significant association between serum concentrations and outcomes; DDE concentration was significantly decreased at 4 years in formula-fed children compared to breastfed children; elevated DDE concentration at birth increased risk of asthma and persistent wheezing at age 6; no significant association between DDE concentration at 4 years and risk of asthma or persistent wheezing	Sunyer et al. (2006)

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 171 participants <sup>b</sup> ; infants examined at 0–3, 4–7, and 8–12 months; breast milk sampled 1–3 days postpartum; Quebec, Canada; 1989–1990	Incidence of acute infectious disease and serum immunology in infants and the effect of breast feeding on these outcomes	NR	Tertile 1: <0.73 Tertile 2: 0.73–1.32 Tertile 3: >1.32 (ppm-milk fat)	NR	NR	No significant association between DDE exposure and immunologic outcomes; At 4–7 months the relative risk of ear infection in the third tertile of DDE exposure was 1.87 times that of the first tertile; at 8–12 months the relative risk of ear infection in the second tertile of DDE exposure was 1.63 times that of the first tertile	Dewailly et al. (2000)  (Cohort was an Inuit population.)
Cohort; 199 participants <sup>b</sup> ; maternal serum sampled at birth, infant serum sampled at median age of 7 months; Quebec, Canada; 1995–2001	Incidence of acute infectious disease	Quartile 1: <0.183 Quartile 2: 0.183–0.281 Quartile 3: 0.281–0.472 Quartile 4: >0.472 (ppm-lipid)	NR	Quartile 1: <0.1 Quartile 2: 0.1–0.355 Quartile 3: 0.355–0.618 Quartile 4: >0.618 (ppm-lipid)	NR	No significant association between incidence of acute infections and maternal or infant concentrations of p,p'-DDE	Dallaire et al. (2004)  (Cohort was an Inuit population.)
Cohort; 802 participants <sup>b</sup> ; maternal serum sampled at birth and 1.5, 3, 6, 12, 18, 24, 36, 48, and 60 months postpartum, breast milk sampled at birth until lactation ceased, infants examined at 6 and 12 months of age; North Carolina, USA; 1978–1982	Neurodevelopmental effects; Bayley Scales of Infant Development scores of children 6 and 12 months of age	NR	0–≥6 (ppm-milk fat, range)	NR	NR	No significant association between Bayley Scales scores at 6 or 12 months and concentrations of DDE at birth or in breast milk	Gladden et al. (1988)  (Breast milk concentration was estimated from all breast milk and serum samples to represent maternal body burden at birth. Median exposure estimate was not reported.)

Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 594 participants <sup>b</sup> ; maternal serum sampled at birth and 1.5, 3, 6, 12, 18, 24, 36, 48, and 60 months postpartum, breast milk sampled at birth until lactation ceased, children surveyed from 10–15 years old; North Carolina, USA; 1978–1982	Height, weight, and pubertal development	NR	2.4 ppm DDE (milk fat, median)	NR	6.2 mg DDE (median lactational exposure index, total DDE over duration of breast-feeding)	No significant association between height, weight, or pubertal development and estimated maternal body burden or estimated lactational exposure to p,p'-DDE	Gladen et al. ( <a href="#">2000</a> )  [This study is a follow-up study on the same cohort examined in Gladen et al. ( <a href="#">1988</a> ). Breast milk concentration was estimated from all breast milk and serum samples to represent maternal body burden at birth. Lactational exposure was estimated from breast milk concentration and duration of breast feeding to represent total consumption by the child.]
Cohort; 670 participants <sup>b</sup> ; maternal serum sampled at birth and 1.5, 3, 6, 12, 18, 24, 36, 48, and 60 months postpartum, breast milk sampled at birth until lactation ceased, children examined at 18 and 24 months; North Carolina, USA; 1978–1982	Neurodevelopmental effects; Bayley Scales of Infant Development scores	NR	0–≥6 (ppm-milk fat, range)	NR	NR	No significant association between Bayley Scales scores at 18 or 24 months and concentrations of DDE at birth or in breast milk	Gladen and Rogan ( <a href="#">1991</a> )  (This study is a follow-up study on the same cohort examined in Gladen et al. ( <a href="#">1988</a> ). Breast milk concentration was estimated from all breast milk and serum samples to represent maternal body burden at birth. Median exposure estimate was not reported.)



Study Design	Effect Examined	Maternal DDT* Concentration, ppm		Infant DDT Concentration*		Conclusion	Reference (Comments)
		Serum <sup>a</sup>	Milk <sup>a</sup>	Serum <sup>a</sup>	Estimated <sup>a</sup>		
Cohort; 645 participants <sup>b</sup> ; maternal serum sampled at birth and 1.5, 3, 6, 12, 18, 24, 36, 48, and 60 months postpartum, breast milk sampled at birth until lactation ceased, children examined at 3, 4, and 5 years of age; North Carolina, USA; 1978–1982	Cognitive function; McCarthy Scales of Children's Abilities	NR	0–≥6 (ppm-milk fat, range)	NR	0–≥17 mg (range of estimated total doses from breast-feeding exposure in the first year of life)	No significant association between McCarthy Scales scores at 3, 4, or 5 years and concentrations of DDE at birth or in breast milk	Rogan and Gladen (1991)  [This study is a follow-up study of Gladen and Rogan (1991). Breast milk concentration was estimated from all breast milk and serum samples to represent maternal body burden at birth. Lactational exposure was estimated from breast milk concentration and duration of breast feeding to represent total consumption by the child.]

\* Refers to DDT unless otherwise noted.

<sup>a</sup> All measurements converted to ppm where possible.

<sup>b</sup> For cohort studies, number of “participants” refers to participating mother-and-child pairs.

BNBAS = Brazelton neonatal behavioral assessment scale; MDI = Mental Developmental Index; NBAS = Neonatal Behavioral Assessment Scale; NR = Not reported; PDI = Psychomotor Development Index

---

#### B.2.4.2. Animal Studies

The animal toxicology literature on controlled exposures to DDT was reviewed for studies presenting data specifically related to exposure occurring during gestation and/or lactation. To provide a comprehensive understanding of the hazard resulting from these specific types of exposures, all relevant studies that could be retrieved were reviewed and are summarized in [Table B-21](#). Several of the reviewed studies administered DDT to pups via injection: an exposure pathway considered irrelevant for lactational exposure. Thus, these studies were omitted from [Table B-21](#). Other studies exposed laboratory animals to mixtures of DDT combined with other pesticides or other organochlorines. It is difficult to establish the role of DDT exposure in producing the effects observed in this type of mixture study. Thus, these studies were also omitted from [Table B-21](#). In [Table B-21](#), the endpoints examined and effects observed are noted, as are potential points of departure (PODs) for each study. For endpoints in the low-dose range, benchmark dose modeling using U.S. EPA's Benchmark Dose Software (BMDS) was considered. However, appropriate litter-specific data were commonly unavailable for critical effects in the low-dose range, making BMD modeling inappropriate for this application. PODs are arrayed in [Figure B-9](#) and [Figure B-10](#) to facilitate comparisons across studies. [Table B-21](#) serves as a reference key for [Figure B-9](#) and [Figure B-10](#).

Studies were classified according to the timing of exposure for the pups. Gestational exposure studies were those in which maternal animals were exposed during gestation and (1) pups were sacrificed prenatally or at parturition, or (2) pups were nursed by unexposed dams. Combined lactational and gestational exposure studies were those in which maternal animals were exposed during gestation or during gestation and lactation and pups were nursed prior to sacrifice. Finally, lactational exposure studies were those in which dams were not dosed until lactation began or pups unexposed during gestation were cross-fostered to exposed mothers.

Developmental endpoints evaluated after gestational exposures, lactational exposures, or both include anomalies in offspring of exposed females, male reproductive effects, and neurological effects. Studies administered o,p'-DDT, p,p'-DDT, and p,p'-DDE in well-characterized animal models such as rats, mice, and rabbits. p,p'-DDE is a highly persistent metabolite of p,p'-DDT that is also thought to be an androgen receptor antagonist ([Kelce et al., 1995](#)). o,p'-DDT has a shorter half-life than p,p'-DDE and is thought to be estrogenic. Developmental effects from DDT exposure vary depending on the timing of exposure, the isomeric form of DDT, and the dose ([ATSDR, 2002a](#)).

**Table B-21. Developmental Effects from Gestational and/or Lactational Exposure to DDT Compounds<sup>a</sup>**

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational Exposure</b>						
p,p'-DDE, Sprague-Dawley rat, 5–7 dams per group exposed on GD 13.5–17.5, gavage, pups and dams examined on GD 19.5	0, 50, 100	<p><b>Measured:</b> maternal body weight gain and clinical toxicity, litter size and sex ratio, fetal body weight, fetal intratesticular testosterone, corticosterone, and luteinizing hormone levels in males, and levels in plasma of fetal male rats, microscopic observation of pooled fetal testicular and adrenal samples; fetal testicular and adrenal steroidogenic acute regulatory protein, cytochrome P450 side change cleavage, 3<math>\beta</math>-hydroxysteroid dehydrogenase/<math>\Delta</math>5-<math>\Delta</math>4 isomerase type 1, and androgen receptor levels</p> <p><b>Observed:</b> reduced number of lipid droplets (weakly osmiophilic and vacuolated) and degeneration of smooth endoplasmic reticulum and mitochondria at 100 mg/kg-day in adrenal cortex, reduced number of lipid droplets (some vacuolated) at 100 mg/kg-day in testicular Leydig cells</p>	Maternal: 100  Develop: 50	Maternal: NA  Develop: 100	Adamsson et al. (2009)	Develop: [A1]

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
p,p'-DDE; Long-Evans hooded and Sprague-Dawley rats; 8–11 dams per group exposed on GD 14–18; gavage; pups examined on PND 2, 10–13, 35, and 300–450	0, 100	<p><b>Measured:</b> maternal weight gain and clinical toxicity; pup body weight, anogenital distance at birth, presence of areolas with and without nipple buds; male pup preputial separation, external malformations, prostate weight and angogenesis, testis weight and angogenesis/atrophy, and epididymal weight and angogenesis</p> <p><b>Observed Long Evans rat:</b> reduced maternal weight gain; increased number of observed areolas and retained nipples, reduced prostate weight, and atrophy of the epididymides in male pups</p> <p><b>Observed Sprague-Dawley rat:</b> reduced maternal weight gain; reduced anogenital distance at birth and increased number of hypospadias, observed areolas and retained nipples in male pups; male pup reduced glans penis, cauda epididymis, and prostate weight</p>	<p>Maternal: NA</p> <p>Develop: NA</p>	<p>Maternal: 100</p> <p>Develop: 100</p>	<p>Gray et al. (<a href="#">1999</a>)</p> <p>(Authors stated that Sprague-Dawley rats appeared to be more sensitive than Long Evans rats.)</p>	Develop: [B1]

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
p,p'-DDE, Long-Evans Hooded and Sprague-Dawley rat, 8-11 dams per group exposed on GD 14–18, gavage, 3–8 dams per group examined on GD 20 and PND 21, pups examined on PND 2–57	0, 10, 100	<p><b>Measured:</b> maternal blood, brain, liver, fat, and placenta tissue concentrations; pup blood, liver, and fat tissue concentrations; pup body weight and anogenital distance; male pup thoracic nipple retention, preputial separation, serum testosterone, immunohistochemistry of androgen receptor, and mRNA analysis of androgen receptor in testicular tissues; female pup vaginal opening</p> <p><b>Observed Long Evans rats:</b> significantly reduced anogenital distance in males on PND 2, significantly increased thoracic nipple retention, and twofold increase of androgen receptor mRNA in testicular tissues of males at 100 mg/kg-d, elevated testosterone level at 100 mg/kg-d</p> <p><b>Observed Sprague-Dawley rats:</b> significantly increased thoracic nipple retention at <math>\geq 10</math> mg/kg-d; marginally reduced anogenital distance in males on PND 2 and decreased immunohistochemical androgen receptor staining in the testis, epididymis, and prostate at 100 mg/kg-d at PND 57, elevated testosterone level at 100 mg/kg-d at PND 57</p>	<p>Maternal: 100</p> <p>Develop Long Evans rats: 10</p> <p>Develop Sprague-Dawley rats: NA</p>	<p>Maternal: NA</p> <p>Develop Long Evans rats: 100</p> <p>Develop Sprague-Dawley rats: 10</p>	<p>You et al. (1998)</p> <p>(Reported serum concentrations were higher in Long-Evans Hooded rats than Sprague-Dawley rats. Authors did not report any maternal effects.)</p>	<p>Develop Long Evans rat: [E1]</p> <p>Develop Sprague-Dawley rat: [E2]</p>

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
p,p'-DDE, Jcl:SD rat, 23–24 dams per group exposed on GD 6–19, gavage, dams and 346–388 pups per group examined on GD 20	0, 5, 25, 100	<p><b>Measured:</b> maternal body weight, body weight gain, food consumption, and clinical observations; number of implants and fetal mortality, fetal and placental weight, anogenital distance, internal and skeletal abnormalities, and gonocyte count in testes (100 mg/kg-d only), gonocytes in tubule sections counted, fetal skeletons examined</p> <p><b>Observed:</b> significantly decreased maternal food consumption and body weight gain and increased clonic convulsions at 100 mg/kg-d, non chemical-related increased incidence of fetal supernumerary ribs at ≥5 mg/kg-d, increased incidence of 27 presacral vertebrae at 100 mg/kg-d</p>	<p>Maternal: NA</p> <p>Develop: 25</p>	<p>Maternal: 100</p> <p>Develop: 100</p>	<p>Takahashi et al. (2001)</p> <p>(Authors noted that the Jcl:SD strain of rat has a reportedly higher background incidence of supernumerary ribs. Historical control values are comparable to experimental values.)</p>	Develop: [F1]
p,p'-DDE, Kbl:JW rabbit, 22 does per group exposed on GD 6–27 by gavage, dams and 212–151 pups examined on GD 28	0, 5, 20, 80	<p><b>Measured:</b> maternal body weight, body weight gain, food consumption, and clinical observations; number of implants and fetal mortality, fetal and placental weight, internal and skeletal abnormalities</p> <p><b>Observed:</b> nonsignificant decrease in maternal body weight gain and food consumption, abortion/premature delivery, and increased clonic convulsion at 80 mg/kg-d; significantly increased corpora lutea at 80 mg/kg-d, no effects noted in the fetus</p>	<p>Maternal: NA</p> <p>Develop: 80</p>	<p>Maternal: 80</p> <p>Develop: NA</p>	<p>Takahashi et al. (2001)</p> <p>(Authors did not assume the increase in number of corpora lutea to be treatment-related because ovulation occurred before p,p'-DDT treatment.)</p>	Develop: [F2]

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
o,p'-DDT, CF-1 mouse, 6–10 dams per group exposed on GD 11–17, gavage, litters culled to 8 pups on PND 1, pups examined on PNDs 2, 5, and 10	0, 0.02, 0.2, 2, 20, 100	<p><b>Measured:</b> maternal body weight; litter size and sex ratio; pup anogenital distance, body weight, righting reflex, cliff drop aversion, and day of eye opening</p> <p><b>Observed:</b> no effects were dose-related, <b>increased litter size and decreased sex ratio</b> at 0.02 and 0.2 mg/kg-d, significantly decreased pup body weight at 0.2 and 100 mg/kg-d, decreased male body weight at birth at 2 mg/kg-d, increased pup body weight on PND 2 at 100 mg/kg-d and PND 5 at 20 and 100 mg/kg-d, and increased anogenital distance in male pups at 0.2 and 100 mg/kg-d and female pups at 100 mg/kg-d</p>	<p>Maternal: 100</p> <p>Develop: NA</p>	<p>Maternal: NA</p> <p>Develop: 0.02</p>	Palanza et al. ( <a href="#">2001</a> )	Develop: [C1]

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational and Lactational Exposure</b>						
o,p'-DDT, CD-1 mouse, 15 dams per group exposed on GD 11–17, gavage, litters culled to 8 pups on PND 1, pups examined at PND 90 and 180	0, 0.018, 0.18	<p><b>Measured:</b> male pup infanticide behavior, inter-male aggression (number of attacks, latency of attack, time spent attacking, social investigation, tail rattling, self-grooming, and defense), and testes and preputial gland weights</p> <p><b>Observed:</b> significantly decreased bite frequency and total attack time at 0.018 and 0.180 mg/kg-d at PND 90, significantly decreased tail rattling at 0.180 mg/kg-d, significantly decreased testes weight at 0.018 mg/kg-d at PND 180</p>	Maternal: ND  Develop: NA	Maternal: ND  Develop: 0.02 <sup>d</sup>	Palanza et al. (1999)  (Diethylstilbestrol was also tested as a positive control.)	Develop: [D1]
DDT, CF <sub>1</sub> mouse, 1 dam per group, GD 1-PND 21, dietary exposure, cross-fostering at birth, 17-20 pups per litter, maze trial at PND 49	200 µg/g in the diet; dose not quantified	<p><b>Measured:</b> pup mortality, T-maze performance by time, number of errors, and maze navigation improvement over time.</p> <p><b>Observed:</b> significantly more errors made by mice exposed during both gestation and lactation compared to controls; significant improvement not shown by mice exposed during gestation and lactation; mice exposed during gestation only made significantly fewer errors than controls; mice exposed during gestation and lactation made more errors than controls (significant at 10% level). <b>Pup mortality observed in controls (5.8%), lactation only (10%), and gestation and lactation (38.8%) groups.</b> No mortality observed in gestation only exposure.</p>	Maternal/Develop: NA	Maternal/Develop: NA	Graig and Ogilvie (1974)  Authors note that it was not possible to quantify exposure during gestation and lactation due to the study design.	Not included in array due to dosimetry.



Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
p,p'-DDE, Crl:CD(SD)IGS rat, 10 dams per group, exposed on GD 6–PND 20, gavage, litters culled to 8 pups on PND 4, dams examined on PND 22, 4 pups per litter examined at PND 84, 4 pups per litter examined after mating	0, 5, 15, 50	<p><b>Measured:</b> maternal body weight; maternal liver, kidney, ovary, and thyroid weight; fetal mortality; pup sex ratio and external anomalies; pup viability, clinical observations, body weight, anogenital distance, and vaginal opening and preputial separation; histopathology of liver, kidneys, testes, epididymides, uterus, ovaries, vagina, pituitary, and thyroids; number and location of retained nipples, cleft phallus, vaginal pouch, hypospadias, vaginal cytology, ectopic or atrophic testes, epididymides and sex accessory glands, agenesis of gubernaculum, and epididymal granulomas; uterus, ovary, testes, epididymides, ventral prostate, seminal vesicles levator ani and bulbocavernosus muscles, brain, liver, adrenals, kidney, thyroid, and pituitary organ weights; pup copulation, fertility, and second generation fetal mortality</p> <p><b>Observed:</b> significantly increased maternal liver weights at 50 mg/kg-d, significantly decreased pup viability, prolonged preputial separation and reduced vaginal opening, increased seminal vesicle weights in males, increased adrenal weights in females, and decreased copulation and fertility at 50 mg/kg-d; <b>significantly increased relative liver weights in males</b> at ≥5 mg/kg-d and in females at 50 mg/kg-d</p>	<p>Maternal: 15</p> <p>Develop: NA</p>	<p>Maternal: 50</p> <p>Develop: 5</p>	<p>Yamasaki et al. (<a href="#">2009</a>)</p> <p>(A preliminary dose-ranging test was also conducted at 0 and 75 mg/kg-d that led to increased liver weights in dams and their offspring and decreased pup viability.)</p>	Develop: [H1]

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
p,p'-DDT, Dutch-Belted rabbit, 7–9 does per group, exposed on GD 15–PND 28, gavage, female pups culled on PND 21, 7–9 pups examined on PND 84 and 6–9 pups examined on PND 168	0, 8.85, 88.5	<p><b>Measured:</b> maternal pregnancy outcome; pup body weights; pup serum levels of gonadotropin-releasing hormone (GnRH) and levels of follicle-stimulating hormone and luteinizing hormone after GnRH challenge; male pup sexual interest, status of penile erection, number of mounts to accomplish ejaculation, and time to ejaculation; sperm concentration and morphology of male pup semen; gross histopathology and organ weights liver, kidneys, testes, epididymides, accessory sex glands; microscopic histopathology of testes of male pups</p> <p><b>Observed:</b> impaired testicular descent at 8.85 and 88.5 mg/kg-d, failure to achieve penile erection in one pup at 88.5 mg/kg-d, significantly <b>increased levels of</b> luteinizing hormone at 88.5 mg/kg-d and <b>testosterone</b> at 8.85 and 88.5 mg/kg-d after GnRH challenge on PND 84 (no increases on PND 168), significantly decreased percentage of normal sperm at 88.5 mg/kg-d, significantly increased incidence of morphological sperm defects at 88.5 mg/kg-d, testicular carcinoma in situ cells observed at 88.5 mg/kg-d, significantly increased kidney weight at 88.5 mg/kg-d on PND 168</p>	Maternal: 88.5  Develop: NA	Maternal: NA  Develop: 8.85	Veeramacheni et al. (2007)	Develop: [G1]

Study Design	Dose <sup>c</sup> (mg/kg-d)	Toxicological Endpoints <sup>b</sup>	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Postnatal Exposure</b>						
DDT (unspecified), NMRI mouse, ≥12 pups per group exposed on PND 3, 10, or 19, gavage, pups examined on PND 120 and 127	0.5 (single administration)	<p><b>Measured:</b> spontaneous behavior; locomotion, rearing, and total activity; muscarinic receptor density and proportion of muscarinic high-affinity and low-affinity binding sites; body weight; clinical observations</p> <p><b>Observed:</b> significantly increased behavioral deviations in PND 10 (but not PND 3 or 19) exposure, significant decrease in the amount of muscarinic receptor binding sites in the cerebral cortex in PND 10 exposure</p>	NA	NA	<p>Eriksson et al. (<a href="#">1992</a>)</p> <p>(Authors noted that PND 10 exposure coincides with peaks in rapid brain growth during development.)</p>	NA

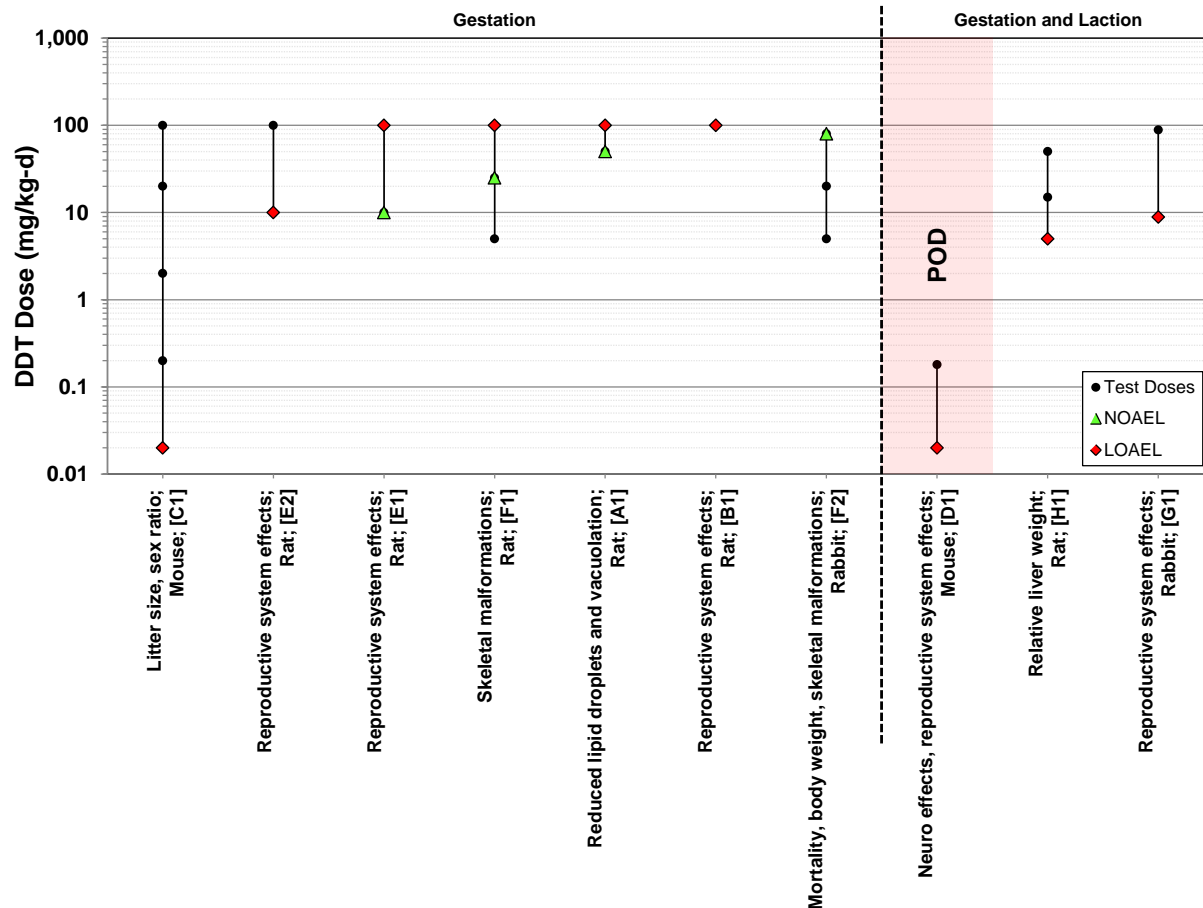
<sup>a</sup> No data were suitable for BMD modeling.

<sup>b</sup> Bold text indicates the endpoint(s) on which the LOAEL is based.

<sup>c</sup> Doses estimated using default body weight and food intake values([U.S. EPA, 1988](#)).

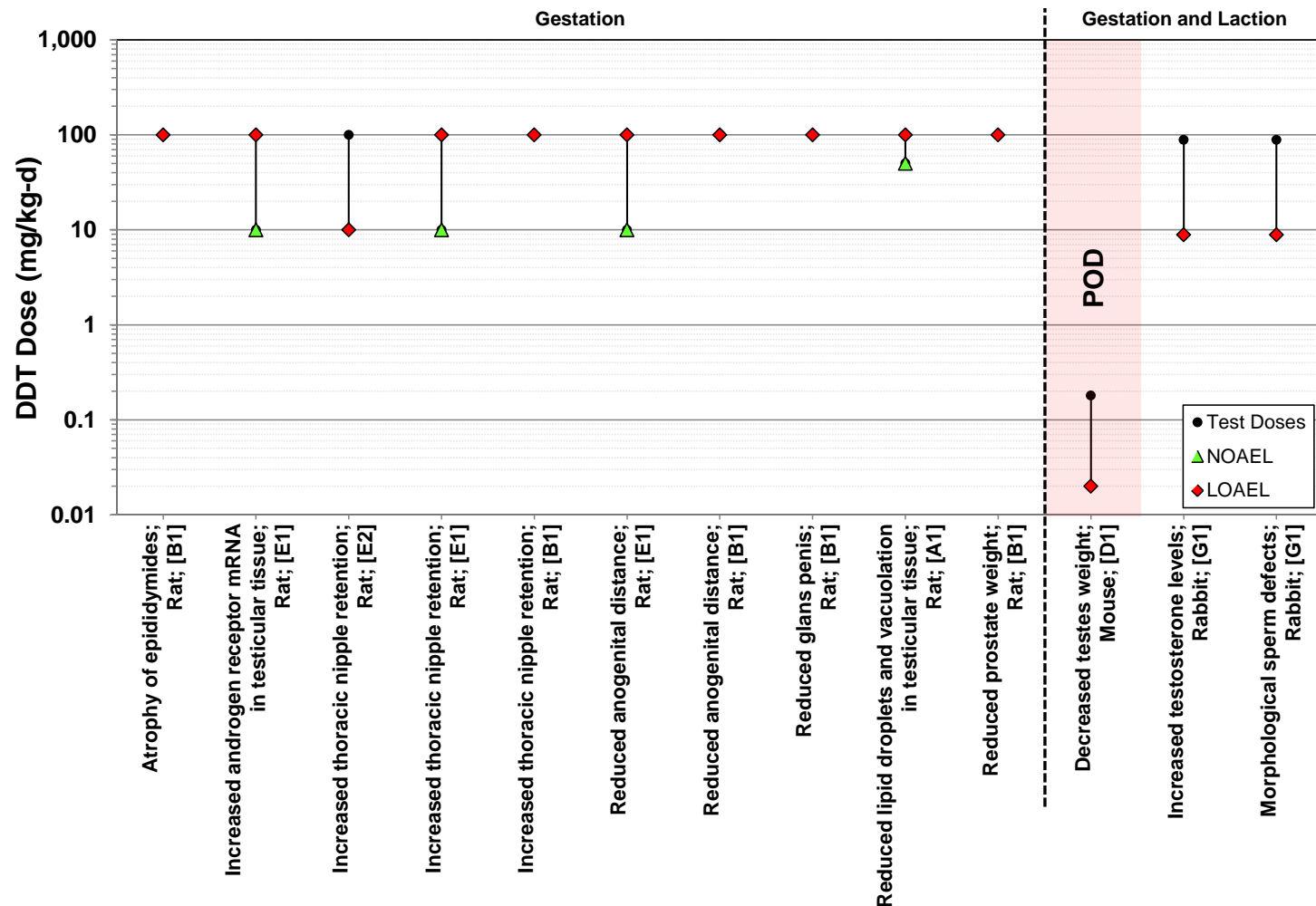
<sup>d</sup> Dose was rounded to nearest one-hundredth for the NOAEL determination.

NA = Not applicable; ND = No data.



Note: Each array element represents the dose-response data for a unique study design. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. The endpoint “reproductive system effects” includes one or more of the following endpoints: decreased testes weight, increased thoracic nipple retention, reduced anogenital distance, increase of androgen receptor mRNA in testicular tissue, atrophy of epididymes, reduced glans penis, reduced prostate weight, and morphological sperm defects. The endpoint “neuro effects” includes one or more of the following endpoints: clonic convulsions, decreased bite frequency and attack time. [Figure B-10](#) details the reproductive system effects observed in male offspring. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-21](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level

**Figure B-9. Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to DDT Compounds**



Note: Each array element represents the dose-response data for each unique study design. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-21](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level

**Figure B-10. Exposure-Response Array Showing All Developmental Effects in Male Pups after Gestational or Gestational and Lactational Exposure to DDT Compounds**

In 5 studies, dosing occurred during gestation, and effects were assessed at various postnatal times ([Palanza et al., 2001](#); [Gray et al., 1999](#); [Palanza et al., 1999](#); [You et al., 1998](#); [Fahim et al., 1970](#)). The most sensitive effects from these studies were effects on the sex ratio of litters, pup weight, anogenital distance, aggression in males, and testes weights in CD-1 and CF-1 mice following administration of 0.02 mg/kg o,p'-DDT to dams from gestational day (GD) 11-17 ([Palanza et al., 2001](#); [Palanza et al., 1999](#)). One study found a dose-dependent decrease in growth rates in rat pups nursed by dams exposed to DDT ([Fahim et al., 1970](#)). Some of the endpoints assessed were clearly a result of gestational exposure (e.g. anogenital distance, altered sex ratio); therefore, these could not be attributed to exposure that occurred via nursing. However, other endpoints were assessed during lactation (e.g., aggression in males, testes weights, and growth rates). Given the known persistence of DDT, it can be assumed that there was ongoing exposure to the offspring during lactation even though dosing to the dams was terminated during gestation.

In two studies, dams were exposed during gestation and lactation, and neurologic and endocrine effects were measured in offspring ([Yamasaki et al., 2009](#); [Veeramachaneni et al., 2007](#)). In one study, a single, oral dose was administered to pups postnatally ([Eriksson et al., 1992](#)). Again, several studies employed direct dosing to neonates via subcutaneous or intraperitoneal injection. These studies were not included because the injection route of exposure is not relevant to the lactational exposure pathway.

Palanza et al. ([1999](#)) reported increased aggression at 3 months of age and decreased testes weight at 6 months of age in male mice following maternal exposure to 0.02 mg/kg-day o,p'-DDT via gavage on GD 11–17. BMD modeling was attempted, but adequate model fit was not achieved. Therefore, the LOAEL of 0.02 mg/kg-day is used as an example POD to assess the impact that accumulated exposure to a mother may have on lactational exposure and postnatal development of her offspring. The POD is used in [Section B.2.6](#), along with results from PBPK models, to estimate a HED. The choice of the POD and HED presented in this packet are intended only as examples to stimulate discussion for the workshop participants.

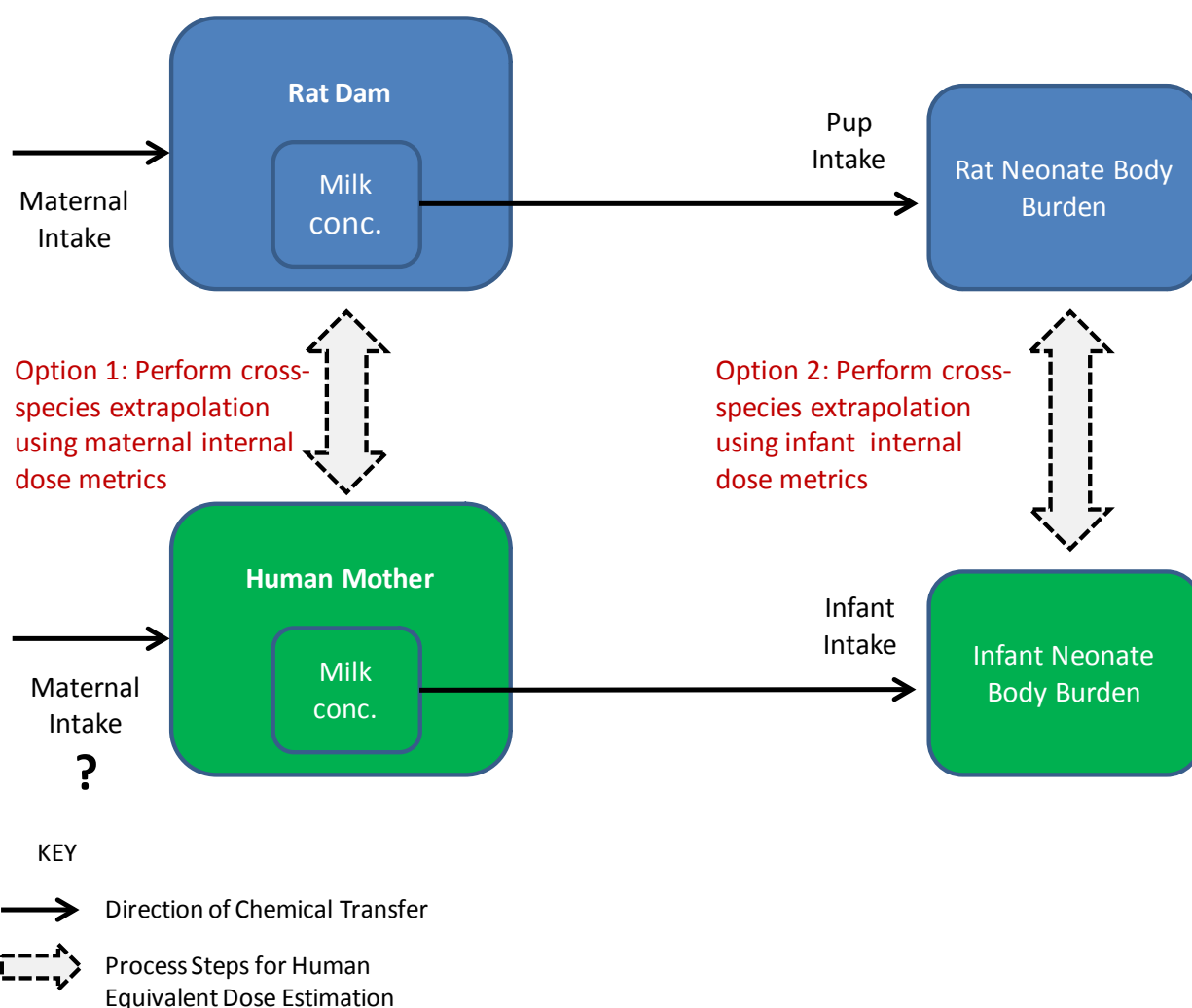
---

### **B.2.5. Human Equivalent Dose (HED) Estimation**

Once the POD has been selected, the next step is estimation of the HED. This HED is intended to quantify the maternal continuous daily exposure in humans that may result in an adverse developmental effect similar to that observed in animal offspring as described in [Section B.2.4](#).

The information provided in this section is designed to promote discussion amongst the workshop attendees as to how best to perform the HED estimation. To provide an example to facilitate discussion, one method is chosen and carried through in this document for demonstration purposes only. The HED can be estimated in a number of different ways. Generally speaking, an internal dose metric is selected for the human and the animal, and then these two metrics are assumed to result in the same effect. The decision must be made as to which internal dose metric is appropriate to serve as an indicator of the associated adverse effect.

For developmental effects resulting from lactational exposure, [Figure B-11](#) provides an example of the pathways that may be chosen to perform cross-species extrapolation to estimate the HED. The assumption is made that the maternal or infant animal intake is known, and the HED is the unknown human continuous daily exposure (depicted with a “?” in the figure). Because exposure occurs through the mother and then passes to the offspring by nursing, cross-species extrapolation may be performed by selecting either an internal maternal dose metric or an internal infant dose metric. Performing the extrapolation on internal maternal dose metrics will not account for species differences regarding infant feeding habits and infant kinetics, while performing the extrapolation on the infants requires more input data and more judgment about how to appropriately link very young members of both species. The overall choice will depend on judgment as to the reliability of the models available and the input data driving the models, balanced against the desire for increased biological accuracy.



**Figure B-11. Process diagram for performing cross-species extrapolation**

### Discussion Points for DDT HED Estimation

- ? DDT is a mixture. How should the relationship between human and animal dose metrics for each constituent be effectively addressed?
- ? The constituents of DDT are metabolized following exposure. How should differences in metabolism between animals and humans be effectively addressed?
- ? What pharmacokinetic data or relevant chemical-specific information are available for the DDT constituents and metabolites in animals and humans to facilitate model implementation?
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ? What PBPK or other simpler biokinetic models are available for DDT?
- ? **What is the best method for estimating the HED using the proposed POD?**

The main questions considered in derivation of an HED from an animal-derived POD are shown in the text box below (“Discussion Points for DDT HED Estimation”). This section discusses some of the chemical-specific properties that affect (1) the selection of the modeling method, (2) the kinetic information available for DDT, and (3) the types of models available for assessing DDT exposure in animals and humans. Summary statements for each of the discussion points are provided in the appropriate sections.

#### B.2.5.1. Chemical-Specific Properties Affecting HED Estimation

Technical DDT is a mixture composed of up to fourteen chemical compounds, of which 65–80% is the pesticide active ingredient, p,p'-DDT. The other components include 15–21% of o,p'-DDT, up to 4% p,p'-DDD, and up to 1.5% 1-(p-chlorophenyl)-2,2,2-trichloroethanol [Metcalf (1995) as reported in ATSDR (2002a)]. The o,p'-DDT component is nearly inactive as a pesticide, and the DDD and DDE constituents are metabolites of DDT. Once released to the environment, the different constituents of DDT may degrade, be taken in by animals and plants, and are incorporated in the food chain at different rates. In the general population, the primary exposure pathway to DDT constituents is through food intake of DDT and DDE, with DDE intakes being higher (ATSDR, 2002a).

#### DDT is a Mixture

- ? How should the relationship between human and animal dose metrics for each constituent be effectively addressed?
- ✓ **To perform cross-species extrapolation, it is necessary to understand the relative toxicity and kinetic properties of each constituent if more sophisticated HED methods are chosen.**



There is a high degree of variability among species in the metabolism of DDT and DDE over time ([WHO, 2011](#)). Following exposure in rats, DDT is metabolized in the liver to DDE and DDD, two intermediary metabolites ([WHO, 2011](#)). DDE and DDD are converted to bis(p-chlorophenyl) acetic acid (DDA), with conversion from DDD being the relatively faster pathway. In mice and hamsters, the primary metabolic pathway for formation of DDA is from DDD. Some evidence shows that DDE is not converted to DDA in mice ([Gingell and Wallcave, 1974](#)). It has been shown that mice had much higher levels of DDE in the liver than hamsters following lifetime exposure to DDT ([Gingell and Wallcave, 1974](#)).

#### DDT Metabolism

- ? How should differences in metabolism between animals and humans be effectively addressed?
- ✓ **If the cross-species extrapolation is performed on the infants, there is a greater burden on understanding the metabolism, excretion, and toxicity of each metabolite.**

The metabolism and kinetics of DDT after oral exposure in humans are complex, and the proportions of the different metabolic pathways are not well understood ([WHO, 2011](#)). Following ingestion, humans metabolize DDT to DDD, which is further degraded and excreted as DDA ([ATSDR, 2002a](#)). The conversion of DDT directly to DDE is very limited in humans compared to rodents, and is a slow metabolic process. The limited amount of DDE created in humans persists for long periods of time in the fat, and DDE is not metabolized to DDA in humans. Levels of DDA in human urine are thus indicative of direct exposure to DDT. Due to the long residence time of DDE in adipose tissue and the relatively faster metabolism of DDT to DDD (and ultimately DDA), people recently exposed to DDT have a higher body burden ratio of DDT to DDE compared to those not recently exposed ([WHO, 2011](#)).

Because DDT is metabolized and toxicity is related to the metabolites, DDT models should account for metabolic rates and for excretion of the metabolites, if possible. Interspecies differences in metabolism and elimination kinetics between humans and animals.

#### B.2.5.2. Available Pharmacokinetic Data for DDT

Before discussing the potential modeling techniques for DDT, the available database of pharmacokinetic data is summarized. This database should help to determine which modeling techniques may be most appropriate and subject to the least uncertainty.

Potential parameters that may be useful in estimating HED by a variety of methods are presented in [Table B-22](#). A discussion of the parameters and their sources follows the table. The various applications and uses of these parameters are discussed in the context of different modeling techniques in [Section B.1.5.4](#).

In the absence of specific information on DDT in humans, the input values presented here were patterned after those in the EPA's Methodology for Assessing Health Risks Associated with Multiple

Pathways of Exposure to Combustor Emissions ([U.S. EPA, 1998](#)). Chapter 9 of this methodology (hereafter “Combustor Assessment”) presents a framework for evaluating exposure to persistent lipophilic contaminants in breast milk. Thus, the assumptions made in that document are applied to DDT when values specific to DDT could not be located. The **maternal absorption fraction** for DDT was not available in the reviewed literature. An absorption fraction value of 1 is used in the current calculations to serve as the most conservative estimate, assuming complete absorption of the chemical ([U.S. EPA, 1998](#)). Quantitative information about the **fraction of DDT in the body that is stored in fat** also could not be found in the literature. The reviewed literature generally described DDT as highly lipophilic. The Combustor Assessment states that for highly lipophilic compounds, >90% of the compound may be stored in the fat ([U.S. EPA, 1998](#)). In the absence of more specific information, this parameter was set to 0.9 in the example calculations.

DDT is a mixture, which complicates the estimation of **half-life**. Different components of the mixture are eliminated at different rates, with DDE eliminated more slowly than DDT. Half-life for DDT in adipose tissue of mice was found to be 165 days ([Gingell and Wallcave, 1974](#)). In humans, the half-life of DDT in fat was determined by Wolff ([1999](#)) to be 5.7 years while the DDE half-life in serum was 13-15 years ([Wolff et al., 2000](#)). Ritter et al. ([2009](#)) estimated an intrinsic half-life for DDT, where the intrinsic half-life takes into account any temporal trends in exposure or body-weight to estimate a half-life consistent with background exposure. When Ritter et al. ([2009](#)) accounted for the temporal change in exposure, they estimated a total body half-life of 6.2 years for *p-p'* DDE and 2.2 years for *p-p'* DDT for a population in Sweden, with similar estimates for a population in the United Kingdom. The Swedish estimates from Ritter et al. ([2009](#)) are used in the example calculations in this briefing packet since the RfD estimation assumes constant chronic exposure.

Animal parameters were determined from values found in the literature where

available. The search focused on mice, since mice are the species studied in the candidate POD study ([Palanza et al., 1999](#)). Studies on *p,p'*-DDT indicate 50% absorption in mice following oral administration ([Gingell and Wallcave, 1974](#)). Therefore, 0.5 is used as the **maternal absorption fraction**. No absorption

#### Pharmacokinetic Data

- ? What pharmacokinetic data or relevant chemical-specific information are available for the DDT constituents and metabolites in animals and humans to facilitate model implementation?
- ✓ **Octanol-water partition coefficient,  $K_{ow}$**
- ✓ **Half-lives in humans and mice**
- ✓ **Absorption fractions and fraction of chemical stored in fat (assumptions can be made based on other PBT chemicals)**
- × **No available *in vitro*-derived partition coefficients in humans or mice; partition coefficients can be derived from  $K_{ow}$**
- × **No available metabolism rates**

fractions could be found in the literature for mouse pups, and the same absorption fraction used for dams (0.5) was assumed for pups.

The **fraction of DDT stored in fat** in mice could not be found in the literature. The value for humans (0.9) was applied to the mouse in the absence of species-specific information.

**Table B-22. Available Pharmacokinetic Parameters for DDT in Humans and Mice**

Parameter (units)	Variable	Value	Note/source
Chemical-Specific Only			
Octanol-water partition coefficient	log(K <sub>ow</sub> )	6.91	Hansch et al. ( <a href="#">1995b</a> )
Human Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	U.S. EPA ( <a href="#">1998</a> ) (default)
Biological elimination constant for DDT (day <sup>-1</sup> )	k <sub>elim</sub>	0.000306 (DDE)	Estimated as ln(2)/t <sub>1/2</sub>
Half-life in humans for DDT and DDE (days)	t <sub>1/2</sub>	DDT – 2.2 years = 803 days DDE – 6.2 years =2,263 days	Ritter et al. ( <a href="#">2009</a> )
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Partition coefficients- in different human body compartments for the DDT constituents	Can be estimated from K <sub>ow</sub>		
Metabolic rates for DDT constituents	None identified in the literature		
Mouse Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the mouse pup (dimensionless)	f <sub>ai</sub>	0.5	Assumed
Fraction of ingested contaminant that is absorbed by the mouse dam (dimensionless)	f <sub>am</sub>	0.5	Gingell and Wallcave ( <a href="#">1974</a> )
Fraction of contaminant that is stored in mouse dam/maternal fat (dimensionless)	f <sub>f</sub>	0.9	Assumed same as human, U.S. EPA ( <a href="#">1998</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.0042	Estimated as ln(2)/t <sub>1/2</sub>
Half-life of <i>p,p'</i> -DDT contaminant in mice (days)	t <sub>1/2</sub>	165	Gingell and Wallcave ( <a href="#">1974</a> )
Partition coefficients in different mouse compartments for the DDT constituents	None identified in the literature		
Metabolic rates for DDT constituents	None identified in the literature		

\* Assumptions made for lipophilic organics in the Combustor Assessment ([U.S. EPA, 1998](#))

### B.2.5.3. Appropriate Dose Metric for Selected Point of Departure (POD)

Adverse developmental effects may be associated with DDT exposure during a critical period of development. Metrics may be selected to look at either peak or average maternal or infant exposure, depending on the effect in question and the dosing protocol in the animal study. For developmental effects, if a critical window can be determined, then the model solutions during the critical window may be used for the cross-species extrapolation; otherwise, peak or average concentrations from different exposure metrics (see below) may be used based on the judgment of the assessor as to what is most appropriate. For the proposed POD presented here, no critical window could be determined. The developmental effects were noted with exposure in both the gestational and lactational periods, and the exact critical window is uncertain.

#### Dose Metric

- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ✓ **Peak or average concentrations from maternal or infant dose metrics**
- × **The critical window could not be defined**

Based on the studies presented and discussed in [Section B.2.5.3](#), the critical exposure window for DDT-induced developmental effects may fall during the gestational period. If this is true, the contribution of lactational exposure to DDT to developmental health effects could be minor. Despite this possibility, DDT is presented as a case study in this briefing packet because methods proposed by workshop participants, using DDT as an example, may be useful in the future assessment of other chemicals with pharmacokinetic databases and/or biochemical properties similar to DDT that do cause adverse health effects as a result of postnatal exposure.

Possible dose metrics for specified lactation durations include:

- The average maternal body burden,
- The peak maternal body burden,
- The average maternal fat concentration,
- The peak maternal fat concentration,
- The average milk concentration,
- The peak milk concentration,
- The average infant body burden, and
- The peak infant body burden.

#### B.2.5.4. Available Models for Estimating DDT Internal Dose

The following section discusses potential models that may be used to estimate internal dose metrics based on maternal intake. The section starts with the most biologically-robust class of models, physiologically-based pharmacokinetic (PBPK) models. The section then discusses simpler first-order kinetic modeling techniques and a biotransfer method for estimating maternal exposure. Thus, the section generally progresses from the most biologically robust to the more simple models. Where possible, the parameters needed for each technique have been collected and presented. The final choice in technique depends on weighing both the biological sophistication of the method and the uncertainty in the model parameters, as discussed in [Section B.1.6](#).

##### *Multi-Compartment PBPK Models*

Generally speaking, the most biologically complete kinetic model for a given chemical is a multi-compartment, data-validated, PBPK model. There is a PBPK model for DDE in rats although no human or mouse models are available. Thus, this section discusses how the existing models could be applied or adapted for DDT and DDE. Of primary consideration is fairly rapid metabolism of DDT to DDE in humans and rats and the different metabolic pathway in mice. The available models can simulate either exposure to DDT or DDE (humans and rats) or to DDT (mice). However, the link between exposure to DDT and the subsequent metabolism and internal exposure to DDE is not fully captured in the models.

##### *Human Models*

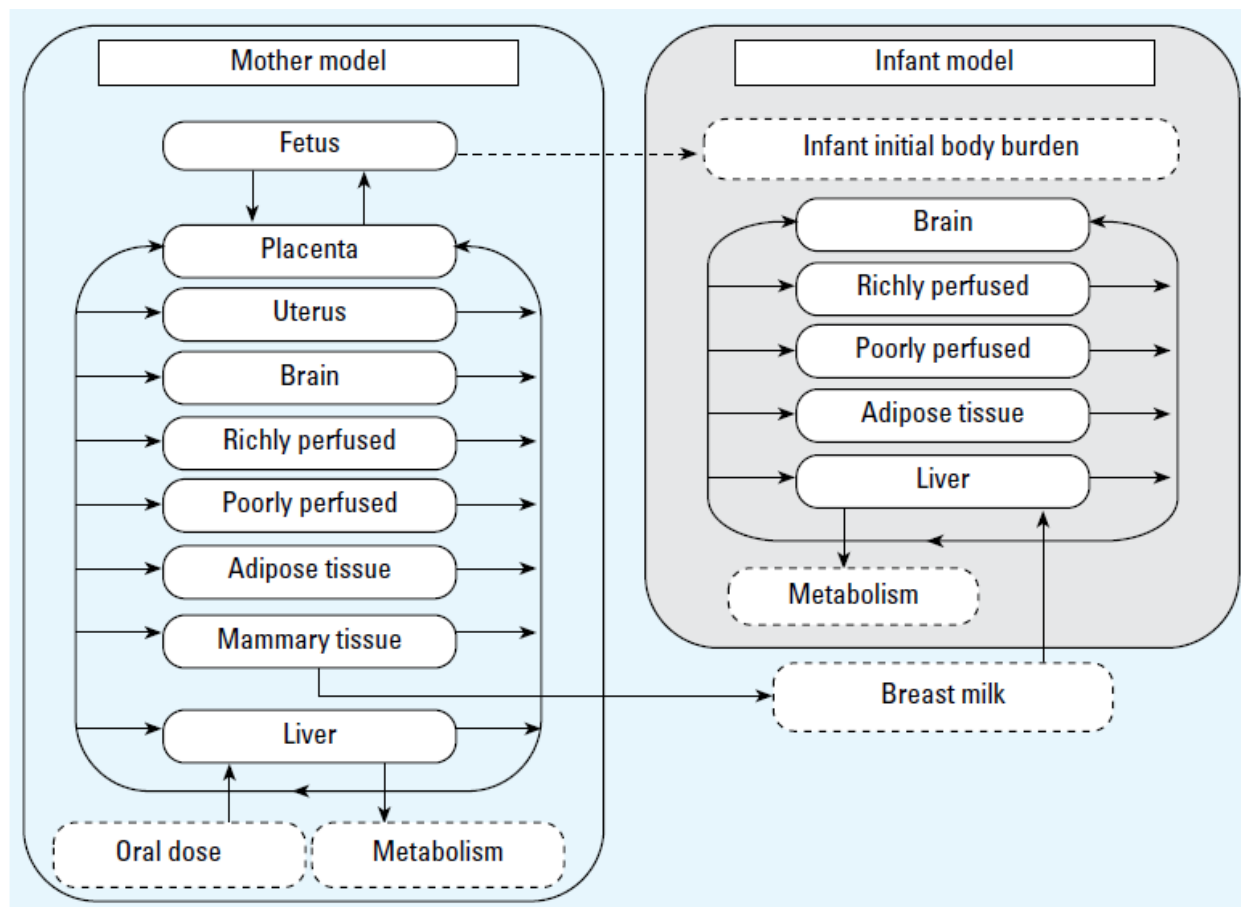
A generic maternal-infant multi-compartment model for persistent organic pollutants (POPs) has been developed ([Verner et al., 2009](#); [Verner et al., 2008](#)). The primary utility of this model for DDT is that the chemical-specific parameters can be estimated using only the half-life and octanol-water partition coefficient ( $K_{ow}$ ) of the compound. As discussed below, the model has been validated for seven different POPs including DDT and DDE.

The full maternal and infant model ([Verner et al., 2009](#)) is intended to simulate the entire life-cycle of a woman up to age 55 years and includes nine maternal tissue compartments and five infant tissue compartments, as shown in [Figure B-12](#). The oral dose is modeled as being directly absorbed into the liver and assumed to be fully bioavailable. First-order hepatic metabolism is included and is intended to represent all excretion in the absence of breastfeeding or childbirth. The elimination rate constant is determined for the chemical from the adult whole-body half-life. The concentrations in each

##### **Available Models**

- ? What PBPK or other simpler biokinetic models are available for DDT?
- × **No human PBPK model validated against human data is available**
- × **No mouse PBPK model is available**
- ✓ **Simple first order models are available**
- ✓ **Simple biotransfer methods are available**
- ?

compartment are determined using partition coefficients, and the chemical-specific partition coefficients are estimated using an equation based on  $K_{ow}$  (Poulin and Krishnan, 1996) and using the fraction of blood and tissue that are lipid and water (Price et al., 2003). Physiological parameters such as body weight and fat volume fraction are assumed to vary in time to capture changes over the life of the woman.



**Figure B-12. Maternal and Infant POP Model (Verner et al., 2009)**

The model has been validated against human cord blood, breast milk, and infant blood concentrations for seven POPs, including DDT and DDE. The authors found strong Spearman correlations for the compounds with longer half-lives (7 years and greater), including p,p'-DDE ( $r = 0.91, 0.93, \text{ and } 0.90$  for cord blood, breast milk, and infant blood, respectively). Correlations were lower for p,p'-DDT and compounds with a shorter half-life (under 7 years;  $r = 0.78, 0.75, \text{ and } 0.77$  for cord blood, breast milk, and infant blood, respectively). In addition, no attempt was made to simulate the metabolism of DDT to DDE in the mother or infant.

The computer code was not provided in the publication although a number of details about the parameters and equations are included in the main papers and in supplemental information. Considerable time would be needed in order to further evaluate the potential for this model to be used for DDT, including model implementation and manipulation in modeling software.

### ***Animal Models***

The candidate POD study by Palanza et al. ([1999](#)) investigates developmental effects in mice. However, no direct lactational DDT model is available for mice. Existing mouse models for other chemicals and their potential adaptation to DDT are discussed in this section.

Developmental effects of DDT exposure have also been observed in rats. Thus, since DDT is metabolized to DDE, the existing DDE rat model is discussed in this section.

As stated in [Section B.2.5.1](#), humans and rats rapidly metabolize DDT to DDE, and toxicity is linked to exposures to either DDT or DDE. Metabolic pathways are different in mice. Because of the species difference in metabolic pathways, the application of the PBPK models is complicated as discussed below.

#### ***Mouse Models: Applicable to Proposed POD Presented in this Briefing Packet***

No direct lactation mouse model of DDT exposure is available. Lactational models are available for other persistent bioaccumulative and toxic substances including PCBs ([Lee et al., 2009](#); [Lee et al., 2002](#)) and methylmercury ([Lee et al., 2009](#)). These models could potentially be adapted to DDT if input parameters and data suitable for model validation were available.

The rat model discussed below could be adapted to mice since mouse physiological parameters are readily available in the literature.

#### ***Rat model: Applicable to PODs Derived from Studies Using Rats***

You et al. ([1999](#)) constructed a PBPK model based on the structure of O'Flaherty et al. ([1992](#)) to simulate the distribution and elimination of p-p' DDE during gestation and lactation in rats. They focused on DDE because it is a primary metabolite of DDT and has a longer half-life than DDT. The transfer of DDE is assumed to be flow-limited between tissue and blood in each compartment except the fat compartment. The fat compartment is assumed to be diffusion limited; in addition, to adequately match the measured rat data, an additional "deep fat" compartment was added, where the diffusion coefficient from the fat to the deep fat compartment is assumed to be higher than from the deep fat to the fat compartment. The deep fat compartment essentially sequesters DDE where it is not readily available for elimination. In addition, DDE is assumed to diffuse from the mammary gland to the milk compartment so that the milk compartment is not in equilibrium with the blood. Observational data were used to fit the oral absorption rate, the fecal excretion rates, and the tissue:blood partition coefficients.



The code is not provided in the publication, although the authors do provide details about the parameters and equations used. Considerable time would be needed in order to further evaluate the potential for this model to be used for DDT, including model implementation and manipulation in modeling software. In particular, it would be necessary to estimate the parent compound (total DDT) intake based on the metabolite (DDE) exposure. This remains an uncertain step and requires further assumptions about the metabolism of DDT in rats.

### ***First-Order Steady-State Single-Compartment Model (U.S. EPA, 1998)***

A first-order single-compartment model is an intermediate step between a full, multi-compartment PBPK model and simpler dosimetric conversions or biotransfer models. The model presented here is patterned after the first-order models presented in the Combustor Assessment (U.S. EPA, 1998) and the dioxin reassessment (U.S. EPA, 2012b) and is adapted to fully incorporate time-dependence during lactation. The general model equations are presented first, and then the model is applied to both humans and mice in the next section. Mice are the focus here since that is the species in the candidate POD study. Because the equations are applied for mice and humans as an example in this briefing packet, they are presented in greater detail than the equations in the PBPK models discussed in the Section on [Multi-Compartment PBPK Models](#). However, this is not meant to imply that the first-order models are superior for DDT.

The single body compartment represented by the model is generally defined as “body burden,” or the total average concentration of contaminant in the body. In a first-order model, the elimination from the body is represented as a rate constant multiplied by the total body burden. This rate constant can be estimated from the whole-body half-life, which is often readily available in the literature. The input to the body is the dose, assumed to be normalized by the total body weight. Thus, the simple model can be represented by the differential equation<sup>7</sup>:

$$\frac{\partial BB(t)}{\partial t} = f_{am} DI_{mat}(t) - k_{elim} \times BB(t)$$

Appendix Equation 12

Where:

BB(t)	=	the time-dependent total body burden (ng/kg),
k <sub>elim</sub>	=	the first order elimination rate (days <sup>-1</sup> ) = ln(2)/half-life (days),
DI <sub>mat</sub> (t)	=	the time-dependent maternal dose (ng/kg-day), and
f <sub>am</sub>	=	the fraction of ingested contaminant absorbed by the mother (dimensionless).

<sup>7</sup> Strictly speaking, this equation is only correct if the maternal body weight is not changing in time. However, this approximation is sufficient given the overall uncertainty in using a first-order model.

This equation can be converted to a difference equation and iterated in time:

$$BB_{t+\Delta t} = BB_t + \Delta t(f_{am} DI_{mat,t} - k_{elim} \times BB_t)$$

Appendix Equation 13

Where:

- $BB_{t+\Delta t}$  = the total body burden at the next time step (ng/kg),
- $BB_t$  = the total body burden at the current time step (ng/kg),
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{mat,t}$  = the maternal dose at the current time step (ng/kg-day),
- $f_{am}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless), and
- $\Delta t$  = the time step (days).

This equation will approximate the solution to the differential equation. To help eliminate errors introduced by converting the differential equation to an algebraic equation, the time step ( $\Delta t$ ) should be much smaller than the elimination time scale.

This equation allows approximation of the maternal body burden; however, additional assumptions can be made to estimate the time dependent infant intake from breast milk. First, the concentration in the maternal milk fat is assumed to be equal to the concentration in the maternal fat and can be estimated as:

$$C_{milk\ fat,t} = \frac{BB_t \times f_f}{f_{fm}}$$

Appendix Equation 14

Where:

- $C_{milk\ fat,t}$  = the concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_f$  = the fraction of contaminant that is stored in maternal fat (dimensionless), and
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW).

Although nonpolar compounds partition to some degree into the aqueous compartment, the most commonly analyzed organic contaminants, including DDT, are highly concentrated in the lipid phase; the concentration of DDT in breast milk is not expected to be significantly underestimated by ignoring the aqueous phase.

To account for the fact that lactation acts as an additional removal mechanism from the mother, the elimination rate during lactation is increased to equal:

$$k_{\text{elac}} = k_{\text{elim}} + \frac{CR_{\text{milk},t} \times f_f \times f_{\text{mbm}}}{f_{\text{fm}} \times BW_{\text{Mat}}}$$

Appendix Equation 15

Where:

$k_{\text{elac}}$	=	the maternal elimination rate during lactation (days <sup>-1</sup> ),
$k_{\text{elim}}$	=	the first order elimination rate (days <sup>-1</sup> ) = ln(2)/half-life (days),
$CR_{\text{milk},t}$	=	the time-dependent infant ingestion rate of milk (kg/day),
$f_f$	=	the fraction of contaminant that is stored in maternal fat (dimensionless)
$f_{\text{mbm}}$	=	the fraction of fat in milk (dimensionless),
$f_{\text{fm}}$	=	the fraction of the mother's weight that is fat (kg fat/kg BW), and
$BW_{\text{Mat}}$	=	the maternal body weight (kg).

Then, the time-dependent infant intake can be estimated as:

$$DI_{\text{INF},t} = \frac{(C_{\text{milk fat},t} \times f_{\text{mbm}}) \times CR_{\text{milk},t}}{BW_{\text{INF},t}}$$

Appendix Equation 16

Where:

$DI_{\text{INF},t}$	=	the time-dependent infant daily ingestion (ng/kg-day),
$C_{\text{milk fat},t}$	=	the time-dependent concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
$f_{\text{mbm}}$	=	the fraction of fat in milk (dimensionless),
$CR_{\text{milk},t}$	=	the time-dependent infant ingestion rate of milk (kg/day), and
$BW_{\text{INF},t}$	=	the time-dependent infant's body weight (kg).

The time-dependent body weights and ingestion rates are incorporated in the models by using different point estimates over the course of the simulation (see the sections on [Input Parameters and Application to DDT in Humans](#) and [Input Parameters and Application to DDT in Mice](#)). Finally, if the infant is modeled using a first-order model as well, the infant body burden can be estimated as:

$$BB_{INF,t+\Delta t} = BB_{INF,t} + \Delta t (f_{ai} DI_{INF,t} - k_{elim,INF} \times BB_{INF,t})$$

Appendix Equation 17

Where:

$BB_{INF,t+\Delta t}$	=	the infant total body burden at the next time step (ng/kg),
$BB_{INF,t}$	=	the infant total body burden at the current time step (ng/kg),
$k_{elim,INF}$	=	the first order elimination rate in the infant ( $\text{days}^{-1}$ ) = $\ln(2)/\text{half-life (days)}$ ,
$DI_{INF,t}$	=	the infant dose at the current time step (ng/kg-day),
$f_{ai}$	=	the fraction of ingested contaminant absorbed by the infant (dimensionless), and
$\Delta t$	=	the time step (days).

The above equations approximate the maternal body burden and exposure to a lactating infant. The model does not, however, include any fetal exposure *in utero*.

This model is generic and not species-specific. For purposes of illustration, the model was implemented in Excel and time integrated through the life of the mother (see “Human Time Dependent Model.xlsm” and “Rodent Time Dependent Model.xlsm” provided to workshop participants). Then, the time-dependent solution can be used to estimate the internal dose metrics in [Section B.2.6](#).

The above model was parameterized so that the maternal dose, the infant body weight, and the infant ingestion rate all vary with time. In the case of the maternal dose, this allows the model to follow dosing protocols from animal studies. In the case of the body weight and ingestion rate, this allows the model to more accurately simulate effects on infants during very early exposure when body weight is changing rapidly and when critical windows for developmental effects may occur.

### ***Input Parameters and Application to DDT in Humans***

For humans, the model was implemented using a time step of 1 week. This represents a balance between a time step short enough to capture changes in the infant during lactation and long enough so that data are not applied beyond their measurement range. Since the Exposure Factors Handbook ([U.S. EPA, 2011a](#)) provides infant estimates on a monthly basis (see below), values across weeks within a single month were kept equal.

The input parameters used for DDT for humans are shown in [Table B-23](#). In addition to using mid-range (i.e., mean) values in the model, low and high end estimates were also used for some parameters, where available, to determine the approximate range of human variability. Values for the parameters that are not chemical-specific were retained from the Combustor Assessment ([U.S. EPA, 1998](#)) or taken from the Exposure Factors Handbook ([U.S. EPA, 2011a](#)). DDT-specific values are the same as those presented and discussed in [Section B.1.5.2](#). Because DDE has a longer half-life than DDT and because DDT is metabolized to DDE, the DDE half-life was used in the model estimates.

**Table B-23. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model**

Parameter (units)	Variable	Low	Mid	High	Note/source
Human Parameters (not chemical-specific)					
Maternal age at pregnancy (years)	Age	18	25	40	Assumptions
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 1 (kg)	BW <sub>INF,t</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 2-3 (kg)		5.9			
Infant body weight, Month 4-6 (kg)		7.4			
Infant body weight, Month 7-12 (kg)		9.2			
Infant ingestion rate, Month 1 (kg milk/day)	CR <sub>milk,t</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, Month 2-3 (kg milk/day)		0.69			
Infant ingestion rate, Month 4-6 (kg milk/day)		0.77			
Infant ingestion rate, Month 7-12 (kg milk/day)		0.62			
Fraction of mother’s weight that is fat (kg maternal fat/kg BW) <sup>b</sup>	f <sub>fm</sub>	0.2	0.3	0.4	Timson and Coffman ( <a href="#">1984</a> ); U.S. EPA ( <a href="#">1998</a> )
Fraction of fat in breast milk (dimensionless) <sup>c</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Human Chemical-Specific Parameters					
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1			U.S. EPA ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	1			U.S. EPA ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9			U.S. EPA ( <a href="#">1998</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.000306			Calculated as ln(2) / t <sub>1/2</sub>
Half-life in humans (days) (DDE)	t <sub>1/2</sub>	2,263			Ritter et al. ( <a href="#">2009</a> )

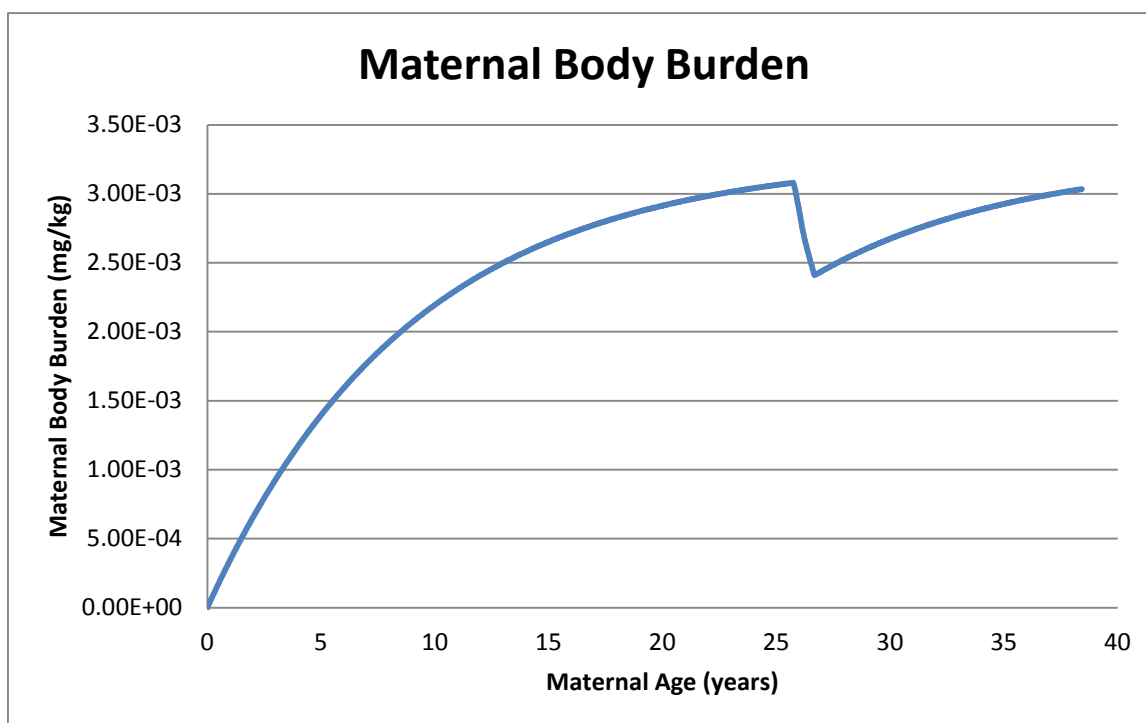
<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 < 50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents mean ± standard deviation.

<sup>c</sup> Represents the typical range and midpoint of the range.

An example figure showing the maternal body burden assuming pregnancy at age 25 years and a daily intake of 1 ng/kg-day is shown in [Figure B-13](#). Because the model is linear with respect to dose, this intake level is chosen for illustrative purposes and the body burden would scale linearly with any change in intake. The dip at age 25 years and 9 months is due to the additional elimination during lactation.

One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, assuming the “mid” parameters and pregnancy at age 25 years, the average infant to maternal dose ratio was estimated to be 29.2 (1 month lactation duration) and 21.8 (12 month lactation duration). The overall range of the ratio, accounting for human variability and including the peak estimates, was from 10.8 to 65.0 (see [Table B-24](#)).



**Figure B-13. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day**

**Table B-24. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model<sup>a</sup>**

Infant Nursing Duration	Pregnancy at 18 years; Low parameters	Pregnancy at 25 years; Mid parameters	Pregnancy at 40 years; High parameters
1 month, Peak Infant Intake	13.7	29.3	61.3
1 month, Average Infant Intake	13.6	29.2	60.5
12 months, Peak Infant Intake	14.9	31.7	65.0
12 months, Average Infant Intake	10.8	21.8	41.0
<sup>a</sup> Calculated using “Human Time Dependent Model.xlsm” provided to workshop participants			

### *Input Parameters and Application to DDT in Mice*

For mice, the model was implemented using a time step of one half day (one week used for humans) to adequately resolve the time evolution of the maternal body burden. The input parameters used for DDT are shown in [Table B-25](#). The age of the mice at the beginning of gestation was not reported in the candidate POD study, so the age at which mice become sexually mature (4 weeks) was assumed for this analysis. In addition, a gestation time of 3 weeks and lactation duration of 3 weeks were assumed for the analysis as representative typical values. A litter size parameter was added so that the nursing mouse fed 8 pups at a time, rather than the single infant assumed in the human model. This litter size, which matches the number of pups per litter in the candidate POD study, increased the maternal lactational elimination rate.

Aside from the average maternal body weight during gestation and lactation (53 g), the physiological parameters needed for the model were not presented in the candidate POD study. The newborn pup weight was taken from Johnson et al. ([2001](#)). This was the same study used to estimate the suckling rate (see below), which allowed for correlations between pup weight and pup milk intake rate. The average pup weight at birth was found to be 1.7 g, and this was used for the pup weight during the first week post-parturition. Pup weight at three weeks of age in the same study was 7.9 g. Then, the weight at two weeks was estimated as the midpoint between the weight at birth and the weight at three weeks (4.8 g).

The suckling rate of neonatal mice was determined from Johnson et al. ([2001](#)). The study measured the milk production in 12 dams with litter sizes varying between 9 and 15 pups. Figure 7 from the paper was digitized using the GetData<sup>®</sup> digitizer, and the milk production of each dam was divided by the litter size to get the average intake per pup. Then, per pup intake was averaged across the 12 litters to give an estimate of 1.26 mL/day. Assuming a milk density close to that of water, this estimate equates to an intake rate of 0.00126 kg/day for each pup. In order to avoid overestimating peak exposures to the pup, this 3-week average was further broken down into estimates for each week separately. It was assumed that the average was 0.00126, but the intake each week scaled as a function of the body weight. For the purposes of this briefing packet, the intake was scaled linearly with the body weight. This can be expressed as:

$$\frac{CR_{milk,1\text{ week}} + CR_{milk,2\text{ weeks}} + CR_{milk,3\text{ weeks}}}{3} = 0.00126$$

or

$$\frac{CR \times BW^1_{1\text{ week}} + CR \times BW^1_{2\text{ weeks}} + CR \times BW^1_{3\text{ weeks}}}{3} = 0.00126$$



Where:

$CR_{\text{milk, X weeks}}$  = the milk intake at X weeks,  
 $CR$  = the milk intake linear multiplier, and  
 $BW_{\text{, X weeks}}$  = the pup body weight at X weeks.

Solving this equation gives intake rates of 0.000276, 0.00126, and 0.00224 kg/day for weeks 1, 2, and 3, respectively.

Fat as a percentage of dam body weight (0.056) was determined from a paper by Brown et al. ([1997](#)).

The same chemical-specific values as presented in [Section B.1.5.2 \(Table B-22\)](#) were used: maternal absorption fraction, the fraction of contaminant stored in the fat relative to the total body burden, and the half-life.

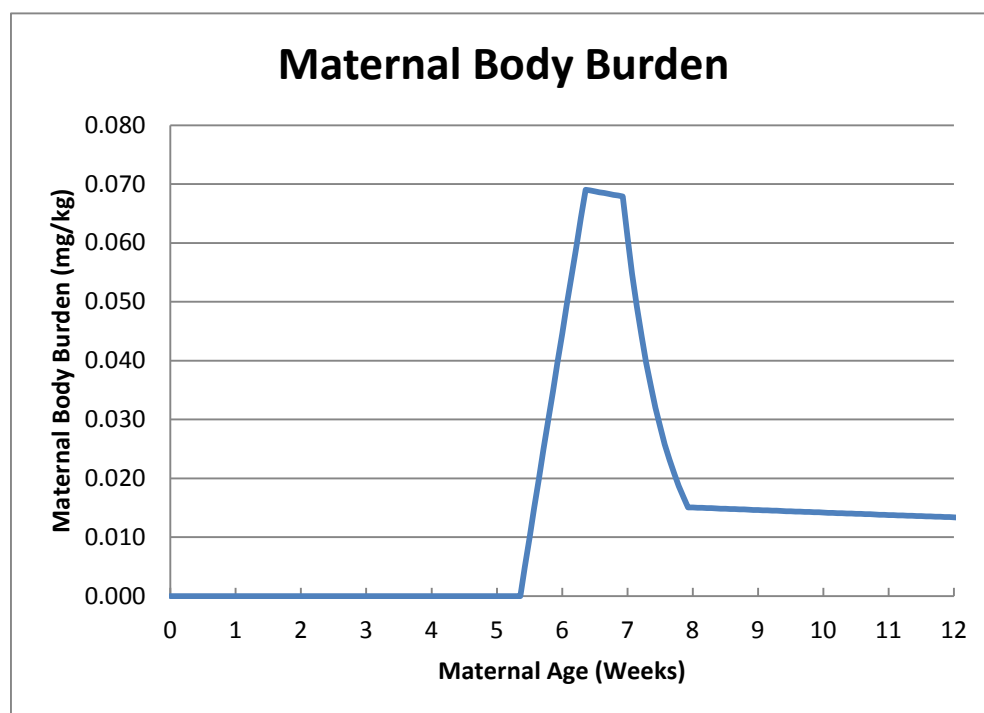
**Table B-25. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Mouse**

Parameter (units)	Variable	Estimated Value	Note/source
<b>Mouse Parameters (not chemical-specific)</b>			
Dam age at pregnancy (weeks)	Age	4	Assumption
Dam weight during lactation (kg)	BW <sub>Mat</sub>	0.053	Palanza et al. (1999)
Litter size (number of pups)	Litter	8	Palanza et al. (1999)
Pup body weight, Week 1 (kg)	BW <sub>INF,t</sub>	0.0017	Rogers et al. (2003) and Jackson Mouse Phenome Database (2012)
Pup body weight, Week 2 (kg)		0.0048	
Pup body weight, Week 3 (kg)		0.0079	
Pup ingestion rate, Week 1 (kg milk/day)	CR <sub>milk,t</sub>	0.000276	Johnson et al. (2001)
Pup ingestion rate, Week 2 (kg milk/day)		0.00126	
Pup ingestion rate, Week 3 (kg milk/day)		0.00224	
Fraction of dam's weight that is fat (kg maternal fat/kg BW)	f <sub>fm</sub>	0.056	Brown et al. (1997)
Fraction of fat in maternal milk (dimensionless)	f <sub>mbm</sub>	0.298	Gors et al. (2009)
<b>Mouse Chemical-Specific Parameters</b>			
Fraction of ingested contaminant that is absorbed by the pup (dimensionless)	f <sub>ai</sub>	0.5	Assumed
Fraction of ingested contaminant that is absorbed by the dam (dimensionless)	f <sub>am</sub>	0.5	Gingell and Wallcave (1974)
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	Assumed
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.0042	Calculated as ln(2) / t <sub>1/2</sub>
Half-life in mice (days)	t <sub>1/2</sub>	165	Gingell and Wallcave (1974)

The dosing protocol in the POD study (Palanza et al., 1999) was applied to this mouse model. In the study, the dams were dosed daily from GD 11 until GD 17. To implement this in the current model, dosing was specified to occur on those days during gestation. The model is able to capture transfer to the nursing pup during lactation, but it does not model any infant body burden during gestation.

An example figure showing the maternal body burden using the candidate POD dose (0.02 mg/kg-day) from GD 11 to 17 (as in the POD study) is shown in Figure B-14. The model predicts very rapid elimination of the chemical from the dam once lactation begins.

One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, the average infant to maternal dose ratio was estimated to be 1.3 (1 week lactation duration) and 0.5 (3 week lactation duration, see [Table B-26](#)). The average infant intake is less than the maternal intake since the maternal dose was only administered during gestation and the maternal body burden rapidly decreases during lactation.



**Figure B-14. Mouse Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study ([Palanza et al., 1999](#)) and an Administered Dose of 0.02 mg/kg-day (POD)**

**Table B-26. Estimated Mouse Pup-to-Maternal Dose Ratio Based on First-Order Model<sup>a</sup>**

Pup Nursing Duration	Ratio
1 week, Peak Pup Intake	2.4
1 week, Average Pup Intake	1.3
3 weeks, Peak Pup Intake	2.4
3 weeks, Average Pup Intake	0.5

<sup>a</sup> Calculated using "Rodent Time Dependent Model.xlsm" provided to workshop participants

### ***Biotransfer Method***

The simplest technique for linking maternal and infant intake involves estimating the transfer of DDT to milk using a biotransfer factor. This method is discussed in the Combustor Assessment ([U.S. EPA, 1998](#)). The technique is based on a study by Travis and Arms ([1988](#)) and assumes that the milk fat contaminant concentration is proportional to maternal contaminant intake, with the proportionality constant represented by a biotransfer factor:

$$C_{\text{milk fat}} = DI_{\text{MAT}} \times BTF_m \times BW_{\text{Mat}}$$

Appendix Equation 18

Where:

- $C_{\text{milkfat}}$  = the concentration of contaminant in the maternal milk fat (ng/kg milk fat),
- $DI_{\text{MAT}}$  = daily maternal intake of contaminant (ng/kg BW-day),
- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $BW_{\text{MAT}}$  = the maternal body weight (kg).

The biotransfer factor is then estimated using a regression equation based on the octanol-water partition coefficient ( $K_{ow}$ ) using the expression:

$$BTF_m = 0.00062 \times K_{ow}$$

Appendix Equation 19

Where:

- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $K_{ow}$  = the octanol-water partition coefficient (unitless).

The regression equation was estimated using six highly lipophilic compounds with  $\log(K_{ow})$  in the range of 5.16 to 6.5. According to the Combustor Assessment ([U.S. EPA, 1998](#)), the model tends to over-predict concentrations. Thus, the Combustor Assessment suggests the equation should only be used if parameters for more sophisticated kinetic models cannot be found in the literature and if the  $K_{ow}$  of the chemical in question is within the range of the  $K_{ow}$  used to fit the model. This method is included for consideration.

The daily infant contaminant dose can then be estimated for the biotransfer method using the equation:

$$DI_{INF} = \frac{(C_{milk\ fat} \times f_{mbm}) \times CR_{milk} \times ED_{INF}}{BW_{INF} \times AT}$$

Appendix Equation 20

Where:

- $DI_{INF}$  = the daily infant contaminant dose (ng/kg-day),
- $C_{milkfat}$  = the concentration of contaminant in the maternal milk fat (ng/kg milk fat),
- $f_{mbm}$  = the fraction of fat in milk (dimensionless),
- $CR_{milk}$  = the infant ingestion rate of milk (kg/day),
- $ED_{INF}$  = the infant exposure duration (year),
- $BW_{INF}$  = the infant's body weight (kg), and
- $AT$  = the averaging time (year).

Using the above equations, the ratio between the infant and maternal intake can be estimated as:

$$\frac{DI_{INF}}{DI_{MAT}} = \frac{0.00062 \times K_{OW} \times f_{mbm} \times CR_{milk} \times ED_{INF} \times BW_{Mat}}{BW_{INF} \times AT}$$

Appendix Equation 21

### *Input Parameters and Application to DDT in Humans*

The input parameters used for DDT are shown in [Table B-27](#). In addition to using mid-range (e.g., mean) values in the model, low and high end estimates were also used for some parameters where available to determine the approximate range of human variability. The  $ED_{INF}$  and AT values are equal to each other (either 1 month or 12 months) and cancel from the equation.

**Table B-27. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age**

Parameter (units)	Variable	Low	Mid	High	Note/source
Maternal weight during lactation (kg) <sup>a</sup>	$BW_{Mat}$	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, 1 month (kg)	$BW_{INF, 1}$	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, average over 12 months (kg)	$BW_{INF, 12}$	7.8			Weighted mean, U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, 1 month (kg milk/day)	$CR_{milk,1}$	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, average over 12 months (kg milk/day)	$CR_{milk,12}$	0.66			Weighted mean, U.S. EPA ( <a href="#">2011a</a> )
Fraction of fat in breast milk (dimensionless) <sup>b</sup>	$f_{mbm}$	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Octanol-water partition coefficient	$\log(K_{ow})$	6.91			Hansch et al. ( <a href="#">1995b</a> )

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 < 50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents the typical range and midpoint of the range.

Based on the  $K_{ow}$  value and using [Appendix Equation 19](#), the biotransfer factor for DDT is 8,128,305.

Using this  $BTF_m$ , the average infant to maternal dose ratio was estimated for infants at 1 and 12 months of age and is presented in [Table B-28](#). For the model parameterization presented here, these ratios tend to be several orders of magnitude larger than the estimates from the simple one-compartment kinetic model.



**Table B-28. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method**

Age	Low	Mid	High
1 month	803.2	1204.8	1606.4
12 months	639.6	959.5	1279.3

### **B.2.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation**

The previous sections discuss the tools and input data available to estimate internal dose metrics for DDT in mice and humans. The next step is to select a method for performing cross-species extrapolation by weighing biological sophistication against methodology and data uncertainty. [Table B-29](#) presents potential dose metrics and methods for performing the cross-species extrapolation. This list may not be exhaustive but is intended to foster discussion at the workshop. For illustrative purposes in this briefing packet, one method for HED calculation was selected (shown in bold type), and sample calculations were performed.

**Table B-29. Potential Dose Metrics and Methods for HED Estimation**

Should the Cross-Species Extrapolation Be Performed on Maternal or Offspring Dose Metric?	What Technique Is Used to Estimate the Dose Metrics?	What Dose Metric Should Be Used for Cross-Species Extrapolation?
<b>Maternal Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in mice and humans</li> <li>• <b>First order kinetic maternal model in mice and humans</b></li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Maternal average or peak body burden during lactation</li> <li>• Maternal average or peak fat concentration during lactation</li> <li>• <b>Maternal average or peak milk concentration during lactation</b></li> </ul>
<b>Offspring Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in mice and humans</li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Infant average or peak body burden during lactation</li> </ul>
<b>Other?</b>	<ul style="list-style-type: none"> <li>• PBPK model in mice and humans</li> <li>• First order kinetic maternal model in mice and humans</li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Other?</li> </ul>

In selecting the dose metric to use for cross-species extrapolation and the technique to use to estimate the dose metrics, the discussion points listed in the text boxes throughout this section should be considered. This information is synthesized in the following text box.

As an example, the points were considered, and an example was provided to foster discussion. The chemical-specific information available should be considered to help guide the decision on whether a maternal or offspring dose metric should be utilized. Humans are primarily exposed to DDT and DDE in food, with DDE exposure being higher than DDT ([ATSDR, 2002a](#)). Then, once DDT and DDE enter the body, they are further metabolized to multiple metabolic products, as discussed in [Section B.2.5.1](#). Thus, the infant will be exposed to multiple metabolites in breast milk. In contrast, in the POD mouse study, the maternal mice were exposed to only o-p' DDT, and the metabolic pathways are different in mice and humans. The available kinetic models for DDT do not account for this difference in metabolism and the differences in the exposures (e.g., constituents). Thus, in the example provided here, the decision was



made to perform the cross-species extrapolation on the maternal concentrations because the mothers receive the initial dose.

Next, a model was selected to characterize the dose metrics. No full PBPK model was available for mice and humans although half-life values for mice and humans were found in the literature. For this reason, the maternal portion of the first-order model was judged to be sufficiently parameterized to justify its use instead of the simpler biotransfer method. Thus, the first-order, time-dependent model in MS Excel was used to estimate the human and mouse maternal concentrations. Again, the dynamic metabolic elimination and the effects of DDT's chemical composition as a mixture are not captured in this simple model.

Finally, the maternal dose metric was selected. As discussed in [Section B.2.5.3](#), no critical window could be determined for the developmental effect in the proposed POD. This is due to the fact that the developmental effects were noted following exposure in both the gestational and lactational periods, and therefore the exact critical window is uncertain.

As discussed previously, the toxicological database for DDT suggests that the critical exposure window for developmental effects may likely fall during the gestational period. Based on this conclusion, the contribution of lactational DDT exposure to developmental health effects is uncertain. Despite that, the methods developed in this briefing packet may be useful in the assessment of postnatal exposure to chemicals with similar biochemical or pharmacokinetic properties. The average maternal concentration was selected to account for the fact that the effect may occur at any point during lactation. For the dose metric, the maternal milk was selected to capture differences in body fat percentages and milk fat concentrations in the different species.

The selections described in the preceding paragraphs were applied to the candidate POD study. The dose was administered between GD 11 and 17. The average milk concentration in the maternal mouse during lactation at the POD dose (0.02 mg/kg-day) was predicted to be 0.055 mg/kg. The human first-order model was used to find the maternal continuous daily exposure that resulted in the same average maternal milk concentration, assuming "mid" parameters for the human model (e.g., pregnancy at age 25). Continuous daily exposure was used to reflect a chronic maternal intake. In this example, the resulting HED is  $2.3 \times 10^{-4}$  mg/kg-day.

### Discussion Points for DDT HED Estimation

- ? DDT is a mixture. How can the relationship between human and animal dose metrics for each constituent be effectively addressed?
  - To perform cross-species extrapolation, it is necessary to understand the relative toxicity and kinetic properties of each constituent if more sophisticated HED methods are chosen.
- ? Constituents of DDT are metabolized. How should differences in metabolism between animals and humans be effectively addressed?
  - If the cross-species extrapolation is performed on the infants, there is a greater burden on understanding the metabolism, excretion, and toxicity of each metabolite.
- ? What pharmacokinetic data or relevant chemical-specific information are available for the DDT constituents and metabolites for both animals and humans to facilitate model implementation?
  - ✓ Octanol-water partition coefficient,  $K_{ow}$
  - ✓ Half-lives in humans and mice
  - ✓ Absorption fractions and fraction of chemical stored in fat (assumptions can be made based on other PBT chemicals)
  - ✗ No available *in vitro*-derived partition coefficients in humans or mice; partition coefficients can be derived from  $K_{ow}$
  - ✗ No available metabolism rates
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
  - ✓ Peak or average concentrations from maternal or infant dose metrics
  - ✗ The critical window could not be defined
- ? What PBPK or other simpler biokinetic models are available for DDT?
  - ✗ No human PBPK model validated against human data is available
  - ✗ No mouse PBPK model is available
  - ✓ Simple first order models are available
  - ✓ Simple biotransfer methods are available
- ? What is the best method for estimating the human equivalent dose using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

## B.2.7. References

- [Adamsson, A; Salonen, V; Paranko, J; Toppari, J.](#) (2009). Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) on steroidogenesis in the fetal rat testis and adrenal gland. *Reprod Toxicol* 28: 66-74. <http://dx.doi.org/10.1016/j.reprotox.2009.03.002>
- [Ando, M.](#) (1978). Transfer of 2,4,5,2',4',5'-hexachlorobiphenyl and 2,2-bis-(p-chlorophenyl),1,1,1-trichloroethane (p,p'-DDT) from maternal to newborn and suckling rats. *Arch Toxicol* 41: 179-186.
- [Ando, M; Wakisaka, I.](#) (1978). [Transfer of 2,4,5,2',4',5'-hexachlorobiphenyl and p,p'-DDT to new born rats from the mother] [Abstract]. *Nippon Eiseigaku Zasshi* 33: 216.
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2002a). Toxicological profile for DDT, DDE, and DDD. Atlanta, GA. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=81&tid=20>
- [Bhatia, R; Shiau, R; Petreas, M; Weintraub, JM; Farhang, L; Eskenazi, B.](#) (2005). Organochlorine pesticides and male genital anomalies in the child health and development studies. *Environ Health Perspect* 113: 220-224.
- [Bouwman, H; Becker, PJ; Cooppan, RM; Reinecke, AJ.](#) (1992). Transfer of DDT used in malaria control to infants via breast milk. *Bull World Health Organ* 70: 241-250.
- [Brown, RP; Delp, MD; Lindstedt, SL; Rhomberg, LR; Beliles, RP.](#) (1997). Physiological parameter values for physiologically based pharmacokinetic models [Review]. *Toxicol Ind Health* 13: 407-484.
- [Chen, J; Gao, J.](#) (1993). The Chinese total diet study in 1990. Part I. Chemical contaminants. *J AOAC Int* 76: 1193-1205.
- [Craan, AG; Haines, DA.](#) (1998). Twenty-Five Years of Surveillance for Contaminants in Human Breast Milk. *Arch Environ Contam Toxicol* 35: 702-710. <http://dx.doi.org/10.1007/s002449900434>
- [Craig, GR; Ogilvie, DM.](#) (1974). Alteration of t-maze performance in mice exposed to DDT during pregnancy and lactation. *Environ Physiol Biochem* 4: 189-199.
- [Dallaire, F; Dewailly, E; Muckle, G; Vézina, C; Jacobson, SW; Jacobson, JL; P, A.](#) (2004). Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik. *Environ Health Perspect* 112: 1359-1364. <http://dx.doi.org/10.1289/ehp.7255>
- [Damgaard, IN; Skakkebaek, NE; Toppari, J; Virtanen, HE; Shen, H; Schramm, KW; Petersen, JH; Jensen, TK; Main, KM.](#) (2006). Persistent pesticides in human breast milk and cryptorchidism. *Environ Health Perspect* 114: 1133-1138.
- [Devanathan, G; Subramanian, A; Someya, M; Sudaryanto, A; Isobe, T; Takahashi, S; Chakraborty, P; Tanabe, S.](#) (2009). Persistent organochlorines in human breast milk from major metropolitan cities in India. *Environ Pollut* 157: 148-154. <http://dx.doi.org/10.1016/j.envpol.2008.07.01>
- [Dewailly, E; Ayotte, P; Bruneau, S; Gingras, S; Belles-Isles, M; Roy, R.](#) (2000). Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. *Environ Health Perspect* 108: 205-211. <http://dx.doi.org/10.1289/ehp.00108205>
- [Eriksson, P; Ahlbom, J; Fredriksson, A.](#) (1992). Exposure to DDT during a defined period in neonatal life induces permanent changes in brain muscarinic receptors and behaviour in adult mice. *Brain Res* 582: 277-281. [http://dx.doi.org/10.1016/0006-8993\(92\)90144-X](http://dx.doi.org/10.1016/0006-8993(92)90144-X)
- [Eskenazi, B; Marks, AR; Bradman, A; Fenster, L; Johnson, C; Barr, DB; Jewell, NP.](#) (2006). In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. *Pediatrics* 118: 233-241. <http://dx.doi.org/10.1542/peds.2005-3117>
- [Fahim, MS; Bennett, R; Hall, DG.](#) (1970). Effect of DDT on the nursing neonate [Letter]. *Nature* 228: 1222-1223. <http://dx.doi.org/10.1038/2281222a0>

- Fang, SC; Fallin, E; Freed, VH. (1977). Maternal transfer of 14<sup>1</sup>-DDT via placenta and milk and its metabolism in infant rats. *Arch Environ Contam Toxicol* 5: 427-536. <http://dx.doi.org/10.1007/BF02220922>
- Fenster, L; Eskenazi, B; Anderson, M; Bradman, A; Hubbard, A; Barr, DB. (2007). In utero exposure to DDT and performance on the Brazelton neonatal behavioral assessment scale. *Neurotoxicology* 28: 471-477. <http://dx.doi.org/10.1016/j.neuro.2006.12.009>
- Frank, R; Braun, HE; Thorpe, B. (1993). Comparison of DDE and PCB residues in the general diet and in human blood--Ontario 1986-87. *Bull Environ Contam Toxicol* 51: 146-152. <http://dx.doi.org/10.1007/BF00201013>
- Frank, R; Rasper, J; Smout, MS; Braun, HE. (1988). Organochlorine residues in adipose tissues blood and milk from Ontario, Canada residents 1976-1985. *Can J Public Health* 79: 150-158.
- Fried, GF; Marrow, GS; Gordon, CH. (1969). Comparative excretion and retention of DDT analogs by dairy cows. *J Dairy Sci* 52: 1800-1805. [http://dx.doi.org/10.3168/jds.S0022-0302\(69\)86844-X](http://dx.doi.org/10.3168/jds.S0022-0302(69)86844-X)
- Fries, GF; Marrow, GS. (1974). Hexachlorobenzene excretion into milk of cows. *J Anim Sci* 39: 155.
- Fujii, Y; Haraguchi, K; Harada, KH; Hitomi, T; Inoue, K; Itoh, Y; Watanabe, T; Takenaka, K; Uehara, S; Yang, HR; Kim, MY; Moon, CS; Kim, HS; Wang, P; Liu, A; Hung, NN; Koizumi, A. (2011). Detection of dicofol and related pesticides in human breast milk from China, Korea and Japan. *Chemosphere* 82: 25-31. <http://dx.doi.org/10.1016/j.chemosphere.2010.10.036>
- Galassi, S; Bettinetti, R; Neri, MC; Falandysz, J; Kotecka, W; King, I; Lo, S; Klingmueller, D; Schulte-Oehlmann, U. (2008). pp'DDE contamination of the blood and diet in central European populations. *Sci Total Environ* 390: 45-52. <http://dx.doi.org/10.1016/j.scitotenv.2007.09.029>
- Galván-Portillo, M; Jiménez-Gutiérrez, C; Torres-Sánchez, L; López-Carrillo, L. (2002). Food consumption and adipose tissue DDT levels in Mexican women. *Cad Saude Publica* 18: 447-452.
- Gingell, R; Wallcave, L. (1974). Species differences in the acute toxicity and tissue distribution of DDT in mice and hamsters. *Toxicol Appl Pharmacol* 28: 385-394.
- Gladen, BC; Ragan, NB; Rogan, WJ. (2000). Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. *J Pediatr* 136: 490-496. <http://dx.doi.org/10.1067/mpd.2000.103505>
- Gladen, BC; Rogan, WJ. (1991). Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. *J Paediatr Child Health* 119: 58-63.
- Gladen, BC; Rogan, WJ; Hardy, P; Thullen, J; Tingelstad, J; Tully, M. (1988). Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene transplacentally and through human milk. *J Pediatr* 113: 991-995. [http://dx.doi.org/10.1016/S0022-3476\(88\)80569-9](http://dx.doi.org/10.1016/S0022-3476(88)80569-9)
- Görs, S; Kucia, M; Langhammer, M; Junghans, P; Metges, CC. (2009). Technical note: Milk composition in mice--methodological aspects and effects of mouse strain and lactation day. *J Dairy Sci* 92: 632-637. <http://dx.doi.org/10.3168/jds.2008-1563>
- Gray, LE, Jr; Price, M; Lambright, C; Wolf, C; Hotchkiss, A; Parks, L; Ostby, J. (1999). Environmental antiandrogens: the malformation pattern varies with the mechanism of antiandrogenic action. *Biol Reprod* 60: 201.
- Hansch, C; Leo, A; Hoekman, D. (1995b). Dibutyl phthalate. In SR Heller (Ed.), *Exploring QSAR Hydrophobic, electronic, and steric constants* (pp. 144). Washington, DC: American Chemical Society.
- Haraguchi, K; Koizumi, A; Inoue, K; Harada, KH; Hitomi, T; Minata, M; Tanabe, M; Kato, Y; Nishimura, E; Yamamoto, Y; Watanabe, T; Takenaka, K; Uehara, S; Yang, HR; Kim, MY; Moon, CS; Kim, HS; Wang, P; Liu, A; Hung, NN. (2009). Levels and regional trends of persistent organochlorines and polybrominated diphenyl ethers in Asian breast milk demonstrate POPs signatures unique to individual countries. *Environ Int* 35: 1072-1079. <http://dx.doi.org/10.1016/j.envint.2009.06.003>

- [Hayes, WJ.](#) (1976). Dosage relationships associated with DDT in milk. *Toxicol Appl Pharmacol* 38: 19-28. [http://dx.doi.org/10.1016/0041-008X\(76\)90156-3](http://dx.doi.org/10.1016/0041-008X(76)90156-3)
- [Hernik, A; Góralczyk, K; Struciński, P; Czaja, K; Kucharska, A; Korcz, W; Snopczyński, T; Ludwicki, JK.](#) (2011). Polybrominated diphenyl ethers, polychlorinated biphenyls and organochlorine pesticides in human milk as markers of environmental exposure to these compounds. *Ann Agric Environ Med* 18: 113-118.
- [Hui, LL; Hedley, AJ; Kypke, K; Cowling, BJ; Nelson, EA; Wong, TW; van Leeuwen, FX; Malisch, R.](#) (2008). DDT levels in human milk in Hong Kong, 2001-02. *Chemosphere* 73: 50-55. <http://dx.doi.org/10.1016/j.chemosphere.2008.05.045>
- [Johnson, MS; Thomson, SC; Speakman, JR.](#) (2001). Limits to sustained energy intake. I. Lactation in the laboratory mouse *Mus musculus*. *J Exp Biol* 204: 1925-1935.
- [Kalra, RL; Chawla, RP; Joia, BS; Tiwana, MS.](#) (1986). Excretion of DDT residues into milk of the Indian buffalo, *Bubalus bubalis* (L.) after oral and dermal exposure. *Pestic Sci* 17: 128-134. <http://dx.doi.org/10.1002/ps.2780170209>
- [Kapoor, SK; Kalra, RL.](#) (1993). Comparative excretion of DDT analogues into milk of Indian buffalo, *Bubalus bubalis* L. following oral administration. *Pestic Sci* 37: 261-266. <http://dx.doi.org/10.1002/ps.2780370305>
- [Kashyap, R; Iyer, LR; Singh, MM.](#) (1994). Evaluation of daily dietary intake of dichloro-diphenyl-trichloroethane (DDT) and benzene hexachloride (BHC) in India. *Arch Environ Health* 49: 63-66. <http://dx.doi.org/10.1080/00039896.1994.9934417>
- [Kelce, WR; Stone, CR; Laws, SC; Gray, LE; Kemppainen, JA; Wilson, EM.](#) (1995). Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature* 375: 581-585. <http://dx.doi.org/10.1038/375581a0>
- [Konishi, Y; Kuwabara, K; Hori, S.](#) (2001). Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in Osaka, Japan. *Arch Environ Contam Toxicol* 40: 571-578. <http://dx.doi.org/10.1007/s002440010212>
- [Krauthacker, B; Votava-Raić, A; Herceg Romanić, S; Tjesić-Drinković, D; Tjesić-Drinković, D; Reiner, E.](#) (2009). Persistent organochlorine compounds in human milk collected in Croatia over two decades. *Arch Environ Contam Toxicol* 57: 616-622. <http://dx.doi.org/10.1007/s00244-009-9301-3>
- [Kumar, A; Dayal, P; Shukla, G; Singh, G; Joseph, PE.](#) (2006). DDT and HCH residue load in mother's breast milk: a survey of lactating mother's from remote villages in Agra region. *Environ Int* 32: 248-251. <http://dx.doi.org/10.1016/j.envint.2005.08.019>
- [Lee, SK; Hamer, D; Bedwell, CL; Lohitnavy, M; Yang, RSH.](#) (2009). Effect of PCBs on the lactational transfer of methyl mercury in mice: PBPK modeling. *Environ Toxicol Pharmacol* 27: 75-83. <http://dx.doi.org/10.1016/j.etap.2008.08.014>
- [Lee, SK; Ou, YC; Yang, RSH.](#) (2002). Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in nonpregnant mice, lactating mice, and suckling pups. *Toxicol Sci* 65: 26-34. <http://dx.doi.org/10.1093/toxsci/65.1.26>
- [Leng, JH; Kayama, F; Wang, PY; Nakamura, M; Nakata, T; Wang, Y.](#) (2009). Levels of persistent organic pollutants in human milk in two Chinese coastal cities, Tianjin and Yantai: Influence of fish consumption. *Chemosphere* 75: 634-639. <http://dx.doi.org/10.1016/j.chemosphere.2009.01.008>
- [Matsumoto, H; Murakami, Y; Kuwabara, K; Tanaka, R; Kashimoto, T.](#) (1987). Average daily intake of pesticides and polychlorinated biphenyls in total diet samples in Osaka, Japan. *Bull Environ Contam Toxicol* 38: 954-958. <http://dx.doi.org/10.1007/BF01609079>
- [Mes, J; Davies, DJ; Doucet, J; Weber, D; McMullen, E.](#) (1993). Levels of chlorinated hydrocarbon residues in Canadian human breast milk and their relationship to some characteristics of the donors. *Food Addit Contam* 10: 429-441. <http://dx.doi.org/10.1080/02652039309374166>

- Metcalfe, RL. (1995). Insect control technology. In J Kroschwitz; M Howe-Grant (Eds.), Kirk-Othmer encyclopedia of chemical technology: Volume 14: Imaging technology to lanthanides (4th ed., pp. 524-602). New York, NY: John Wiley & Sons.
- Morales, E; Sunyer, J; Castro-Giner, F; Estivill, X; Julvez, J; Ribas-Fitó, N; Torrent, M; Grimalt, JO; de Cid, R. (2008). Influence of glutathione s-transferase polymorphisms on cognitive functioning effects induced by p,p'-ddt among preschoolers. *Environ Health Perspect* 116: 1581-1585.  
<http://dx.doi.org/10.1289/ehp.11303>
- Newsome, WH; Davies, D; Doucet, J. (1995). PCB and organochlorine pesticides in Canadian human milk-1992. *Chemosphere* 30: 2143-2153. [http://dx.doi.org/10.1016/0045-6535\(95\)00086-N](http://dx.doi.org/10.1016/0045-6535(95)00086-N)
- Niessen, KH; Ramolla, J; Binder, M; Gruegmann, G; Hofmann, U. (1984). Chlorinated hydrocarbons in adipose tissue of infants and toddlers: Inventory and studies on their association with intake of mothers milk. *Eur J Pediatr* 142: 238-243. <http://dx.doi.org/10.1007/BF00540242>
- O'Flaherty, EJ; Scott, W; Schreiner, C; Beliles, RP. (1992). A physiologically based kinetic model of rat and mouse gestation: disposition of a weak acid. *Toxicol Appl Pharmacol* 112: 245-256.  
[http://dx.doi.org/10.1016/0041-008X\(92\)90194-W](http://dx.doi.org/10.1016/0041-008X(92)90194-W)
- Palanza, P; Parmigiani, S; Liu, H; vom Saal, FS. (1999). Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and o,p'-DDT alters aggressive behavior of male and female house mice. *Pharmacol Biochem Behav* 64: 665-672.
- Palanza, P; Parmigiani, S; vom Saal, FS. (2001). Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'-DDT, and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. *Horm Behav* 40: 252-265. <http://dx.doi.org/10.1006/hbeh.2001.1697>
- Poulin, P; Krishnan, K. (1996). A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. *Toxicol Appl Pharmacol* 136: 126-130.  
<http://dx.doi.org/10.1006/taap.1996.0015>
- Price, K; Haddad, S; Krishnan, K. (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol Environ Health A* 66: 417-433.  
<http://dx.doi.org/10.1080/15287390306450>
- Rawn, DF; Cao, XL; Doucet, J; Davies, DJ; Sun, WF; Dabeka, RW; Newsome, WH. (2004). Canadian Total Diet Study in 1998: pesticide levels in foods from Whitehorse, Yukon, Canada, and corresponding dietary intake estimates. *Food Addit Contam* 21: 232-250. <http://dx.doi.org/10.1080/02652030310001655470>
- Ribas-Fito, N; Cardo, E; Sala, M; Eulalia De Muga, M; Mazon, C; Verdu, A; Kogevinas, M; Grimalt, JO; Sunyer, J. (2003). Breastfeeding, exposure to organochlorine compounds, and neurodevelopment in infants. *Pediatrics* 111: e580-e585.
- Ritter, R; Scheringer, M; Macleod, M; Schenker, U; Hungerbühler, K. (2009). A multi-individual pharmacokinetic model framework for interpreting time trends of persistent chemicals in human populations: application to a postban situation. *Environ Health Perspect* 117: 1280-1286. <http://dx.doi.org/10.1289/ehp.0900648>
- Rogan, WJ; Gladen, BC. (1991). PCBs, DDE, and child development at 18 and 24 months. *Ann Epidemiol* 1: 407-413.
- Rogers, EH; Hunter, ES; Rosen, MB; Rogers, JM; Lau, C; Hartig, PC; Francis, BM; Chernoff, N. (2003). Lack of evidence for intergenerational reproductive effects due to prenatal and postnatal undernutrition in the female CD-1 mouse. *Reprod Toxicol* 17: 519-525.
- Subramanian, A; Ohtake, M; Kunisue, T; Tanabe, S. (2007). High levels of organochlorines in mothers' milk from Chennai (Madras) city, India. *Chemosphere* 68: 928-939.  
<http://dx.doi.org/10.1016/j.chemosphere.2007.01.041>

- [Sunyer, J; Torrent, M; Garcia-Esteban, R; Ribas-Fitó, N; Carrizo, D; Romieu, I; Antó, JM; Grimalt, JO.](#) (2006). Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. *Clin Exp Allergy* 36: 1236-1241. <http://dx.doi.org/10.1111/j.1365-2222.2006.02560.x>
- [Szyrwińska, K; Lulek, J.](#) (2007). Exposure to specific polychlorinated biphenyls and some chlorinated pesticides via breast milk in Poland. *Chemosphere* 66: 1895-1903. <http://dx.doi.org/10.1016/j.chemosphere.2006.08.010>
- [Takahashi, KL; Aoyama, H; Hojo, H; Shimizu, N; Kikuta, M; Kushida, H; Kawakatsu, H; Ebino, K; Teramoto, S.](#) (2001). Prenatal developmental toxicity studies of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT) in rats and rabbits. *Congenit Anom* 41: 321-328. <http://dx.doi.org/10.1111/j.1741-4520.2001.tb00842.x>
- [Telford, HS; Guthrie, JE.](#) (1945). Transmission of the toxicity of DDT through the milk of white rats and goats. *Science* 102: 647.
- [The Jackson Laboratory.](#) (2012). Mouse phenome database. Available online at <http://phenome.jax.org/db/q?rtn=meas/catlister&req=Dreproductionqqq245>
- [Timson, BF; Coffman, JL.](#) (1984). Body composition by hydrostatic weighing at total lung capacity and residual volume. *Med Sci Sports Exerc* 16: 411-414.
- [Torres-Sanchez, L; Rothenberg, SJ; Schnaas, L; Cebrian, ME; Osorio, E; Hernandez, MD; Garcia-Hernandez, RM; del Rio-Garcia, C; Wolff, MS; Lopez-Carrillo, L.](#) (2007). In utero p,p'-DDE exposure and infant neurodevelopment: A perinatal cohort in Mexico. *Environ Health Perspect* 115: 435-439. <http://dx.doi.org/10.1289/ehp.9566>
- [Torres-Sánchez, L; Schnaas, L; Cebrián, ME; Hernández, M; Valencia, EO; García Hernández, RM; López-Carrillo, L.](#) (2009). Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and neurodevelopment: a follow-up from 12 to 30 months of age. *Neurotoxicology* 30: 1162-1165. <http://dx.doi.org/10.1016/j.neuro.2009.08.010>
- [Travis, CC; Arms, AD.](#) (1988). Bioconcentration of organics in beef, milk, and vegetation. *Environ Sci Technol* 22: 271-274. <http://dx.doi.org/10.1021/es00168a005>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1988). Recommendations for and documentation of biological values for use in risk assessment [EPA Report]. (EPA/600/6-87/008). Cincinnati, OH. <http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=34855>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1998). Methodology for assessing health risks associated with multiple pathways of exposure to combustor emissions [EPA Report]. (EPA 600/R-98/137). Washington, DC. <http://cfpub.epa.gov/ncea/CFM/recorddisplay.cfm?deid=55525>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2011a). Exposure factors handbook 2011 edition (final) [EPA Report]. (EPA/600/R-09/052F). <http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=236252>
- [U.S. EPA.](#) (2012b). EPA's reanalysis of key issues related to dioxin toxicity and response to NAS comments (CAS No. 1746-01-6) [EPA Report]. (EPA/600/R-10/038F). Washington, DC.
- [Veeramachaneni, DN; Palmer, JS; Amann, RP; Pau, KY.](#) (2007). Sequelae in male rabbits following developmental exposure to p,p'-DDT or a mixture of p,p'-DDT and vinclozolin: cryptorchidism, germ cell atypia, and sexual dysfunction. *Reprod Toxicol* 23: 353-365. <http://dx.doi.org/10.1016/j.reprotox.2007.01.001>
- [Verner, MA; Ayotte, P; Muckle, G; Charbonneau, M; Haddad, S.](#) (2009). A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. *Environ Health Perspect* 117: 481-487.
- [Verner, MA; Charbonneau, M; López-Carrillo, L; Haddad, S.](#) (2008). Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: A new tool in breast cancer epidemiologic studies. *Environ Health Perspect* 116: 886-892. <http://dx.doi.org/10.1289/ehp.10917>



- WHO (World Health Organization). (2005). Frequently asked questions on DDT use for disease vector control. Geneva, Switzerland. [http://www.chem.unep.ch/DDT/documents/WHO\\_FAQonDDT.pdf](http://www.chem.unep.ch/DDT/documents/WHO_FAQonDDT.pdf)
- WHO. (2011). DDT in indoor residual spraying: Human health aspects [WHO EHC]. In Environmental Health Criteria. (Environmental health criteria: 241). Geneva, Switzerland. <http://www.who.int/ipcs/publications/ehc/ehc241.pdf>
- Witt, JM; Whiting, FM; Brown, WH; Stull, JW. (1966). Contamination of milk from different routes of animal exposure to DDT. J Dairy Sci 49: 370-380. [http://dx.doi.org/10.3168/jds.S0022-0302\(66\)87877-3](http://dx.doi.org/10.3168/jds.S0022-0302(66)87877-3)
- Wolff, MS. (1999). Letter to the Editor. Arch Environ Contam Toxicol 36: 504-504. <http://dx.doi.org/10.1007/PL00006624>
- Wolff, MS; Zeleniuch-Jacquotte, A; Dubin, N; Toniolo, P. (2000). Risk of breast cancer and organochlorine exposure. Cancer Epidemiol Biomarkers Prev 9: 271-277.
- Yamasaki, K; Okuda, H; Takeuchi, T; Minobe, Y. (2009). Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicol Lett 189: 14-20. <http://dx.doi.org/10.1016/j.toxlet.2009.04.023>
- You, L; Casanova, M; Archibeque-Engle, S; Sar, M; Fan, LQ; Heck, HA. (1998). Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to p,p'-DDE. Toxicol Sci 45: 162-173.
- You, L; Gazi, E; Archibeque-Engle, S; Casanova, M; Conolly, RB; Heck, HA. (1999). Transplacental and lactational transfer of p,p'-DDE in Sprague-Dawley rats. Toxicol Appl Pharmacol 157: 134-144.
- Zhou, P; Wu, Y; Yin, S; Li, J; Zhao, Y; Zhang, L; Chen, H; Liu, Y; Yang, X; Li, X. (2011). National survey of the levels of persistent organochlorine pesticides in the breast milk of mothers in China. Environ Pollut 159: 524-531. <http://dx.doi.org/10.1016/j.envpol.2010.10.014>
- Zietz, BP; Hoopmann, M; Funcke, M; Huppmann, R; Suchenwirth, R; Gierden, E. (2008). Long-term biomonitoring of polychlorinated biphenyls and organochlorine pesticides in human milk from mothers living in northern Germany. Int J Hyg Environ Health 211: 624-638. <http://dx.doi.org/10.1016/j.ijheh.2008.04.001>



### B.3. Hexachlorobenzene Briefing Packet



## Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk

### Workshop Briefing Packet: Hexachlorobenzene

#### Prepared for

National Center for Environmental Assessment  
U.S. EPA  
109 T.W. Alexander Drive  
Durham, NC 27711

#### Prepared by

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713

#### Notice

Although the research described in this briefing packet has been supported by the U. S. Environmental Protection Agency through EP-C-09-009 to ICF International, it does not necessarily reflect the views of the Agency and no official endorsement should be inferred.



## Contents B.3

<b>Appendix B. Original Briefing Packets, as Distributed to Workshop Attendees .....</b>	<b>B-1</b>
B.3. Hexachlorobenzene Briefing Packet.....	B-140
B.3.1. Introduction.....	B-142
B.3.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans.....	B-144
B.3.2.1. Mothers' Exposure to HCB .....	B-145
B.3.2.2. Is HCB Sequestered in Breast Milk or Other Human Tissues? .....	B-147
B.3.2.3. How Much HCB Passes from Mother to Infant via Breast Milk? .....	B-148
B.3.3. Maternal Dose vs. Nursing Offspring Dose in Animals.....	B-149
B.3.3.1. Maternal Exposure and Elimination in Milk .....	B-149
B.3.4. Developmental Effects of HCB .....	B-153
B.3.4.1. Human Studies .....	B-153
B.3.4.2. Animal Studies.....	B-153
B.3.4.3. Exposure-Response Arrays for Developmental Effects .....	B-166
B.3.5. Human Equivalent Dose (HED) Estimation.....	B-168
B.3.5.1. Chemical-Specific Properties Affecting HED Estimation.....	B-170
B.3.5.2. Available Pharmacokinetic Data for HCB.....	B-171
B.3.5.3. Appropriate Dose Metric for Selected Point of Departure (POD) .....	B-174
B.3.5.4. Available Models for Estimating HCB Internal Dose.....	B-175
B.3.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation.....	B-191
B.3.7. References.....	B-199

## List of Figures

Figure B-15. Exposure-Response Array Showing All Developmental Effects, Sorted by Exposure Route, in Animals after Exposure to HCB .....	B-166
Figure B-16. Exposure-Response Array Showing All Developmental Effects, Sorted by Effect Type, in Animals after Exposure to HCB .....	B-167
Figure B-17. Process Diagram for Performing Cross-Species Extrapolation .....	B-169
Figure B-18. Maternal and Infant POP Model (Verner et al., 2009) .....	B-177
Figure B-19. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day .....	B-184
Figure B-20. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Mendoza et al., 1977) and an Administered Dose of 6.7 mg/kg-day (POD).....	B-187

## List of Tables

Table B-30. Intake of HCB as Estimated by FDA Total Diet Study, Select Years, 1982-1994 .....	B-146
Table B-31. HCB Residues in Breast Milk and Blood During Lactation .....	B-148
Table B-32. HCB Concentrations in Mother's Milk and Fecal Excretion of HCB in the Breastfed Infant at 1 and 5 Months of Age .....	B-149
Table B-33. Concentration of HCB in Blood and Fat of Exposed Dams and Their Pups.....	B-151
Table B-34. Mean HCB Concentrations (µg/g wet weight) in Serum and Milk of Dam and Infant Rhesus Monkeys, Dams Dosed with 60 mg/kg-day while Nursing .....	B-152
Table B-35. Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene .....	B-155

Table B-36. Available Pharmacokinetic Parameters for HCB in Humans and Rats.....	B-172
Table B-37. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First- Order Single-Compartment Human Model .....	B-183
Table B-38. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-184
Table B-39. Model Parameters and Their Values Used in First-Order Steady State Single- Compartment Model in Rat .....	B-186
Table B-40. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model <sup>a</sup> .....	B-187
Table B-41. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-190
Table B-42. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method.....	B-190
Table B-43. Potential Dose Metrics and Methods for HED Estimation .....	B-191
Table Att. B.3-1. Mean Levels of HCB in Breast Milk as Reported in IPCS (1997b) .....	B-194
Table Att. B.3-2. Mean Levels of HCB in Breast Milk as Reported by ATSDR (2002b).....	B-197

---

### **B.3.1. Introduction**

Hexachlorobenzene (HCB) is a synthetic compound that was widely used in the United States as a pesticide until 1965; use as a fungicide on seeds of onions, sorghum, wheat, and other grains continued until 1984. It was also used to make fireworks, ammunition, military pyrotechnic and ordnance materials, and synthetic rubber ([ATSDR, 2002b](#)). Commercial production of HCB in the U.S. for these direct uses ended in the late 1970s, but HCB is still formed (1) as a by-product of the manufacture of solvents, other chlorine-containing compounds and pesticides (e.g., trichloroethylene, vinyl chloride, pentachlorophenol, atrazine, lindane), (2) during the combustion of municipal waste, and (3) in the waste streams of chlor-alkali and wood-preserving plants ([ATSDR, 2002b](#)).

In this briefing packet, the available literature on gestational and lactational exposure to HCB was reviewed for key findings and data to help estimate the maternal dose and breastfeeding infant dose of HCB in humans. Selected references are summarized and evaluated in this briefing packet in five sections: maternal dose and breastfeeding infant dose of HCB in humans; maternal dose and nursing offspring dose in animals; developmental effects of HCB in humans and animals; human and animal kinetic models; and a final section, which discusses the application of kinetic models to derive an example human equivalent dose (HED).

These briefing packets are intended to stimulate ideas and provide material for discussion regarding innovative approaches to the assessment of risk to breastfeeding infants from HCB and other PBT chemicals in breast milk. The information in this and other briefing packets is provided to help workshop participants discuss and formulate answers to the workshop charge questions and does not reflect Agency opinion or policy regarding HCB. Areas where there exists uncertainty, data gaps, or where there is a lack of understanding in the current literature are highlighted to foster further discussion.

---

### **B.3.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans**

This section discusses how women of child-bearing age may be exposed to HCB; how their bodies sequester HCB in various tissues, and the extent and manner that HCB is eliminated through breast milk, leading to infant exposure. HCB exposure levels vary geographically and may be at least partially dependent on social factors (e.g., dietary choices) and environmental factors (e.g., the amount of residual HCB in the local environment). Concentrations of HCB have been reported in various human tissues, including breast milk, blood plasma, and adipose tissue.

In this briefing packet, measured concentrations of HCB in the diet or environmental media and their relationship with measured body burdens in mothers are presented. Studies are also described that inform the characterization of the relationship between the body burden of HCB

in mothers and intake by breastfeeding infants. However, the relationships between maternal exposure to HCB (i.e., intake from environmental and dietary sources), maternal body burden, and infant dose in humans are not well described in the literature. Attempts have been made to draw connections between studies showing intake and breast milk concentrations in similar geographic areas during similar time periods to illustrate relationships between exposure and body burden of HCB.

---

#### **B.3.2.1. Mothers' Exposure to HCB**

Trace levels of HCB or its metabolites have been measured in nearly all people who have been tested ([ATSDR, 2002b](#)). While direct exposure to HCB is limited in the general population, indirect environmental exposure can occur because HCB released into soil, air, or water remains in the environment for a long time. The half-life in soil is 3-6 years; in surface waters it is 2.7-5.7 years; in ground water it is 5.3-11.4 years; and in air it is 0.63-6.28 years. Releases of HCB can be significant at or near sites where it is generated, such as industrial facilities ([ATSDR, 2002b](#)). HCB does not dissolve easily in water and has been detected at very low to trace levels in drinking water, surface water, and groundwater (below 0.1 parts per trillion) ([ATSDR, 2002b](#)). When HCB is released into a body of water, it is likely to partition into sediment and will likely remain at the bottom of the lake, river, or stream thereby limiting human exposure via surface water. Due to its long half-life in air, HCB released into air can be transported over wide geographic areas. Inhalation of dust particles or dermal contact with contaminated soil/sediment or dust are major routes of exposure, especially for individuals who live near industrial sites where HCB is unintentionally produced as a by-product or disposed of as hazardous waste ([ATSDR, 2002b](#)). Levels up to 440 ng/g have been detected in agricultural soils at sites in analyzed 37 states, and at 15 ng/g in lake sediment. Ambient air measurements have ranged from  $1 \times 10^{-4}$  ng/m<sup>3</sup> to 1.5 ng/m<sup>3</sup>, but indoor air concentrations in a pesticide production facility and industrial plant were much higher: 22,000 ng/m<sup>3</sup> and 150,000 ng/m<sup>3</sup>, respectively ([ATSDR, 2002b](#)). Environmental monitoring of HCB has been focused in the Great Lakes region as historic production of chlorobenzene products was high in this region. Lake sediment levels of HCB have been as high as 97 ng/g in Lake Ontario; mean ambient air levels were 0.03668 ng/m<sup>3</sup> in Villeroy, Quebec ([ATSDR, 2002b](#)).

It has been estimated that about 92% of total HCB intake comes from food, 7% from air, and 1% from drinking water ([IPCS, 1997](#)). Intake studies conducted in various countries (Canada, USA, Germany, Finland, Vietnam, Thailand, India, Japan, Australia, and the Netherlands) have estimated daily intake of HCB from food ranging from approximately 0.0004 to 0.0028 µg/kg-day ([IPCS, 1997](#)). The Fourth National Report on Human Exposure to Environmental Chemicals ([CDC, 2009](#)), did not include any data on HCB. Higher serum HCB levels have been associated with living near agricultural or industrial areas, but background levels in the human population

have declined over the last 20 years. Recent measurements of HCB in human tissues have been near the lipid adjusted limit of detection.<sup>8</sup>

Generally, levels of HCB in foods, human adipose tissue, and breast milk declined from the 1970s to the mid-1990s ([IPCS, 1997](#)). These decreases have been attributed to the discontinued use of HCB in agriculture and industrial applications. Decreased levels of HCB in food were illustrated in the data collected from the U.S. FDA Total Diet Studies ([FDA, 1994](#); [Yess, 1992, 1991](#); [FDA, 1990, 1989](#); [Gunderson, 1988](#)). [Table B-30](#) shows estimated HCB intakes as determined from the FDA Total Diet Studies for select years from 1982 through 1994.

**Table B-30. Intake of HCB as Estimated by FDA Total Diet Study, Select Years, 1982-1994**

Year(s)	Frequency of Detection (%)	Intake for 14-16 year old males (µg/kg-day)	Intake for 60-65 year old females (µg/kg-day)	Intake for 25-30 yr old females (µg/kg-day)	References
1982-1984	9	0.0020	0.0011	0.0013	Gunderson ( <a href="#">1988</a> )
1988	7	0.0011	0.0006	NR	FDA ( <a href="#">1989</a> )
1989	5	0.0009	0.0005	NR	FDA ( <a href="#">1990</a> )
1990	4	0.0005	0.0002	NR	Yess ( <a href="#">1991</a> )
1991	2	0.0004	0.0002	NR	Yess ( <a href="#">1992</a> )
1991-1993	<2	NR	NR	NR	FDA ( <a href="#">1994</a> )

NR = Not reported.

Using methods similar to the FDA Total Diet Study, Nakata et al. ([2002](#)) reported levels of HCB (and other chemicals) in 39 food items collected from Shanghai and Yixing, China in 2000 and 2001. The authors determined that the average daily intake of HCB in China, based on food consumption, was 0.046 µg/person-day. Assuming an average adult body weight of 60 kg, authors calculated that the intake was approximately 0.0007 µg/kg-day, indicating a similar exposure and intake level to the U.S. Total Diet Study a decade prior.

Several studies have investigated intake levels as a result of fish/seafood consumption. Dewailly et al. ([1993](#)) illustrated that the consumption of marine mammal fats was correlated with HCB contamination of human breast milk by comparing HCB concentration in Inuit versus Caucasian women in neighboring regions. Similarly, Ayotte et al. ([1995](#)) found that breast milk levels of organochlorine compounds, including HCB, in Inuit and Caucasian women correlate with omega-3 fatty acid levels in plasma phospholipids, from which they concluded that consumption of

<sup>8</sup> Data discussed here are described in the Biomonitoring Summary for Hexachlorobenzene, from the CDC National Biomonitoring Program. Information available at: [http://www.cdc.gov/biomonitoring/Hexachlorobenzene\\_BiomonitoringSummary.html](http://www.cdc.gov/biomonitoring/Hexachlorobenzene_BiomonitoringSummary.html)

marine organisms serves as a source of HCB exposure and intake ([Ayotte et al., 1995](#)). Newsome et al. ([1995](#)) also reported that higher breast milk levels of HCB were related to eating more than 100 g of fish weekly. More recently, Falco et al. ([2008](#)) estimated intake levels of HCB from marine fish for four age groups (children, adolescents, adults, and seniors) separated by sex. The intake estimated for female adults was 13.3 ng/day, or  $2 \times 10^{-4}$  µg/kg-day, based on an apparent 55 kg bodyweight ([Falcó et al., 2008](#)). The authors did not specify what body weight was used to calculate daily dose, however, using the *Exposure Factors Handbook* reference value of 80 kg ([U.S. EPA, 2011a](#)), the daily dose would be  $1.66 \times 10^{-4}$  µg/kg-day.

Greve ([1986](#)) estimated that the average daily intake of HCB in 1977 to 1978 was 1 µg per person [ $1.25 \times 10^{-5}$  mg/kg-day, assuming average body weight of 80 kg ([U.S. EPA, 2011a](#))], with a maximum value of 12 µg per person ( $2.18 \times 10^{-4}$  mg/kg-day). Intake values were based on a total dietary intake study conducted by analyzing 199 duplicate 24-hour meals for HCB.

---

### **B.3.2.2. Is HCB Sequestered in Breast Milk or Other Human Tissues?**

#### ***HCB in Human Breast Milk***

Several studies were identified that measured HCB concentrations in human milk. Most studies show a decrease in observed HCB concentrations in maternal breast milk and/or neonates over time ([Norén and Meironyté, 2000](#); [Craan and Haines, 1998](#)). The decrease in detected HCB levels has been attributed to the discontinued use of HCB in agriculture and industrial applications ([Hardell et al., 2010](#); [Lackmann, 2002](#); [Vaz et al., 1993](#)).

Findings from several epidemiological studies suggest that HCB levels decrease in breast milk as the number of children breastfed increases, with the percentage of decline ranging from approximately 11% to 50% ([Ennaceur et al., 2007](#); [Kunisue et al., 2006](#); [Kunisue et al., 2004](#); [Newsome et al., 1995](#); [Vaz et al., 1993](#); [Skaare and Polder, 1990](#)). In contrast, Ennaceur et al. ([2007](#)) found HCB concentrations increased 74% for mothers with two children compared to mothers with one child. HCB levels in breast milk may also be related to maternal age and body mass index. Ennaceur et al. ([2007](#)) also observed a trend of increased HCB residues in breast milk of older mothers. Similarly, Lackmann et al. ([1999](#)) showed that HCB concentration in neonatal serum taken prior to the first breast milk feeding was significantly correlated with maternal age ( $p < 0.01$ ), with 2.7-fold higher serum levels in offspring of 40-year-old women as compared with 20-year-old women. Birth order of the neonates was not reported in the study. One study showed a positive relationship between PCB levels in breast milk and decreased body mass index ([Geyer et al., 1986](#)), but another study did not confirm this finding ([Lovelady et al., 1999](#)).

[Attachment B.3-1](#) contains data on breast milk concentrations measured globally from 1970 to 1991, [[Table Att. B.3-1](#) as reported by IPCS ([1997](#))] and from 1967 to 2001 [[Table Att. B.3-2](#), as reported by ATSDR ([2002b](#))].



### *HCB in Other Human Tissues*

In a study by Mes et al. (1984), breast milk samples from 16 women were analyzed for HCB during eight intervals of a 98-day lactation period. Corresponding blood samples were collected the same day as the milk samples. When comparing the blood levels and breast milk levels of HCB (expressed on either a whole milk or milk fat basis), there were no correlations observed (see Table B-31).

**Table B-31. HCB Residues in Breast Milk and Blood During Lactation**

Days following parturition	HCb concentration in breast milk (ng residue/g whole blood) (coefficient of variation)	HCb concentration in maternal blood (ng residue/g whole blood) (coefficient of variation)
7	4.0 (170)	0.3 (167)
14	8.6 (181)	0.3 (167)
28	4.3 (179)	0.3 (167)
42	4.3 (195)	0.3 (167)
56	3.5 (154)	0.3 (167)
70	3.4 (179)	0.3 (167)
84	2.9 (166)	0.1 (100)
98	3.5 (149)	0.1 (100)

Source: Mes et al. (1984)

### **B.3.2.3. How Much HCB Passes from Mother to Infant via Breast Milk?**

Abraham et al. (1994) measured HCB in the feces of one breastfed baby and one formula-fed baby. The infants' stool was collected at the beginning and end of 2.5- and 3-day sampling periods at ages 1 and 5 months, respectively. A corresponding sample of at least 100 mL mother's milk was obtained at the one-month period, and one breast milk sample was obtained for the 5-month period. The results of the study are presented in Table B-32. The study authors concluded that absorption of HCB in the breastfed infant was "almost complete" and that fecal excretion of HCB was generally low.

**Table B-32. HCB Concentrations in Mother's Milk and Fecal Excretion of HCB in the Breastfed Infant at 1 and 5 Months of Age**

Infant age	HCB concentration (ng/g fat)		Infant fecal excretion (total ng) <sup>a</sup>		Concentration ratio <sup>b</sup>
	Milk Sample 1:	Milk Sample 2:	First:	Second (2.5 days later):	
1 month	95	96	17	5	0.1
5 month	Pap (vegetables and carrots):	Milk Sample:	First:	Second (3 days later):	0.7
	<0.4	87	91	72	

<sup>a</sup> Two milk samples were taken 2.5 days apart at the 1 month time period, and one sample of breast milk was taken at the beginning of the 5-month sampling period, along with a food sample of pap, a vegetable and carrot liquid food. The pap was fed once at age 5 months.

<sup>b</sup> Concentration ratio: stool fat/milk fat (at 1 month) and stool fat/dietary fat (at 5 months).

Source: Abraham et al. (1994).

Ribas-Fito et al. (2005) recruited a birth cohort of 92 mother-infant pairs in Spain to measure organochlorine compounds (HCB, dichlorodiphenyl dichloroethane, and polychlorinated biphenyls) in colostrum taken 3 days after delivery, breast milk taken 3 weeks after delivery, and cord and infant serum taken at 13 months of age. At 13 months of age, infants that were breastfed had higher concentrations of HCB in serum than those that were formula-fed (4.26 ng/mL among breastfed infants vs. 2.13 ng/mL among formula-fed infants). Within the group of breastfed infants, those fed for longer durations had higher concentrations of HCB. The authors did not find a correlation between serum concentrations of HCB in the children and concentrations of HCB in colostrum or breast milk.

### B.3.3. Maternal Dose vs. Nursing Offspring Dose in Animals

#### B.3.3.1. Maternal Exposure and Elimination in Milk

In the Environmental Health Criteria for HCB, IPCS (1997) concluded that HCB is transferred from mothers to offspring in a number of species and that lactation seems to be the most important route of transfer. Studies cited by IPCS and others identified for the purposes of this briefing packet are summarized below.

##### *Mice*

Courtney and Andrews (1985) compared HCB levels in CD-1 dams and their nursing pups in full litters and litters reduced to only 2 pups. While lactational transfer from dams to pups resulted

in approximately 95% depletion of HCB burden in dams by postnatal day (PND) 20, litter size did not have a significant effect on HCB levels in dams or pups. Dams were dosed with 0, 10, or 50 mg/kg-day HCB on gestation days (GD) 11 through 17 (the last week of gestation). Some of the dams (n=23) nursed their full litters while some dams (n=14) had litters reduced to 2 pups. All dams nursed their pups until PND 20. HCB concentrations in liver, brain, muscle, skin, fat, and whole blood of dams were depleted almost entirely on PND 20 in all exposure groups regardless of full or reduced litter size; depletion in non-pregnant female mice over the same time period was significantly less. For example, HCB concentrations were reduced in the liver and in fat by about 99% in the highest dose group of lactating mice. In non-pregnant female mice, HCB concentrations were only reduced by about 72% in the liver, and HCB concentrations actually increased about 1% in fat in those mice. Similar HCB levels were observed in pups regardless of whether they were weaned as part of a full or reduced litter; pup body burdens corresponded in a dose-dependent manner with maternal exposure levels.

Andrews and Courtney ([1986](#)) measured HCB levels in groups of CD-1 mouse pups to ascertain the gestational and lactational transfer of HCB from dams who received 50 mg/kg-day from GD 6 through 16. Pups born to unexposed dams and cross-fostered and nursed by exposed dams had higher body burdens than pups born to exposed dams and cross-fostered to control dams. Pups born to and nursed by exposed dams had the highest burdens after 4 days of nursing ([Andrews and Courtney, 1986](#)).

### ***Rats***

Cripps ([1990](#)) showed that both gestational and lactational transfer of HCB occurs in rats. Female rats were orally dosed with 400 or 2,000 µg/g HCB during gestation; offspring were sacrificed at the time of birth or after 2, 4 or 8 weeks. At birth, HCB levels in neonatal liver in the offspring of dams dosed with 2,000 µg/g were 27.3 µg/g; at 5 weeks, these levels had increased to 836 µg/g. Sacrifice and sampling times were not consistent in this study design, and limited additional information was available. Additionally, HCB levels in maternal breast fat of dams dosed with 2,000 µg/g decreased from 2,947 µg/g at the time of delivery to 1,091 µg/g after 3 weeks of nursing ([Cripps, 1990](#)).

Goldey et al. ([1990](#)) reported on the uptake, distribution, and elimination of HCB in dams, fetuses, and nursing pups when dams were exposed to 11 mg/kg HCB over three days at breeding. During pregnancy, fetal blood HCB levels were about 0.9 times maternal blood HCB levels. However, maternal HCB levels decreased as a result of nursing, and HCB levels in pups' liver, kidney, and blood at PND 7 were about 5 times the level in respective maternal tissues.

Mendoza et al. ([1978](#); [1977](#)) performed two cross-foster studies investigating gestational and lactational exposures to HCB in rats; the toxicological results of these studies are discussed in [Section B.3.4.2](#). The authors reported that pups born to unexposed dams and cross-fostered to HCB-exposed dams had significantly higher HCB body burden than pups nursed by unexposed

dams. Pups born to HCB-exposed dams and nursed by unexposed dams had similar HCB body burdens compared to pups born to and nursed by unexposed mothers. These results suggest that lactation was an important route for HCB transfer to pups ([Mendoza et al., 1977](#)).

Lilienthal et al. ([1996](#)) exposed female rats to diets containing 4 to 16 mg/kg HCB starting 90 days prior to mating and continuing throughout mating, gestation and lactation. Dams, unmated rats with the same exposure, and offspring all showed dose-related tissue levels of HCB. However, HCB levels were significantly decreased in dams after nursing compared to unmated rats.

Nakashima et al. ([1997](#)) fed Sprague-Dawley rats 35.1 nmol HCB per 100 g diet *ad libitum* during gestation and lactation. HCB levels were elevated in the blood, subcutaneous fat, and perirenal fat of 2 pregnant dams one day before parturition compared to 3 nursing dams on PND 16 ([Table B-33](#)). Additionally, HCB levels in blood, subcutaneous fat, and perirenal fat were approximately 6, 29, and 15 times higher in the respective tissues of 43 suckling pups compared to their 3 dams. The authors concluded that HCB is transferred from dams to pups during lactation. In a second experiment reported within the same study, Nakashima et al. ([1997](#)) compared placental and lactational transfer of HCB using a cross-foster study design. Lactational transfer during the early days after birth resulted in a significant contribution to HCB burden in pups and reduction of burden in dams, but was not quantified by the authors. The authors estimated that 0.39% of maternal HCB intake was transferred to the fetuses prenatally.

**Table B-33. Concentration of HCB in Blood and Fat of Exposed Dams and Their Pups**

Tissue type	Pregnant dams <sup>a,b</sup> (n=2)	Fetuses <sup>a</sup> (n=26)	Nursing dams <sup>a,c</sup> (n=3)	Nursing pups <sup>a,c</sup> (n=43)
Blood (nmol/L)	12.01, 15.48	ND	3.84 ± 0.24	27.90 ± 2.10*
Subcutaneous fat (pmol/g)	546.2, 909.5	ND	49.10 ± 3.81	1,495 ± 240*
Perirenal fat (pmol/g)	445.6, 358.2	ND	145.9 ± 26.52	2,351 ± 238*
Whole body (pmol/g)	ND	22.72 ± 4.34	ND	ND

<sup>a</sup> Values represent means ± SEM. When n<3, individual values are listed.

<sup>b</sup> Measured one day prior to presumed parturition; dams fed 31.5 nmol HCB/100g diet during gestation and lactation

<sup>c</sup> Measured 16 days post-partum; dams fed 31.5 nmol HCB/100g diet during gestation and lactation

ND = Not determined

\*Statistically significant compared to corresponding dam at P < 0.05

Source: Nakashima et al. ([1997](#)).

Taylor et al. ([1991](#)) also illustrated that HCB is transferred from dams to pups via lactation, quickly depleting maternal body burden. Dams were dosed with either 10 or 100 mg/kg HCB two weeks prior to mating; HCB tissue concentrations remained 10-fold different between the

two groups during the 20-day gestation period. However, during lactation (measured on PND4, 5, 10, and 14), tissue concentrations in both dams and pups were only 2- to 3-fold different between the two dose groups. The authors concluded that pups receive a similar dose of HCB via milk regardless of the maternal dose because HCB stored in the dams' tissues equilibrates with lipoprotein-rich blood during lactation.

### Monkeys

Bailey et al. (1980) exposed nursing rhesus monkey dams with infants between 1 and 3 months of age to HCB; dams received 60 mg/kg-day HCB for 60 days. HCB concentrations in milk and serum were measured 14 and 7 days prior to exposure and on exposure days 0, 1, 2, 4, 8, and then weekly through exposure day 60 (or until infant death as two infants became ill before the end of the study). As shown in Table B-34, the milk concentration of HCB rose rapidly as dosing continued while the serum concentration rose much more slowly. Serum concentrations were consistently higher in infants than in mothers, with a greater amount of HCB concentrating in milk than serum (Bailey et al., 1980).

**Table B-34. Mean HCB Concentrations ( $\mu\text{g/g}$  wet weight) in Serum and Milk of Dam and Infant Rhesus Monkeys, Dams Dosed with 60 mg/kg-day while Nursing**

Day of dosing	Number of dams	Number of infants	Concentration in dam's serum	Concentration in milk	Concentration in infant serum
-14*	3	3	<0.01	<0.01	<0.01
-7*	3	3	<0.01	<0.01	<0.01
1	3	0	0.41 $\pm$ 0.23	7.51 $\pm$ 7.15	--
2	3	3	0.78 $\pm$ 0.14	17.31 $\pm$ 15.49	0.42 $\pm$ 0.18
4	3	0	1.35 $\pm$ 0.31	12.90 $\pm$ 7.30	--
8	3	3	1.86 $\pm$ 0.46	29.37 $\pm$ 11.02	2.62 $\pm$ 1.26
15	3	3	3.37 $\pm$ 0.34	36.47 $\pm$ 6.81	5.82 $\pm$ 2.35
22	3	3	3.43 $\pm$ 0.21	107.13 $\pm$ 37.54	14.32 $\pm$ 1.87
29	2	2	1.86 $\pm$ 0.46	61.20 $\pm$ 20.80	2.62 $\pm$ 1.26
36	2	0	5.73 $\pm$ 2.18	24.75 $\pm$ 10.25	--
43	2	1	8.15 $\pm$ 0.15	186.00 $\pm$ 119.00	28.31
50	1	1	14.40	110.00	28.50
57	1	1	16.16	--	49.44

\*Days listed as lactation days. Negative values refer to measurements taken prior to dosing.

Source: Bailey et al. (1980).

---

### B.3.4. Developmental Effects of HCB

---

#### B.3.4.1. Human Studies

In Anatolia, Turkey in the 1950s, a widespread HCB exposure occurred when a large population ate bread made from grain that was treated with HCB. Studies focused on developmental effects resulting from the exposures include Cripps et al. ([1984](#)), Gocmen et al. ([1989](#)), Peters et al. ([1982a](#); [1982b](#)) and Peters et al. [([1966](#)) as cited in IPCS ([1997](#))]. In addition, the epidemic is described by ATSDR ([2002b](#)) in the Toxicological Profile for Hexachlorobenzene. Doses of HCB from the treated grain were estimated at 0.7 to 2.9 mg/kg-day for a person of “average” size.

In response to this exposure, infants presented with a condition called *pembe yara* (pink sore), which was associated with skin lesions and infant deaths from cardiorespiratory failure ([ATSDR, 2002b](#)). The skin lesions were further diagnosed as *porphyria cutanea tarda*. Porphyria indicates a dysfunction in the normal production of hemoglobin, and HCB exposure has been demonstrated to cause porphyria ([ATSDR, 2002b](#)). *Porphyria cutanea tarda* is a specific form of porphyria that results in tissue damage to the skin. The effects of HCB exposure to infants were irreversible, and there was some evidence that adult women who had been exposed as children had elevated incidences of miscarriages and stillbirths ([ATSDR, 2002b](#)).

In an early report of the grain contamination incident and cases of *pembe yara* [Peters et al. ([1966](#)), as cited in IPCS ([1997](#))], HCB was said to be present in breast milk but was not quantified. Elevated levels of HCB were measured in breast milk 20–30 years after the incident, with a mean level of 510 ng/g-fat in milk from 56 mothers whose children experienced porphyria. The mean HCB level was 70 ng/g fat in milk from 77 women from families without porphyria or from areas outside of the endemic area ([IPCS, 1997](#); [Gocmen et al., 1989](#); [Peters et al., 1982b](#)).

---

#### B.3.4.2. Animal Studies

The animal toxicology literature on controlled exposures to HCB was reviewed for studies presenting data specifically related to exposure occurring during gestation and/or lactation. To provide a comprehensive understanding of the hazard resulting from these specific types of exposures, all relevant studies that could be retrieved were reviewed and are summarized in [Table B-35](#). The endpoints examined and effects observed are noted, as are potential points of departure (PODs) for each study. For endpoints in the low-dose range, benchmark dose modeling using U.S. EPA’s Benchmark Dose Software (BMDS) was considered. However, appropriate litter-specific data were not available for critical effects in the low-dose range, making BMD modeling inappropriate for this application. PODs are arrayed in [Figure B-15](#) and [Figure B-16](#) to facilitate comparisons across studies. [Table B-35](#) serves as a reference key for [Figure B-15](#) and [Figure B-16](#).

Studies were classified according to the timing of exposure for the pups. Gestational exposure studies were those in which maternal animals were exposed during gestation and (1) pups were sacrificed prenatally or at parturition, or (2) pups were nursed by unexposed dams. Combined lactational and gestational exposure studies were those in which maternal animals were exposed during gestation or during gestation and lactation and pups were nursed prior to sacrifice. Finally, lactational exposure studies were those in which dams were not dosed until lactation began or pups unexposed during gestation were cross-fostered to exposed mothers.

HCB induced a number of developmental effects in monkeys, mink, rats, and mice; including fetal abnormalities, neurotoxicity, reproductive effects, immunotoxicity, hepatotoxicity, and mortality. Three studies specifically investigated effects following lactational exposure to the offspring via controlled maternal exposure ([Iatropoulos et al., 1978](#); [Mendoza et al., 1978](#); [Mendoza et al., 1977](#)). Iatropoulos et al. (1978) exposed three rhesus monkey dams to HCB at 64 mg/kg-day for 22, 38, and 60 days. This dose resulted in mortality in two of the three exposed offspring, and HCB levels measured in milk samples and in infant serum support the importance of lactation as a route of exposure. Results of a cross-fostering study in Wistar rats further demonstrate the importance of lactational exposure relative to gestational exposure ([Mendoza et al., 1978](#); [Mendoza et al., 1977](#)). Changes in liver weights and enzyme activities were more significant in pups exposed solely through lactation compared to those exposed solely during gestation. Furthermore, liver weight and enzyme changes were not significantly different in pups exposed solely through lactation compared to those exposed during both gestation and lactation.

One study was selected to serve as the example POD for this briefing packet. Mendoza et al. (1977) observed hepatotoxicity in pups following exposure of lactating Wistar rats to 6.7 mg/kg-day HCB via the diet during lactation. Because this study included only one HCB dose group, BMD modeling was precluded. Therefore, the LOAEL of 6.7 mg/kg-day is used as an example POD to assess the impact that accumulated exposure to a mother may have on lactational exposure of her offspring. This POD is used in [Section B.1.6](#), along with results from PBPK models, to estimate an HED. The choice of the POD and HED presented in this packet are intended only as examples to stimulate discussion for the workshop participants.

**Table B-35. Developmental Effects from Gestational and/or Lactational Exposure to Hexachlorobenzene**

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational Exposure</b>								
Mouse, CD-1, gavage, treated GD 7–11, fetal examination on PND -2	0, 100	<p><b>Measured:</b> Maternal body weight and relative liver weight, average fetal weight, live fetuses per litter, fetal mortality, fetal physical abnormalities including incidence of cleft palate and clubfoot</p> <p><b>Observed:</b> No differences between treatment or control in terms of maternal effects; <b>increased average number and percent of abnormal fetuses per litter</b></p>	Maternal: DU Develop: NA	Maternal: DU Develop: 100	DU	DU	Courtney et al. ( <a href="#">1976</a> )  (The number of dams per dose not reported but 10 litters evaluated; inconsistencies in reporting results in tables and text make the significance of maternal findings impossible to report)	Develop: [A1]



Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<p>Rat, Wistar, ~7-16 dams per group, gavage, GD 6-9, 10-13, 6-16, or 6-21, sacrificed GD 22</p> <p>Authors report that dams were dosed in four groups, as follows: 10–80 mg/kg-d GD 6-9; 10–80 mg/kg-d GD 10-13; 10–60 mg/kg-d GD 6-16; 40–120 mg/kg-d GD 6-21; number of dams per group not reported but number of pregnant rats at term ranged from 7-16; Data presented is pup data, with no information on incidence per litter.</p>	0, 10, 20, 40, 60, 80, 120	<p><b>Measured:</b> Maternal (GD 1, 6-15, and PND 0) and fetal weight; pathological alterations in dams; number of resorptions, deciduomas and fetuses dying late in development; pup viability and external and skeletal malformations; microscopic evaluation of pups' mid-sagittal section</p> <p><b>Maternal Observed: Decreased maternal body weight and fetal body weight (associated with maternal toxicity) and increased incidence of hyperaesthesia, tremors, and convulsions in dams at 80 or 120 mg/kg (dams dosed on GD 6-21)</b></p> <p><b>Developmental Observed: Increased incidence of uni-and bilateral 14th rib in offspring at <math>\geq 10</math> mg/kg-d (dams dosed GD 6-16), at <math>\geq 10</math> mg/kg-d (dams dosed GD10-13), and at <math>\geq 40</math> mg/kg-d (dams dosed GD 6-21) (related to dose and duration of exposure); increased sterna defects (i.e., asymmetrical apposition of sternebrae, hemisternebrae and delayed ossification) at <math>\geq 40</math> mg/kg-d (dams dosed GD 6-21)</b></p>	Maternal: 60  Develop: NA	Maternal: 80  Develop: 10	6.12 (GD 6-16)	10.9 (GD 6-16)	Khera ( <a href="#">1974</a> )	Develop (GD 6-16): [B1] Develop (GD10-13): [B2]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per treated litter cross-fostered to control dams immediately after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Body weights and organ weights of pups on PND 17 or 21  <b>Observed: Decreased absolute brain weight at PND 17 and 21</b>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. (1978)	Develop: [C1]
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per treated litter cross-fostered to control dams immediately after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Mean HCB residue concentration in liver, brain, heart, kidney, and spleen; absolute and relative liver weight; liver enzyme activity towards thiophenyl, p-nitrophenyl, and indophenyl acetate (TPA, PNPA, IPA) hydrolysis  <b>Observed:</b> Significant difference in mean HCB levels in liver, brain, heart, kidney, and spleen in pups nursed by exposed dams compared to control dams. Liver esterase activity in control pups significantly lower than in pups nursed to control dams.	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. (1977)  (Increased HCB residue concentrations found in all examined tissues of gestationally exposed pups [liver, brain, heart, kidney, spleen] compared to control.)	Develop: [D1]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Sprague-Dawley, 20 dams per dose, dosed prior to mating and through three matings (F1a, b and c); F1a, F1b, F1c offspring examined PND1	0, 0.5, 2.5, 12.5	<p><b>Measured:</b> Litters examined on PND 1 for number of pups, number of live and dead pups, number of male and female pups, average pup weight; anogenital distance; gross reproductive tract structure; surface righting (beginning PND1) external malformations; stomach milk and cleft palate; visceral and skeletal evaluation, blood and serum parameters; thyroid hormone determination</p> <p><b>F1 a, b, and F1c on PND1 Observed:</b> Mean anogenital distance increased by 8% in F1a males and by 4% in F1b females at 12.5 mg/kg-d; increased mortality in F1c pups at 12.5 mg/kg-d; average individual pup weight increased by 10% on PND 1 at 2.5 mg/kg-d; anogenital distance increased by 6% in females at 12.5 mg/kg-d; <b>increased incidence of skeletal malformations at 12.5 mg/kg-d for both F1a and F1b litters</b> (31.9% of pups showing variation compared to 8.3% of control for F1a and 25.6% compared to 6.3% for control for F1b); increased average pup weight in F1c males.</p>	Maternal: 5.0  Develop: 0.5	Maternal: 12.5  Develop: 2.5	DU	DU	<p>Wolfe and Pepperl (2005)</p> <p>(Biological significance of decreased number of females with normal cycles at 2.5 and 12.5 mg/kg-d questioned due to comparable female reproductive data between treatment and controls; absolute liver weight increased in dams at 2.5 mg/kg/d reported to be significant, but 9% is not considered to be biologically significant; statistical significance of skeletal malformations not reported)</p>	Develop: [E1]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational and Lactational Exposure</b>								
Rat, Sprague-Dawley, 20 dams per dose, dosed prior to mating and through three matings (F1a, b and c); F1c litters examined at various time points from PND 1 through PND 23.	0, 0.5, 2.5, 12.5	<p><b>Measured:</b> Dam body weight, food consumption clinical observations; full evaluation at necropsy. F1c litter examined on PND 1, 4, 7, 14, and 21 for number of pups, number of live and dead pups, number of male and female pups, total body weight, individual body weight; anogenital distance; gross reproductive tract structure; surface righting (beginning PND1); nipple retention and hypospadias (PND 12 and 13); vaginal thread and cleft clitoris (PND 22 or 23); startle response and functional observation battery (PND 22); immunological evaluation (PND 16); 6 control and 18 low-dose pups sacrificed PND 22-23 for full necropsy.</p> <p><b>F1c Observed:</b> Increased mortality in F1c pups until PND 9 (100% by PND9) at 12.5 mg/kg-d; pup weights decreased by 20% on PND 4 and decreased by ~40% on PND 7 at 12.5 mg/kg-d; <b>increased surface righting reflex and increased hindlimb grip strength (males only) at 2.5 mg/kg-d; delayed day of prepuce separation in males at 2.5 mg/kg-d.</b></p>	Maternal: 5.0  Develop: 0.5	Maternal: 12.5  Develop: 2.5			Wolfe and Pepperl (2005)  (Results from the immunological evaluation were not reported.)	Develop: [E2]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<p>Rat, Sprague-Dawley, unreported number of dams, dams treated 2 weeks prior to breeding by gavage for 4 consecutive days, pups evaluated for neurotoxicity (PND9–120)</p> <p>Blood, fat, liver, brain, and kidneys collected on GD 9, 15, 20 and on PND 4, 7, 10, 14. Milk samples collected during lactation; Brain, blood, and liver tissues collected from 20-day old fetuses</p>	0, 2.5, 25	<p><b>Measured:</b> Overt signs of toxicity in dams and pups; behavioral assays in young pups (~9-16 days): negative geotaxis reflex response, olfactory discrimination/homing test, exploratory and locomotor activity. Behavioral assays in mature offspring: locomotor activity (~60 days), exploratory behavior (100 days), learning behavior (40 days), acoustic startle response (23 days and 120/150 days)</p> <p><b>Observed:</b> No overt toxicity in dams, signs of overt toxicity in pups (severe body weight loss and organ malformations) at 25 mg/kg-d; <b>faster responses in negative geotaxis and olfactory homing assays in offspring at 2.5 and 25 mg/kg-d</b>; increased exploratory behavior and hyperactivity in offspring at 25 mg/kg-day; decrease in amplitude of acoustic startle response of pups (25 mg/kg-day at 23 days; 2.5 and 25 mg/kg-d at 120 days)</p>	<p>Maternal: 25</p> <p>Develop: NA</p>	<p>Maternal: NA</p> <p>Develop: 2.5</p>	DU	DU	Taylor and Goldey (1992)	Develop: [F1]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Sprague-Dawley, 30 dams per group, treated 2 weeks prior to breeding by gavage for 4 consecutive days, litters culled to 8 pups/litter and evaluated at various intervals (PND1–120)	0, 2.5, 25	<p><b>Measured:</b> Length of gestation, number of live and dead pups at PND1; number of days to eye opening; maternal and pup body weight at PND 1, 3, 6, 9, 12, 14, and 28; absolute and relative brain weight in pups at PND 28; negative geotaxis reflex (PND 6, 8, and 10); olfactory discrimination (PND 9–11); exploratory behavior (PND15–20); acoustic startle reflex; reflex modification of ASR (PND 90); visual discrimination learning (PND 40); motor activity in mature offspring (PND 60)</p> <p><b>Observed:</b> No overt toxicity in dams, <b>faster responses in negative geotaxis and olfactory homing assays in offspring, at 2.5 and 25 mg/kg-d; increased exploratory activity on PND 19 and 20 at 2.5 and 25 mg/kg-d;</b> decrease in amplitude of acoustic startle response of pups (PND 23) at 25 mg/kg-day; <b>elevated mean startle amplitude (PND90) in males at 2.5 and 25 mg/kg-d</b> and in females only at the highest dose</p>	Maternal: 25  Develop: NA	Maternal: NA  Develop: 2.5	DU	DU	Goldey and Taylor ( <a href="#">1992</a> )	Develop: [G1]
Mouse, BALB/C, 2 dams per dose group, diet, GD 1-18, offspring weaned at PND 21, immunological assays began on PND 45	0, 0.5, 5.0	<p><b>Measured:</b> Overt signs of toxicity; delayed-type hypersensitivity (DTH) response; hemolytic plaque assay; mitogen-induced blastogenesis; mixed-lymphocyte response</p> <p><b>Observed:</b> No overt toxicity in dams, <b>DTH response to oxazolone severely depressed at 0.5 and 5.0 mg/kg-d in offspring;</b> decreased mixed lymphocyte response level at 5.0 mg/kg-day (ex vivo); increased distribution of splenic T cells at 5.0 mg/kg-day (low-dose group not analyzed); decreased distribution of splenic B cells at 5.0 mg/kg-day (low-dose group not analyzed)</p>	Maternal: 5.0  Develop: NA	Maternal: NA  Develop: 0.5	DU	DU	Barnett et al. ( <a href="#">1987</a> )  (Authors conclude that HCB is capable of impacting development of immune response in mice at the T cell level.)	Develop: [H1]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Mouse, CD-1, 3-7 dams per dose group, gavage, GD 6-17, dams sacrificed PND 1 or 21, pups sacrificed PND 1, 8, 10, 15, or 21.	0, 1, 10, 50	<b>Measured:</b> Lactic dehydrogenase (LDH) and creatine kinase (CK) activities and isozymes profiles in dams and pups; mortality  <b>Observed:</b> Decreased LDH-5 and increased LDH-3 in dams at 50 mg/kg-day on PND 1; <b>increased pup mortality at 50 mg/kg-day (71.7%) and 10 mg/kg-d (27.6%);</b> Increased LDH-5 enzyme in pups at 50 mg/kg-day on PND 21	Maternal: 10  Develop: 1	Maternal: 50  Develop: 10 (FEL)	DU	DU	Courtney et al. ( <a href="#">1984</a> )  (Study was limited to an evaluation of cardiac enzymes.)	Develop: [I1]
Mink, <i>Mustela vison</i> , unreported number of dams, diet, approximately 1 month prior to breeding through gestation and lactation; 6 female kits/dose examined at 16-17 weeks of age	0, 0.2, 1.0 <sup>b</sup>	<b>Measured:</b> Kit weight; HCB analysis of blood, fat, muscle, kidney, liver, and brain; hepatic mixed-function oxidases; renal function (in vitro; p-aminohippurate and tetraethylammonium [TEA]); histological examination of liver and kidney  <b>Observed:</b> <b>Increased mortality in low- (44.10%) and high dose (77.4%) offspring ( 0.2 and 1.0 mg/kg-d);</b> elevated hepatic P-450 concentrations and ethoxyresorufin-O-deethylase at 1.0 mg/kg-d, and decreased TEA accumulation in kidneys at 1.0 mg/kg-d; liver cell cytoplasm occupied by ellipsoidal vesicular profiles	Maternal: NE  Develop: NA	Maternal: NE  Develop: 0.2 <sup>b</sup> (FEL)	DU	DU	Rush et al. ( <a href="#">1983</a> )  (No information on timing of mortality or cause precluding conclusions as to contributing exposure period. Study includes data on tissue distribution of HCB in offspring.)	Not included
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per treated litter remaining with treated mother after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Body weights and organ weights of pups on PND 17 or 21  <b>Observed:</b> <b>Decreased absolute brain weight at PND 17 and 21;</b> decreased absolute kidney weight at PND 17; decreased absolute spleen weight at PND 17; increased relative liver weight at PND 21	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. ( <a href="#">1978</a> )  (No significant difference in liver effects as compared to lactation-only or gestation-only exposure)	Develop: [C2]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating PND 23, litters culled to 10 pups with 5 pups per treated litter remaining with treated mother after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<p><b>Measured:</b> Mean HCB residue concentration in liver, brain, heart, kidney, and spleen; absolute and relative liver weight, liver enzyme activity towards thiophenyl p-nitrophenyl, and indophenyl acetate (TPA, PNPA, IPA) hydrolysis</p> <p><b>Observed:</b> Increased liver enzyme activity toward TPA, PNPA, and IPA hydrolysis, and increased relative liver weight on PND 17 and 21</p>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. (1977)  (No significant difference in liver effects compared to lactationally exposed pups, but increased as compared to gestationally exposed pups. Increased HCB residue concentrations found in all examined tissues of the pups (liver, brain, heart, kidney, spleen) compared to control.)	Develop: [D2]
Rat, Sprague-Dawley, 10 dams per dose, diet, 96 days prior to mating through gestation and lactation (2 successive litters raised); offspring (litters not culled) observed through weaning	0, 5.9, 7.8, 9.8, 11.8, 13.7 <sup>a</sup>	<p><b>Measured:</b> Pup mortality, maternal lung histology after second litter, clinical observations, maternal and pup body weight, litter size, pup brain and liver HCB residues</p> <p><b>Observed:</b> Dose-dependent increase in mortality through weaning (11, 19, 30, 45, 94, and 92% for F1a and 11, 19, 22, 20, 45, 100, and 94% for F1b in the 0, 5.9, 7.8, 9.8, 11.8, 13.7 mg/k-d dose groups, respectively); increased incidence of intra-alveolar foamy histiocytes and hypertrophy and proliferation of the lining endothelial cells in pulmonary venules in treated dams (dose not reported)</p>	Maternal: ND  Develop: NA	Maternal: ND <sup>c</sup>  Develop: 5.9 <sup>a</sup> (FEL)	DU	DU	Kitchin et al. (1982)  (Authors report LD50 for day 21 cumulative mortality from birth = 100 ppm for F1a generation and 104 for F1b generation. Mortality incidence was reported slightly differently in abstract.)	Develop: [K1]



Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Lactational Exposure</b>								
Monkey, Rhesus, 3 dams per dose, gavage; offspring 21, 61, or 118 days old at onset of exposure and exposed for 60, 38, or 22 days, respectively; 5 controls per age group	0, 64	<p><b>Measured:</b> <i>in situ</i> examination of CNS, salivary glands, mammary tissue, liver, stomach, duodenum, jejunum, ileum, colon, pancreas, adrenals, kidneys, urinary bladder, mesenteric lymph nodes, esophagus, trachea, lungs, heart, thyroid, parathyroid, uterus, eyes, ovaries, and tongue</p> <p><b>Observed: Mortality seen in offspring (66%) with onset of exposure at PND 61 or 118</b> (lung edema associated with mortality in both instances with bronchopneumonia reported in one animal, one infant was listless for 24 hours prior to death with engorgement of all central nervous system vessels and decreased body weight, the second infant showed lethargy, depression, and ataxia prior to death); microscopic changes included: mild centrilobular hepatocellular hypertrophy (n=1), diffuse fatty metamorphosis of liver (n=1), slight degree of proximal renal tubular vacuolation (n=2), mild gliosis in the cerebrum (n=1)</p>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 64 (FEL)	DU	DU	<p>Latropoulos et al. (<a href="#">1978</a>)</p> <p>(Authors note that HCB serum concentrations of mothers and infants as well as HCB concentrations in the milk samples are reported in other studies and results indicate: (1) the amount of HCB infants received through milk was equal or greater than the amount given to mothers (2) HCB in the milk samples was very high (3) mean HCB in infant serum at sacrifice was higher than mothers' HCB serum.)</p>	Develop: [L1]

Study Design	Dose (mg/kg-d)	Toxicological Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	BMDL (mg/kg-d)	BMD (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per control litter cross-fostered to treated dams immediately after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Body weights and organ weights of pups on PND 17 or 21  <b>Observed: Increased absolute and relative liver weight at PND 17 and 21;</b> decreased absolute kidney weight at PND 17	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>	DU	DU	Mendoza et al. (1978)  (Significantly increased absolute and relative liver weights compared to pups in gestation-only exposure group at PND 17 and 21.)	Develop: [C3]
Rat, Wistar, 5 dams per group, diet, 2 weeks before mating to PND 23, litters culled to 10 pups with 5 pups per control litter cross-fostered to treated dams immediately after parturition, pups examined at PND 17 and 21	0, 6.7 <sup>a</sup>	<b>Measured:</b> Mean HCB residue concentration in liver, brain, heart, kidney, and spleen; absolute and relative liver weight, liver enzyme activity towards TPA, PNPA, and IPA hydrolysis  <b>Observed: Increased liver enzyme activity toward TPA, PNPA, and IPA hydrolysis, and increased relative liver weight on PND 17 and 21</b>	Maternal: NE  Develop: NA	Maternal: NE  Develop: 6.7 <sup>a</sup>			Mendoza et al. (1977)  (Significantly increased absolute and relative liver weights, and liver enzyme activity compared to gestationally exposed pups at PND 17 and 21. More than 10 fold increase in organ HCB residue compared to gestationally exposed pups.)	Develop: [D3]

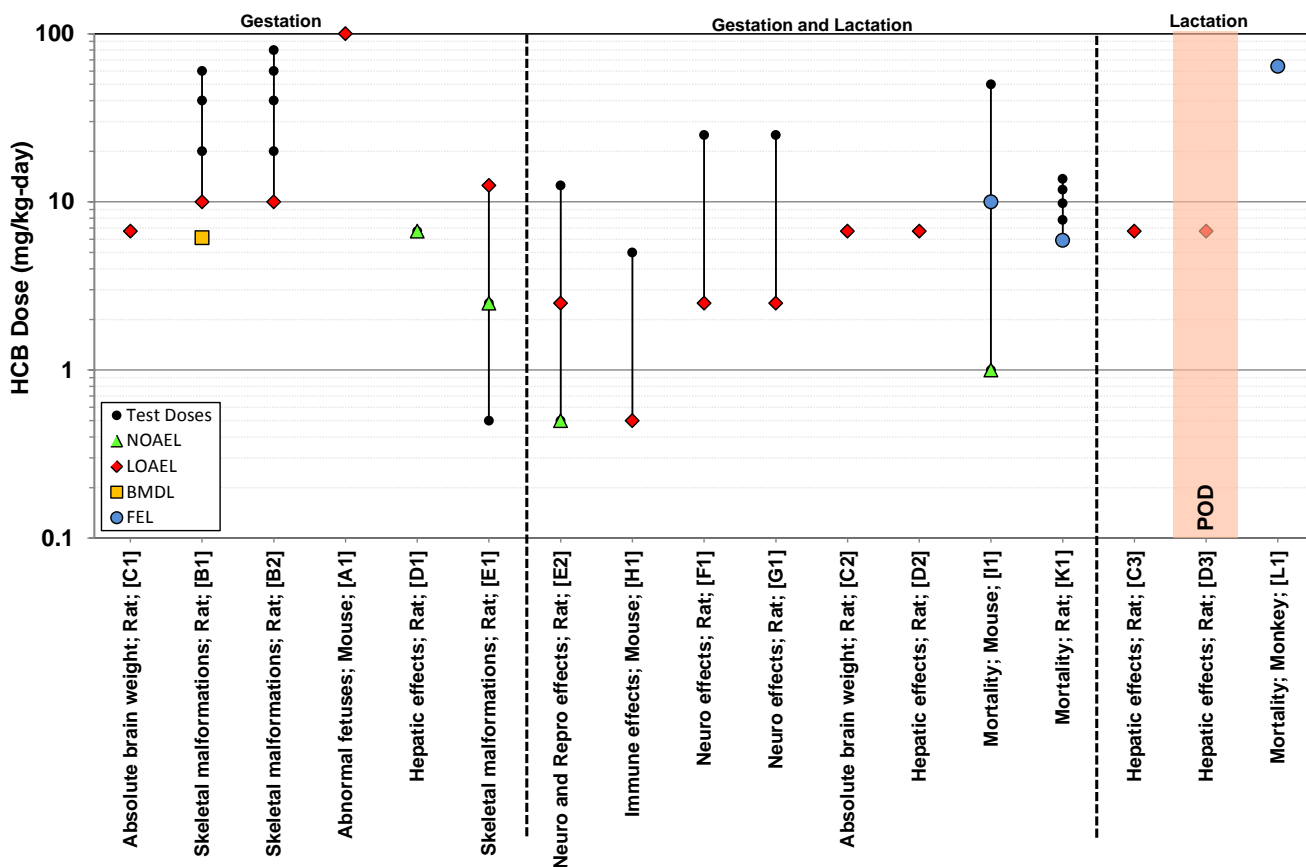
<sup>a</sup> Doses estimated using default body weight and food intake values (U.S. EPA, 1988).

<sup>b</sup> Doses estimated using mink body weight and food intake values reported in Aroclor 1016 IRIS, due to lack of information on mink dosimetry in (U.S. EPA, 1988): <http://www.epa.gov/iris/subst/0462.htm>.

<sup>c</sup> Authors report maternal effects, however the significance of these findings (and the lowest dose at which findings are significant) are not reported.

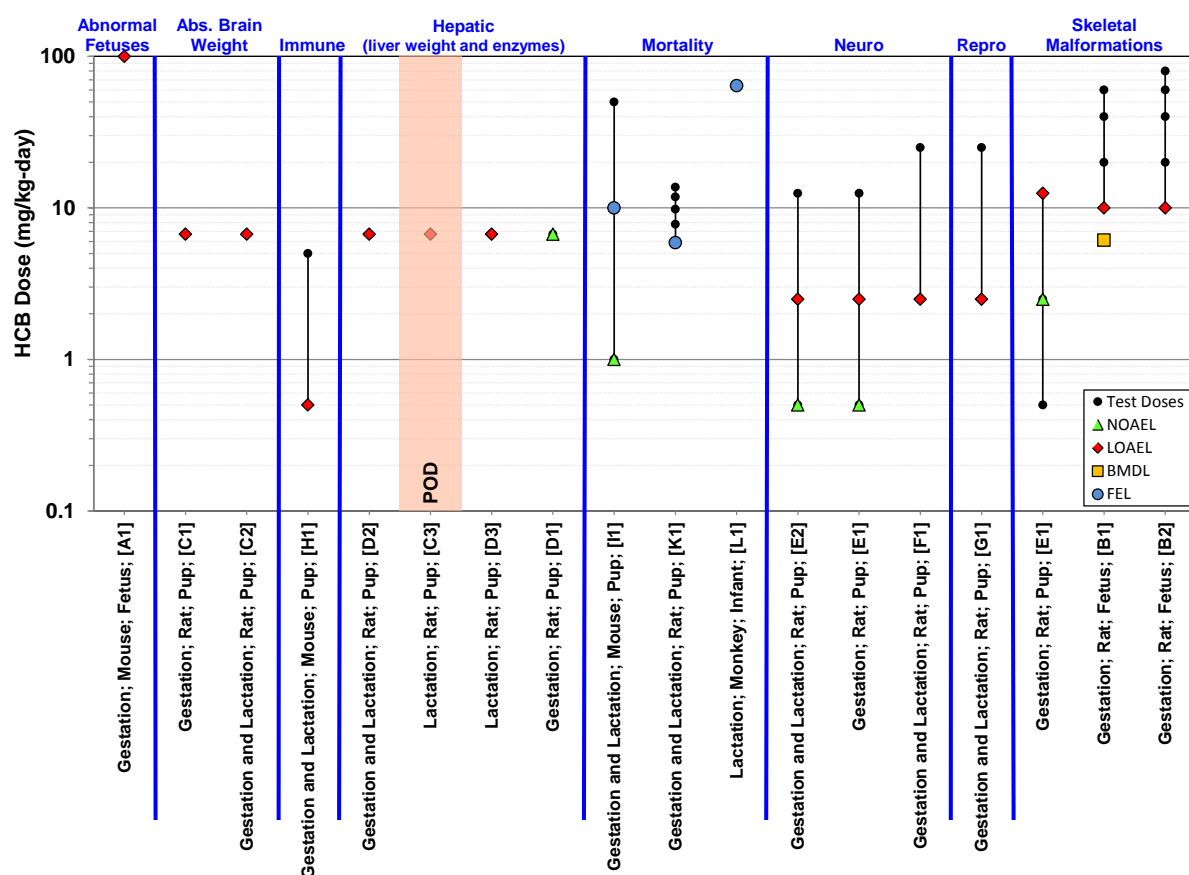
DU = data unsuitable; NA = not applicable; ND = no data; NE = not examined; BMD = benchmark dose; BMDL = the 95% lower bound confidence limit on the BMD; LOAEL = lowest observed adverse effect level; NOAEL = no observed adverse effect level

### B.3.4.3. Exposure-Response Arrays for Developmental Effects



Note: Each array element represents the dose-response data for a unique study design. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. All endpoints pertaining to relative liver weight and liver enzymes are labeled as “Hepatic effects”. “Repro effects” refers to delayed day of prepuce. All endpoints pertaining to negative geotaxis, olfactory homing, surface righting reflex, and hindlimb grip strength are labeled as “Neuro effects”. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-35](#). The study by Rush et al. (1983) is not included in the array due to poor reporting. NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; BMDL = the 95% lower bound confidence limit on the BMD; FEL = frank effect level

**Figure B-15. Exposure-Response Array Showing All Developmental Effects, Sorted by Exposure Route, in Animals after Exposure to HCB**



Note: Each array element represents the dose-response data for a unique study design or endpoint. Elements are labeled with the adverse effects on which NOAEL and LOAEL decisions were based. “Immune” refers to DTH response to oxazolone. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. All endpoints pertaining to negative geotaxis, olfactory homing, surface righting reflex, and hindlimb grip strength are labeled as “Neuro”. “Repro effects” refers to delayed day of prepuce. “Skeletal Malformations” refers to general category (including incomplete ossification, shortened supernumerary rib, or other), or uni- and bilateral 14th rib. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-35](#). The study by Rush et al. ([1983](#)) is not included in the array due to poor reporting. NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; BMDL = the 95% lower bound confidence limit on the BMD; FEL = frank effect level

**Figure B-16. Exposure-Response Array Showing All Developmental Effects, Sorted by Effect Type, in Animals after Exposure to HCB**

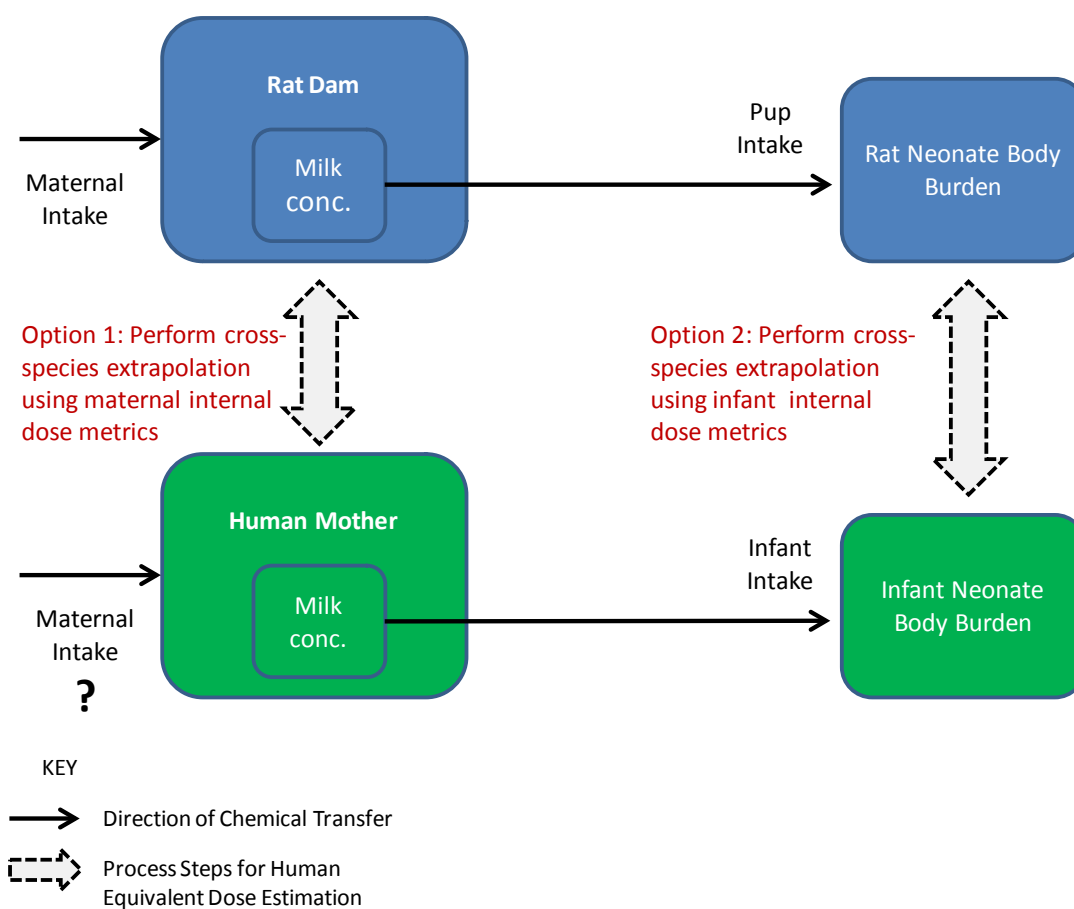
---

### B.3.5. Human Equivalent Dose (HED) Estimation

Once the POD has been selected, the next step is estimation of the HED. This HED is intended to quantify the maternal continuous daily exposure in humans that may result in an adverse developmental effect similar to that observed in animal offspring as described in [Section B.3.4](#).

The information provided in this section is designed to promote discussion amongst the workshop attendees as to how best to perform the HED estimation. To provide an example to facilitate discussion, one method is chosen and carried through in this document for demonstration purposes only. The HED can be estimated in a number of different ways. Generally speaking, an internal dose metric is selected for the human and the animal, and then these two metrics are assumed to result in the same effect. The decision must be made as to which internal dose metric is appropriate to serve as an indicator of the associated adverse effect.

For developmental effects resulting from lactational exposure, [Figure B-17](#) provides an example of the pathways that may be chosen to perform cross-species extrapolation to estimate the HED. The assumption is made that the maternal or infant animal intake is known, and the HED is the unknown human continuous daily exposure (depicted with a “?” in the figure). Because exposure occurs through the mother and then passes to the offspring by nursing, cross-species extrapolation may be performed by selecting either an internal maternal dose metric or an internal infant dose metric. Performing the extrapolation on internal maternal dose metrics will not account for species differences regarding infant feeding habits and infant kinetics, while performing the extrapolation on the infants requires more input data and more judgment about how to appropriately link very young members of both species. The overall choice will depend on judgment as to the reliability of the models available and the input data driving the models, balanced against the desire for increased biological accuracy.



**Figure B-17. Process Diagram for Performing Cross-Species Extrapolation**

### Discussion Points for HCB HED Estimation

- ? Is HCB metabolized? How should metabolism be addressed in the HED estimation?
- ? What pharmacokinetic data or relevant chemical-specific information are available for HCB in animals and humans to facilitate model implementation?
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ? What PBPK or other simpler biokinetic models are available for HCB?
- ? What is the best method for estimating the HED using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

The main questions considered in derivation of an HED from an animal-derived POD are shown in the text box below (“Discussion Points for HCB HED Estimation”). This section discusses some of the chemical-specific properties that affect (1) the selection of the modeling method, (2) the kinetic information available for HCB, and (3) the types of models available for assessing HCB exposure in animals and humans. Summary statements for each of the discussion points are provided in the appropriate sections.

#### B.3.5.1. Chemical-Specific Properties Affecting HED Estimation

HCB is slowly metabolized in animals and humans. The main metabolite in both animals and humans is pentachlorothiophenol (PCP) ([Renner, 1988](#); [Ingebrigtsen et al., 1986](#)), with other metabolites including chlorinated benzenes, chlorophenols, and benzenes ([Koss et al., 1986](#)).

In a study that dosed rhesus monkeys with radiolabeled HCB in the diet for 15 months, the recovered radioactivity in the feces indicated 99% of the excreted HCB was eliminated as parent compound with only 1% consisting of the different metabolites. It is expected that HCB toxicity is at least in part due to the parent compound. For these reasons, accounting for metabolism in the estimation of the HED may not be of high importance. However, if the human equivalent dose is estimated assuming long-term exposure, excluding metabolism will tend to overestimate the human HCB tissue concentrations associated with a given dose.

#### HCB Metabolism

- ? Is HCB metabolized? How should metabolism be addressed in the HED estimation?
- ✓ **HCB is metabolized at a very slow rate.**

### B.3.5.2. Available Pharmacokinetic Data for HCB

Before discussing the potential modeling techniques for HCB, the available database of pharmacokinetic data is summarized. This database should help to determine which modeling techniques may be most appropriate and subject to the least uncertainty.

Potential parameters that may be useful in estimating HED by a variety of methods are presented in [Table B-36](#). A discussion of the parameters and their sources follows the table. The various applications and uses of these parameters are discussed in the context of different modeling techniques in [Section B.1.5.4](#).

#### Pharmacokinetic Data

- ? What pharmacokinetic data or relevant chemical-specific information are available for HCB for both animals and humans to facilitate model implementation?
- ✓  $K_{ow}$
- ✓ Human and rat half-lives
- ✓ Absorption fractions and fraction of chemical stored in fat in humans (assumptions can be made based on other PBT chemicals)
- × No fraction of chemical stored in fat for rats
- ✓ Partition coefficients from PBPK models for rats and humans



**Table B-36. Available Pharmacokinetic Parameters for HCB in Humans and Rats**

Parameter (units)	Variable	Value	Note/source
Chemical-Specific Only			
Octanol-water partition coefficient	log(K <sub>ow</sub> )	5.73	Hansch et al. ( <a href="#">1995a</a> )
Human Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	Assumed same profile for infants as for mother	Schlummer et al. ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	Variable; 85.4% at zero blood concentration and decreasing for increasing blood concentration	Schlummer et al. ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	U.S. EPA ( <a href="#">1998</a> ) (default)
Biological elimination constant for HCB (day <sup>-1</sup> )	k <sub>elim</sub>	0.00322	Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans HCB (days)	t <sub>1/2</sub>	215	Yesair et al. ( <a href="#">1986</a> )
Partition coefficients- in different human body compartments for HCB	Rate constants found in Yesair et al. ( <a href="#">1986</a> ); estimated for rats and applied to humans; can also be estimated using K <sub>ow</sub>		
Metabolic rates for HCB	None identified in the literature		
Animal Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the rat pup (dimensionless)	f <sub>ai</sub>	0.8	Assumed
Fraction of ingested contaminant that is absorbed by the rat dam (dimensionless)	f <sub>am</sub>	0.8	Koss and Koransky ( <a href="#">1975</a> )
Fraction of contaminant that is stored in rat dam/maternal fat (dimensionless)	f <sub>f</sub>	0.9	Assumed
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.087	Estimated as ln(2) / t <sub>1/2</sub>
Half-life of contaminant in rats (days)	t <sub>1/2</sub>	8 days to 1.5 years; 8 days used here	Koss et al. ( <a href="#">1978</a> )
Partition coefficients in different rat compartments for the HCB	Partition coefficients found in Yesair et al. ( <a href="#">1986</a> ) and Lu et al. ( <a href="#">2006</a> )		
Metabolic rates for HCB	None identified in the literature		

Hansch et al. ([1995a](#)) estimated a log octanol-water partition coefficient (**log  $K_{ow}$** ) for HCB of 5.73.

In the absence of specific information on HCB in humans, the input values presented here were patterned after those in the EPA's Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions ([U.S. EPA, 1998](#)). Chapter 9 of this methodology (hereafter "Combustor Assessment") presents a framework for evaluating exposure to persistent lipophilic contaminants in breast milk. Thus, the assumptions made in that document are applied to HCB when values specific to HCB could not be located.

Schlummer et al. ([1998](#)) estimated the **gastrointestinal absorption fraction** for HCB in seven humans ranging in age from 24 to 81 years old. In the younger subjects, absorption was 70-82%; however, absorption decreased in the older subjects fed the same meal, with 1% in a 53 year old and net excretion (rather than absorption) in 76 and 81 year old subjects. In addition, the blood concentrations also varied by age, with increasing blood levels with age. Thus, the authors determined a regression equation that relates absorption to the blood concentration. The relationship predicts 85.4% absorption when the blood concentration is zero and decreases 0.2% for each ng/g lipid of HCB in the blood. For the purposes of this briefing packet, women of child bearing age are expected to be age 21 to 50, although most mothers are expected to be in the lower age range and expected to have the higher absorption reported by Schlummer et al. Infants were assumed to absorb 100% of the dose in the absence of specific information.

Quantitative information about the **fraction of HCB in the body that is stored in fat in humans** could not be found in the literature. The reviewed literature generally described HCB as highly lipophilic. The Combustor Assessment states that for highly lipophilic compounds, >90% of the compound may be stored in the fat ([U.S. EPA, 1998](#)). In the absence of more specific information, this parameter was set to 0.9 in the example calculations.

No direct measures of **HCB half-life in humans** were available in the literature. However, Yang et al. ([1978](#)) estimated half-lives of 91 and 114 days in two monkeys. PBPK modeling by Yesair et al. ([1986](#)) estimated human female half-lives of 215 days when exposed to HCB for 15 weeks at 15 years of age. For this briefing packet, the PBPK estimate of 215 days is used.

For **partition coefficients in humans**, no partition coefficients estimated from human empirical data are available. Yesair et al. ([1986](#)) estimated coefficients in rats using empirical data in the development of a PBPK model and then applied the same coefficients to humans. Coefficients were estimated for the brain, systemic circulation, kidney, liver, intestinal lumen, richly perfused tissue, poorly perfused tissue, breast tissue, and the offspring compartments. Partition coefficients can also be estimated based on  $K_{ow}$  ([Poulin and Krishnan, 1996](#)).

Animal parameters were determined from values found in the literature where available. The search focused on rats, since rats are the species studied in the candidate POD study ([Mendoza et al., 1977](#)). Studies on **HCB absorption in rats** revealed that absorption fractions depend on whether the HCB was administered in oil or in aqueous solution. Koss and Koransky ([1975](#)) found absorption fractions of 0.8 when HCB was administered to rats in oil while absorption from aqueous solutions ranged from 2-5%. In

this briefing packet, an absorption rate of 80% is assumed for the dam. In the absence of specific information, an absorption rate equal to the maternal rate (0.8) is assumed for the pups.

No estimate could be found in the literature for the fraction of absorbed HCB deposited in the adipose tissue of rats (**fraction deposited in fat for rats**). Human studies found a concentration 60 times higher of radioactive HCB in fat compared to blood and 30 times higher in fat than in the liver or the brain, indicating preferential transport to and storage in the fat ([Ingebrigtsen and Nafstad, 1983](#)). In the absence of specific information, the same fraction assumed for humans (90%) was assumed for rats.

**HCB half-life values in rats** tend to increase in time. When rats were dosed every other day for six weeks, the half-life was estimated to be 8 days right after exposure ended, 10 weeks after three months, and 12 months after 1.5 years ([Koss et al., 1978](#)). In the POD study selected in this briefing packet ([Mendoza et al., 1977](#)), the dams were dosed for a total of eight weeks (i.e., two weeks prior to mating, three weeks during gestation, and three weeks during lactation). Dosing did not proceed after weaning. Thus, the 8 day half-life was selected for the half-life in this briefing packet since it represents a half-life following exposure.

**Partition coefficients for rats** were estimated in the study by Lu et al. ([2006](#)) as discussed in the Section on [Multi-Compartment PBPK Models](#). Coefficients are also calculated from empirical data in Yesair et al. ([1986](#)).

#### B.3.5.3. Appropriate Dose Metric for Selected Point of Departure (POD)

Adverse developmental effects may be associated with HCB exposure during a critical period of development. Metrics may be selected to look at either peak or average maternal or infant exposure, depending on the effect in question and the dosing protocol in the animal study. For developmental effects, if a critical window can be determined, then the model solutions during the critical window may be used for the cross-species extrapolation; otherwise, peak or average concentrations from different exposure metrics (see below) may be used based on the judgment of the assessor as to what is most appropriate. For the proposed POD presented here, no critical window could be determined. Possible dose metrics for specified lactation durations include:

##### Dose Metric

- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ✓ **Peak or average concentrations from maternal or infant dose metrics**
- × **The critical window could not be defined**

- The average maternal body burden,
- The peak maternal body burden,
- The average maternal fat concentration,
- The peak maternal fat concentration,
- The average milk concentration,
- The peak milk concentration,
- The average infant body burden, and
- The peak infant body burden.

#### B.3.5.4. Available Models for Estimating HCB Internal Dose

The following section discusses potential models that may be used to estimate internal dose metrics based on maternal intake. The section starts with the most biologically-robust class of models, physiologically-based pharmacokinetic (PBPK) models. The section then discusses simpler first-order kinetic modeling techniques and a biotransfer method for estimating maternal exposure. Thus, the section generally progresses from the most biologically robust to the more simple models. The final choice in technique depends on weighing both the biological sophistication of the method and the uncertainty in the model parameters, as discussed in [Section B.3.6](#).

##### *Multi-Compartment PBPK Models*

Generally speaking, the most biologically complete kinetic model for a given chemical is a multi-compartment, data-validated, PBPK model. Validated models exist for HCB in rats (the species in the candidate POD study), and a non-validated model is also available for HCB in humans. The models are discussed below, with special attention given to their ability to address lactational exposure.

##### **Available Models**

- ? What PBPK or other simpler biokinetic models are available for HCB?
- ✓ **Data-validated rat PBPK model and a non-validated human PBPK model are available that include lactation**
- ✓ **Simple first order models are available**
- ✓ **Simple biotransfer methods are available**

##### *Rat Models*

Two PBPK models for HCB are discussed in this section. The first model by Yesair et al. ([1986](#)) simulates the distribution and excretion of HCB following oral exposure. The model describes gestation and lactation, as well as the nursing pup. Other compartments include intestinal lumen, blood, feces, liver, metabolites, kidney, urine, brain, richly perfused tissues, and poorly perfused tissues. Metabolism was included in the liver compartment by simulating first-order transfer from the liver compartment to the

metabolite compartment. The rate constants for metabolism were estimated from the literature, although the paper does not specify the sources. Metabolites were then eliminated via the feces and urine.

The model was validated using rat data from several studies, including Koss and Koransky ([1975](#)), Koss et al. ([1978](#)), and Courtney et al. ([1979](#)). The model was judged by the authors to agree well with the experimental data in multiple compartments. In addition, data from Kitchin et al. ([1982](#)) were used to compare the lactational transfer in the model to experimental data, and the model results were found to compare well. The model code is not provided and was not requested from the authors. In addition, the model equations are not provided in the study. Thus, implementation of the model would include both coding and testing the model against results in the paper.

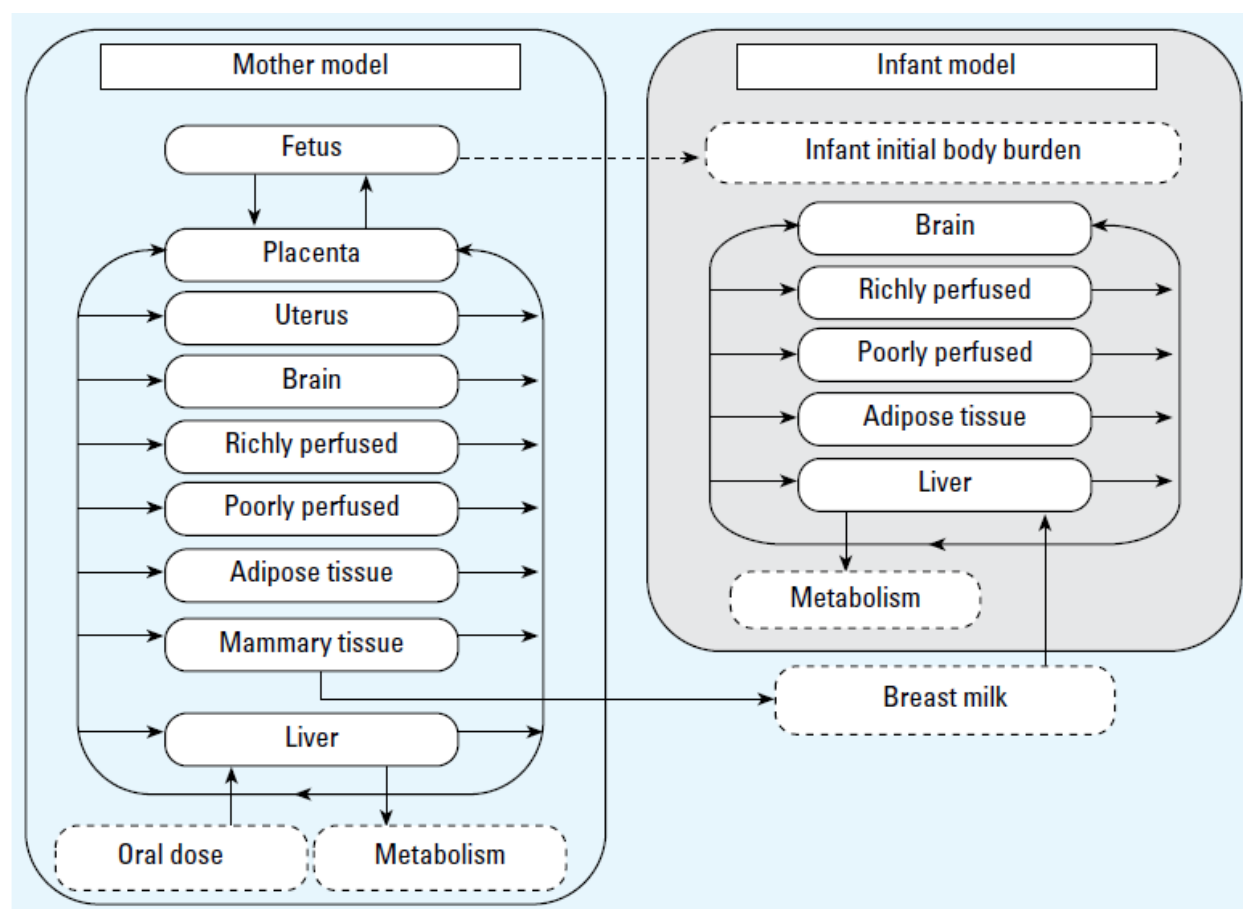
The second model is by Lu et al. ([2006](#)). This study incorporated two additional pharmacokinetic enhancements for HCB in rats. Based on findings from Yang et al. ([1975](#)), HCB binding with erythrocytes and the subsequent effects on sequestration and elimination were included in the model. In addition, passive diffusion from the blood compartment to the gastrointestinal lumen was modeled. These enhancements were judged by the authors to improve the performance of the model in estimating concentrations shortly (i.e., hours) after exposure. However, this model does not include descriptions for lactation and the nursing pup, so these descriptions would have to be added if this model were implemented here. The model code is provided as supplemental material to the paper.

### *Human Models*

After developing and validating the Yesair et al. ([1986](#)) rat model discussed in [Section B.3.5.4](#), the model was applied by the study authors to humans by changing the physiological parameters to reflect human values. However, the model was not validated against available human data.

A generic maternal-infant multi-compartment model for persistent organic pollutants (POPs) has been developed ([Verner et al., 2009](#); [Verner et al., 2008](#)). The primary utility of this model for HCB is that the chemical-specific parameters can be estimated using only the half-life and octanol-water partition coefficient ( $K_{ow}$ ) of the compound.

The full maternal and infant model ([Verner et al., 2009](#)) is intended to simulate the entire life-cycle of a woman up to age 55 years and includes nine maternal tissue compartments and five infant tissue compartments, as shown in [Figure B-18](#). The oral dose is modeled as being directly absorbed into the liver and assumed to be fully bioavailable. First-order hepatic metabolism is included and is intended to represent all excretion in the absence of breastfeeding or childbirth. The elimination rate constant is determined for the chemical from the adult whole-body half-life. The concentrations in each compartment are determined using partition coefficients, and the chemical-specific partition coefficients are estimated using an equation based on  $K_{ow}$  ([Poulin and Krishnan, 1996](#)) and using the fraction of blood and tissue that are lipid and water ([Price et al., 2003](#)). Physiological parameters such as body weight and fat volume fraction are assumed to vary in time to capture changes over the life of the woman.



**Figure B-18. Maternal and Infant POP Model ([Verner et al., 2009](#))**

Although HCB was not included, the model has been validated against human cord blood, breast milk, and infant blood concentrations for seven POPs ( $p,p'$ -DDE,  $p,p'$ -DDT, HDB,  $\beta$ -HCH, PCB-138, PCB-153, and PCB-180). The computer code was not provided in the publication, although a number of details about the parameters and equations are included in the main papers and in supplemental information. Considerable time would be needed in order to further evaluate the potential for this model to be used for HCB, including model implementation and manipulation in modeling software.

#### ***First-Order Steady-State Single-Compartment Model ([U.S. EPA, 1998](#))***

A first-order single-compartment model is an intermediate step between a full, multi-compartment PBPK model and simpler dosimetric conversions or biotransfer models. The model presented here is patterned after the first-order models presented in the Combustor Assessment ([U.S. EPA, 1998](#)) and the dioxin reassessment ([U.S. EPA, 2012b](#)) and is adapted to fully incorporate time-dependence during lactation. The general model equations are presented first, and then the model is applied to both humans and rats in the next section. Rats are the focus here since that is the species in the candidate POD study. Because the equations are readily available, they are presented in greater detail than the

equations in the PBPK models discussed above. However, this is not meant to imply that the first-order models are superior for HCB.

The single body compartment represented by the model is generally defined as “body burden,” or the total average concentration of contaminant in the body. In a first-order model, the elimination from the body is represented as a rate constant multiplied by the total body burden. This rate constant can be estimated from the whole-body half-life, which is often readily available in the literature. The input to the body is the dose, assumed to be normalized by the total body weight. Thus, the simple model can be represented by the differential equation<sup>9</sup>:

$$\frac{\partial BB(t)}{\partial t} = f_{am} DI_{mat}(t) - k_{elim} \times BB(t)$$

Appendix Equation 22

Where:

- $BB(t)$  = the time-dependent total body burden (ng/kg),
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{mat}(t)$  = the time-dependent maternal dose (ng/kg-day), and
- $f_{am}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless).

This equation can be converted to a difference equation and iterated in time:

$$BB_{t+\Delta t} = BB_t + \Delta t (f_{am} DI_{mat,t} - k_{elim} \times BB_t)$$

Appendix Equation 23

Where:

- $BB_{t+\Delta t}$  = the total body burden at the next time step (ng/kg),
- $BB_t$  = the total body burden at the current time step (ng/kg),
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{mat,t}$  = the maternal dose at the current time step (ng/kg-day),
- $f_{am}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless), and
- $\Delta t$  = the time step (days).

<sup>9</sup> Strictly speaking, this equation is only correct if the maternal body weight is not changing in time. However, this approximation is sufficient given the overall uncertainty in using a first-order model.

This equation will approximate the solution to the differential equation. To help eliminate errors introduced by converting the differential equation to an algebraic equation, the time step ( $\Delta t$ ) should be much smaller than the elimination time scale.

This equation allows approximation of the maternal body burden; however, additional assumptions can be made to estimate the time dependent infant intake from breast milk. First, the concentration in the maternal milk fat is assumed to be equal to the concentration in the maternal fat and can be estimated as:

$$C_{\text{milk fat},t} = \frac{BB_t \times f_f}{f_{fm}}$$

Appendix Equation 24

Where:

- $C_{\text{milk fat}, t}$  = the concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_f$  = the fraction of contaminant that is stored in maternal fat (dimensionless), and
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW).

Although nonpolar compounds partition to some degree into the aqueous compartment, the most commonly analyzed organic contaminants, including HCB, are highly concentrated in the lipid phase; the concentration of HCB in breast milk is not expected to be significantly underestimated by ignoring the aqueous phase.

To account for the fact that lactation acts as an additional removal mechanism from the mother, the elimination rate during lactation is increased to equal:

$$k_{\text{elac}} = k_{\text{elim}} + \frac{C_{\text{milk fat},t} \times f_f \times f_{\text{mbm}}}{f_{fm} \times BW_{\text{Mat}}}$$

Appendix Equation 25



Where:

$k_{elac}$	=	the maternal elimination rate during lactation ( $\text{days}^{-1}$ ),
$k_{elim}$	=	the first order elimination rate ( $\text{days}^{-1}$ ) = $\ln(2)/\text{half-life (days)}$ ,
$CR_{milk,t}$	=	the time-dependent infant ingestion rate of milk ( $\text{kg/day}$ ),
$f_f$	=	the fraction of contaminant that is stored in maternal fat (dimensionless)
$f_{mbm}$	=	the fraction of fat in milk (dimensionless),
$f_{fm}$	=	the fraction of the mother's weight that is fat ( $\text{kg fat/kg BW}$ ), and
$BW_{Mat}$	=	the maternal body weight ( $\text{kg}$ ).

Then, the time-dependent infant intake can be estimated as:

$$DI_{INF,t} = \frac{(C_{milk\ fat,t} \times f_{mbm}) \times CR_{milk,t}}{BW_{INF,t}}$$

Appendix Equation 26

Where:

$DI_{INF,t}$	=	the time-dependent infant daily ingestion ( $\text{ng/kg-day}$ ),
$C_{milkfat,t}$	=	the time-dependent concentration of contaminant in the maternal milk fat at the current time step ( $\text{ng/kg milk fat}$ ),
$f_{mbm}$	=	the fraction of fat in milk (dimensionless),
$CR_{milk,t}$	=	the time-dependent infant ingestion rate of milk ( $\text{kg/day}$ ), and
$BW_{INF,t}$	=	the time-dependent infant's body weight ( $\text{kg}$ ).

The time-dependent body weights and ingestion rates are incorporated in the models by using different point estimates over the course of the simulation (see the Sections on [Input Parameters and Application to HCB in Humans](#) and [Input Parameters and Application to HCB in Rats](#)). Finally, if the infant is modeled using a first-order model as well, the infant body burden can be estimated as:

$$BB_{INF,t+\Delta t} = BB_{INF,t} + \Delta t (f_{ai} DI_{INF,t} - k_{elim,INF} \times BB_{INF,t})$$

Appendix Equation 27

Where:

$BB_{INF,t+\Delta t}$	=	the infant total body burden at the next time step (ng/kg),
$BB_{INF,t}$	=	the infant total body burden at the current time step (ng/kg),
$k_{elim,INF}$	=	the first order elimination rate in the infant ( $\text{days}^{-1}$ ) = $\ln(2)/\text{half-life (days)}$ ,
$DI_{INF,t}$	=	the infant dose at the current time step (ng/kg-day),
$f_{ai}$	=	the fraction of ingested contaminant absorbed by the infant (dimensionless), and
$\Delta t$	=	the time step (days).

The above equations approximate the maternal body burden and exposure to a lactating infant. The model does not, however, include any fetal exposure *in utero*.

This model is generic and not species specific. For purposes of illustration, the model was implemented in Excel and time integrated through the life of the mother (see “Human Time Dependent Model.xlsm” and “Rodent Time Dependent Model.xlsm” provided to workshop participants). Then, the time-dependent solution can be used to estimate the internal dose metrics in [Section B.1.6](#).

The above model was parameterized so that the maternal dose, the infant body weight, and the infant ingestion rate all vary with time. In the case of the maternal dose, this allows the model to follow dosing protocols from animal studies. In the case of the body weight and ingestion rate, this allows the model to more accurately simulate effects on infants during very early exposure when body weight is changing rapidly and when critical windows for developmental effects may occur.

### ***Input Parameters and Application to HCB in Humans***

For humans, the model was implemented using a time step of 1 week. This represents a balance between a time step short enough to capture changes in the infant during lactation and long enough so that data are not applied beyond their measurement range. Since the Exposure Factors Handbook ([U.S. EPA, 2011a](#)) provides infant estimates on a monthly basis (see below), input values across weeks within a single month were kept equal.

The input parameters used for HCB for humans are shown in [Table B-37](#). In addition to using mid-range (i.e., mean) values in the model, low and high end estimates were also used for some parameters, where available, to determine the approximate range of human variability. Values for the parameters that are not chemical-specific were retained from the Combustor Assessment ([U.S. EPA, 1998](#)) or taken from the Exposure Factors Handbook ([U.S. EPA, 2011a](#)). HCB-specific values are the same as those presented and discussed in [Section B.3.5.2](#).

As discussed in [Section B.3.5.2](#), the absorption rate of HCB depends on the relative concentration of the chemical in the blood at the time of intake. If the blood concentration is zero, then absorption may be as high as 80% while higher blood concentrations can lead to absorption rates of 1% or less. Because this

simple first-order kinetic model does not simulate the blood concentration, the regression equation for absorption from Schlummer et al. ([1998](#)) was not directly implemented in the model. Instead, the infant absorption rate was assumed to be 80%, which is the maximum absorption reported by Schlummer et al. ([1998](#)) amongst the seven test subjects. An attempt was made to vary the maternal absorption by maternal age; however, when implemented in this way, the maternal body burden decreased after age 25 years, which is inconsistent with experimental evidence of increasing HCB concentrations with age. Thus, the model was implemented assuming low daily exposure and constant absorption at 80% throughout the maternal lifetime. This may tend to overestimate the absorption, particularly in women who get pregnant at an older age.

**Table B-37. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model**

Parameter (units)	Variable	Low	Mid	High	Note/source
Human Parameters (not chemical-specific)					
Maternal age at pregnancy (years)	Age	18	25	40	Assumptions
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 1 (kg)	BW <sub>INF,t</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 2-3 (kg)		5.9			
Infant body weight, Month 4-6 (kg)		7.4			
Infant body weight, Month 7-12 (kg)		9.2			
Infant ingestion rate, Month 1 (kg milk/day)	CR <sub>milk,t</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, Month 2-3 (kg milk/day)		0.69			
Infant ingestion rate, Month 4-6 (kg milk/day)		0.77			
Infant ingestion rate, Month 7-12 (kg milk/day)		0.62			
Fraction of mother’s weight that is fat (kg maternal fat/kg BW) <sup>b</sup>	f <sub>fm</sub>	0.2	0.3	0.4	Timson and Coffman ( <a href="#">1984</a> ); U.S. EPA ( <a href="#">1998</a> )
Fraction of fat in breast milk (dimensionless) <sup>c</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Human Chemical-Specific Parameters					
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	0.80			Schlummer et al. ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	0.80			Schlummer et al. ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9			U.S. EPA ( <a href="#">1998</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.00322			Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans (days)	t <sub>1/2</sub>	215			Yesair et al. ( <a href="#">1986</a> )

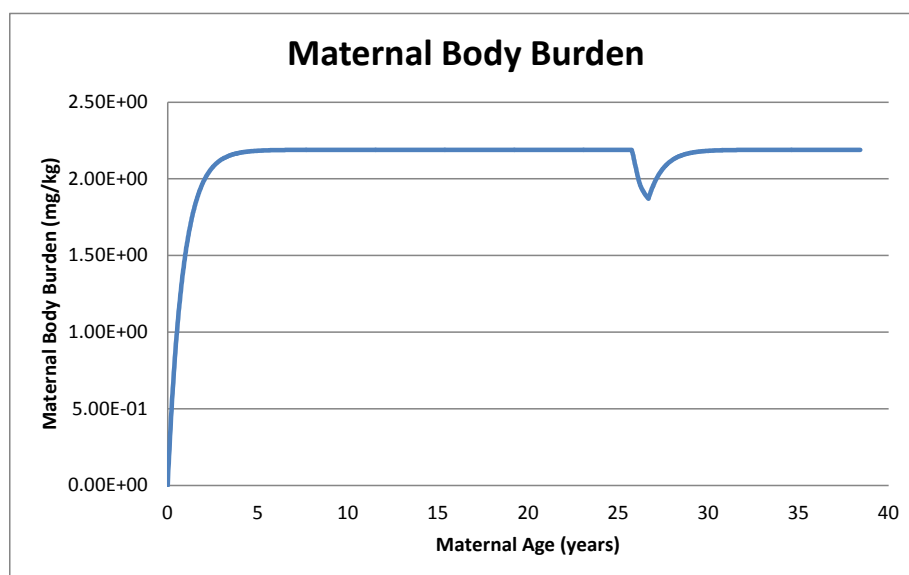
<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents mean ± standard deviation.

<sup>c</sup> Represents the typical range and midpoint of the range.

An example figure showing the maternal body burden assuming pregnancy at age 25 years and a daily intake of 1 ng/kg-day is shown in [Figure B-19](#). Because the model is linear with respect to dose, this intake level is chosen for illustrative purposes and the body burden would scale linearly with any change in intake. The dip at age 25 years and 9 months is due to the additional elimination during lactation.

One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, assuming the “mid” parameters and pregnancy at age 25 years, the average infant to maternal dose ratio was estimated to be 2.3 (1 month lactation duration) and 1.8 (12 month lactation duration). The overall range of the ratio, accounting for human variability and including the peak estimates, was from 0.9 to 5.0 (see [Table B-38](#)).



**Figure B-19. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day**

**Table B-38. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model<sup>a</sup>**

Infant Nursing Duration	Pregnancy at 18 years; Low parameters	Pregnancy at 25 years; Mid parameters	Pregnancy at 40 years; High parameters
1 month, Peak Infant Intake	1.2	2.4	4.7
1 month, Average Infant Intake	1.2	2.3	4.6
12 months, Peak Infant Intake	1.3	2.6	5.0
12 months, Average Infant Intake	0.9	1.8	3.3

<sup>a</sup> Calculated using “Human Time Dependent Model.xlsm” provided to workshop participants

### *Input Parameters and Application to HCB in Rats*

For rats, the model was implemented using a time step of one half day (one week used for humans). This was deemed sufficient to resolve changes during the typical three-week lactation period. The input parameters used for HCB are shown in [Table B-39](#). A gestation time of 3 weeks and lactation duration of 3 weeks were assumed for the analysis as representative typical values. A litter size parameter was added so that the nursing rat fed 10 pups at a time, rather than the single infant assumed in the human model. This litter size, which matches the number of pups per litter in the candidate POD study, increased the maternal lactational elimination rate.

The physiological parameters needed for the model were not presented in the candidate POD study. The suckling rate in rat litters was assumed to be equal to the milk production in the dam. Experimentally determined milk production values of 0.0009, 0.0016, 0.0018, and 0.0016 liters/hr were measured on lactation days 3, 10, 17, and 23, respectively ([Knight et al., 1984](#)). The values for day 3, 10, and 17 were used for weeks 1, 2, and 3, respectively, assuming a milk density of 1 kg/liter, dividing by the total number of pups to get a per pup ingestion rate, and normalizing by the pup body weight. The body weight normalization reflects the assumption that intake per kg body weight is similar across rat strains. Knight et al. ([1984](#)) also reported average pup body weights corresponding to these suckling rates of 8.5, 21 and 31 g for weeks 1, 2 and 3, respectively.

Knight et al. ([1984](#)) reported the weight of lactating Wistar dams at day 2, 7, 14 and 21. The average across these days for the control group (273 g) was used in the model. Fisher et al. ([1990](#)) reported fat as a percentage of body weight to be 6.0 to 12.0% in lactating F344 dams. A midpoint of 9.0% was used for this application. The fraction of fat in milk was reported to be 15.0% in Fisher et al. ([1990](#)), compared to a value of 4% in humans ([Welch and Findlay, 1981](#)).

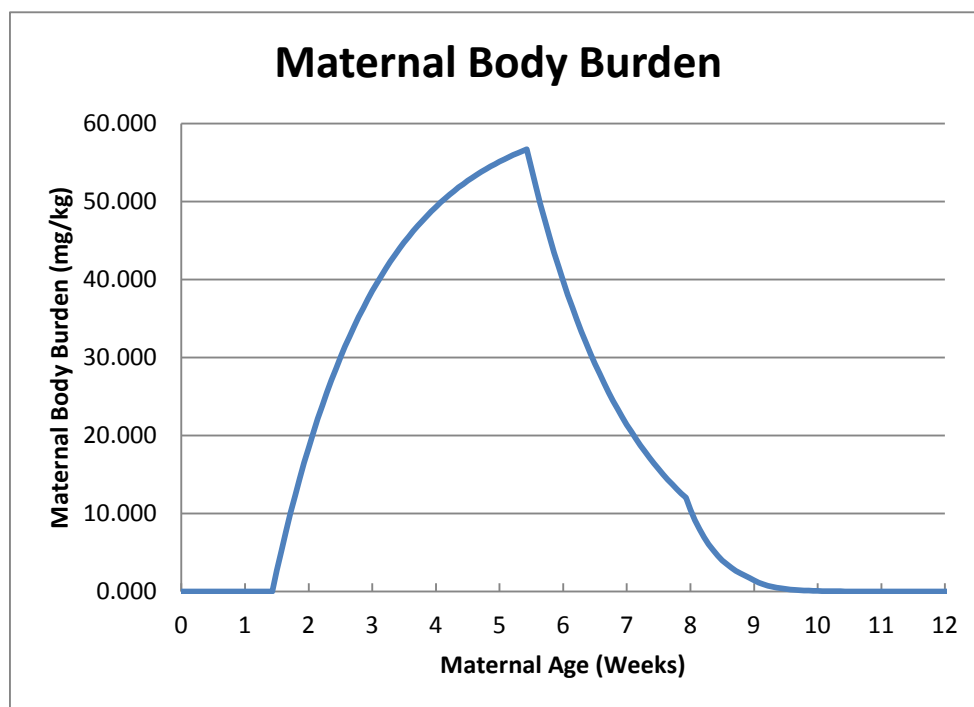
The same chemical-specific values as presented in [Section B.3.5.2 \(Table B-36\)](#) were used: maternal absorption fraction, the fraction of contaminant stored in the fat relative to the total body burden, and the half-life.

**Table B-39. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat**

Parameter (units)	Variable	Estimated Value	Note/source
<b>Rat Parameters (not chemical-specific)</b>			
Dam age at pregnancy (weeks)	Age	5	Assumption
Dam weight during lactation (kg)	BW <sub>Mat</sub>	0.273	Fisher et al. ( <a href="#">1990</a> )
Litter size (number of pups)	Litter	10	Mendoza et al. ( <a href="#">1978</a> ; <a href="#">1977</a> )
Pup body weight, Week 1 (kg)	BW <sub>INF,t</sub>	0.0085	Knight et al. ( <a href="#">1984</a> )
Pup body weight, Week 2 (kg)		0.021	
Pup body weight, Week 3 (kg)		0.031	
Pup ingestion rate, Week 1 (kg milk/day)	CR <sub>milk,t</sub>	0.0031	Knight et al. ( <a href="#">1984</a> ), adjusted for litter size
Pup ingestion rate, Week 2 (kg milk/day)		0.0054	
Pup ingestion rate, Week 3 (kg milk/day)		0.0061	
Fraction of dam's weight that is fat (kg maternal fat/kg BW)	f <sub>fm</sub>	0.09	Fisher et al. ( <a href="#">1990</a> )
Fraction of fat in maternal milk (dimensionless)	f <sub>mbm</sub>	0.15	Welch and Findlay ( <a href="#">1981</a> )
<b>Rat Chemical-Specific Parameters</b>			
Fraction of ingested contaminant that is absorbed by the pup (dimensionless)	f <sub>ai</sub>	0.8	Assumed
Fraction of ingested contaminant that is absorbed by the dam (dimensionless)	f <sub>am</sub>	0.8	Koss and Koransky ( <a href="#">1975</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	Assumed
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.087	Calculated as ln(2) / t <sub>1/2</sub>
Half-life in rats (days)	t <sub>1/2</sub>	8 days to 1.5 years; 8 days used here	Koss et al. ( <a href="#">1978</a> )

The dosing protocol in the POD study ([Mendoza et al., 1977](#)) was applied to this rat model. In the study, the dams were dosed daily from two weeks before mating until PND 21. The model is able to capture transfer to the nursing pup during lactation, but it does not model any infant body burden during gestation. An example figure showing the maternal body burden using the candidate POD dose (6.7 mg/kg-day) is shown in [Figure B-20](#).

One useful metric from the model is the estimate of the ratio between the pup and maternal intake. For the model parameterized as discussed above, the average pup to maternal dose ratio was estimated to be 0.4 (1 week lactation duration) and 0.1 (3 week lactation duration), see [Table B-40](#).



**Figure B-20. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study ([Mendoza et al., 1977](#)) and an Administered Dose of 6.7 mg/kg-day (POD)**

**Table B-40. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model<sup>a</sup>**

Pup Nursing Duration	Ratio
1 week, Peak Pup Intake	0.9
1 week, Average Pup Intake	0.4
3 weeks, Peak Pup Intake	0.9
3 weeks, Average Pup Intake	0.1

<sup>a</sup> Calculated using "Rodent Time Dependent Model.xlsm" provided to workshop participants

### ***Biotransfer Method***

The simplest technique for linking maternal and infant intake involves estimating the transfer of HCB to breast milk using a biotransfer factor. This method is discussed in the Combustor Assessment ([U.S. EPA](#),



[1998](#)). The technique is based on a study by Travis and Arms ([1988](#)) and assumes that the milk fat contaminant concentration is proportional to maternal contaminant intake, with the proportionality constant represented by a biotransfer factor:

$$C_{\text{milk fat}} = DI_{\text{MAT}} \times BTF_m \times BW_{\text{Mat}}$$

Appendix Equation 28

Where:

- $C_{\text{milk fat}}$  = the concentration of contaminant in the maternal milk fat (ng/kg milk fat),
- $DI_{\text{MAT}}$  = daily maternal intake of contaminant (ng/kg BW-day),
- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $BW_{\text{Mat}}$  = the maternal body weight (kg).

The biotransfer factor is then estimated using a regression equation based on the octanol-water partition coefficient ( $K_{ow}$ ) using the expression:

$$BTF_m = 0.00062 \times K_{ow}$$

Appendix Equation 29

Where:

- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $K_{ow}$  = the octanol-water partition coefficient (unitless).

The regression equation was estimated using six highly lipophilic compounds with  $\log(K_{ow})$  in the range of 5.16 to 6.5. According to the Combustor Assessment ([U.S. EPA, 1998](#)), the model tends to over-predict concentrations. Thus, the Combustor Assessment suggests the equation should only be used if parameters for more sophisticated kinetic models cannot be found in the literature and if the  $K_{ow}$  of the chemical in question is within the range of the  $K_{ow}$  used to fit the model. This method is included for consideration.

The daily infant contaminant dose can then be estimated for the biotransfer method using the equation:

$$DI_{\text{INF}} = \frac{(C_{\text{milk fat}} \times f_{\text{mbm}}) \times CR_{\text{milk}} \times ED_{\text{INF}}}{BW_{\text{INF}} \times AT}$$

Appendix Equation 30

Where:

- $DI_{INF}$  = the daily infant contaminant dose (ng/kg-day),  
 $C_{milkfat}$  = the concentration of contaminant in the maternal milk fat (ng/kg milk fat),  
 $f_{mbm}$  = the fraction of fat in milk (dimensionless),  
 $CR_{milk}$  = the infant ingestion rate of milk (kg/day),  
 $ED_{INF}$  = the infant exposure duration (year),  
 $BW_{INF}$  = the infant's body weight (kg), and  
 $AT$  = the averaging time (year).

Using the above equations, the ratio between the infant and maternal intake can be estimated as:

$$\frac{DI_{INF}}{DI_{MAT}} = \frac{0.00062 \times K_{OW} \times f_{mbm} \times CR_{milk} \times ED_{INF} \times BW_{MAT}}{BW_{INF} \times AT}$$

Appendix Equation 31

### *Input Parameters and Application to HCB in Humans*

The input parameters used for HCB in the biotransfer model are shown in [Table B-41](#). In addition to using mid-range (e.g., mean) values in the model, low and high end estimates were also used for some parameters where available to determine the approximate range of human variability. The  $ED_{INF}$  and AT values are equal to each other (either 1 month or 12 months) and cancel from the equation.

**Table B-41. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age**

Parameter (units)	Variable	Low	Mid	High	Note/source
Maternal weight during lactation (kg) <sup>a</sup>	$BW_{Mat}$	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, 1 month (kg)	$BW_{INF, 1}$	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, average over 12 months (kg)	$BW_{INF, 12}$	7.8			Weighted mean, U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, 1 month (kg milk/day)	$CR_{milk,1}$	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, average over 12 months (kg milk/day)	$CR_{milk,12}$	0.66			Weighted mean, U.S. EPA ( <a href="#">2011a</a> )
Fraction of fat in breast milk (dimensionless) <sup>b</sup>	$f_{mbm}$	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Octanol-water partition coefficient	$\log(K_{ow})$	5.73			Hansch et al. ( <a href="#">1995a</a> )

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents the typical range and midpoint of the range.

Based on the  $K_{ow}$  value and using [Appendix Equation 29](#), the biotransfer factor for HCB is 333. Using this  $BTF_m$ , the average infant to maternal dose ratio was estimated for infants at 1 and 12 months of age and is presented in [Table B-42](#). For the model parameterization presented here, these ratios tend to be one order of magnitude higher than the estimates from the simple one-compartment kinetic model.

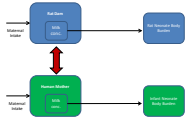

**Table B-42. Estimated Infant-to-Maternal Dose Ratio Based on Biotransfer Method**

Age	Low	Mid	High
1 month	53.1	79.6	106.1
12 months	42.3	63.4	84.5

### B.3.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation

The previous sections discuss the tools and input data available to estimate internal dose metrics for HCB in rats and humans. The next step is to select a method for performing cross-species extrapolation by weighing biological sophistication against methodology and data uncertainty. Cross-species extrapolation is used to estimate a human equivalent dose, or the daily dose likely to lead to the same developmental effect in humans as that observed in animals. [Table B-43](#) presents potential dose metrics and methods for performing the cross-species extrapolation. This list may not be exhaustive but is intended to foster discussion at the workshop. For illustrative purposes in this briefing packet, one method for HED calculation was selected (shown in bold type), and sample calculations were performed.

**Table B-43. Potential Dose Metrics and Methods for HED Estimation**

Should the Cross-Species Extrapolation Be Performed on Maternal or Offspring Dose Metric?	What Technique Is Used to Estimate the Dose Metrics?	What Dose Metric Should Be Used for Cross-Species Extrapolation?
<b>Maternal Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Maternal average or peak body burden during lactation</li> <li>• Maternal average or peak fat concentration during lactation</li> <li>• Maternal average or peak milk concentration during lactation</li> </ul>
<b>Offspring Concentration</b> 	<ul style="list-style-type: none"> <li>• <b>PBPK model in rats and humans</b></li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Infant average or peak body burden during lactation</b></li> </ul>
Other?	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Other?</li> </ul>

In selecting the dose metric to use for cross-species extrapolation and the technique to use to estimate the dose metrics, the discussion points listed in the text boxes throughout this section should be considered. This information is synthesized in the following text box.

As an example, the points were considered, and an example was provided to foster discussion. The chemical-specific information available should be considered to help guide the decision on whether a maternal or offspring dose metric should be utilized. Both a human and rat PBPK model for HCB exists with the necessary inputs although the human model is not validated. The chemical is metabolized in the body, but metabolism is slow. For these reasons, the database was considered sufficient to support selection of infant/pup dose metrics to perform the cross-species extrapolation.

Next, a model was selected to characterize the dose metrics. Both human and rat HCB PBPK models are available. The Yesair et al. ([1986](#)) model would have to be coded, tested, and validated for humans, while the Lu et al. ([2006](#)) would need to be coded and tested for rats. For the first-order model, some of the necessary parameters are unavailable for rats and humans, including the fraction of contaminant stored in fat. For this reason, the PBPK models are selected in this example as the method to estimate the dose metrics in rats and humans.

Finally, the infant/pup dose metric was selected. Increased liver weight and enzyme activity were more pronounced in the cross-fostered animals exposed during lactation only. For this reason, lactation is likely an important exposure route for the effect. However, increased liver weight and enzyme activity are not effects on development, per se. Therefore, it is unlikely that their occurrence is determined by exposure during a specific window of susceptibility. Thus, the average infant body burden may be the most appropriate dose metric for cross-species extrapolation.

The selections described above were not applied in this briefing packet due to the significant effort necessary to implement and run the PBPK models.

### Discussion Points for HCB HED Estimation

- ? Is HCB metabolized? How should metabolism be addressed in the HED estimation?
  - ✓ HCB is metabolized at a very slow rate.
- ? What pharmacokinetic data or relevant chemical-specific information are available for HCB for both animals and humans to facilitate model implementation?
  - ✓  $K_{ow}$
  - ✓ Human and rat half lives
  - ✓ Absorption fractions and fraction of chemical stored in fat in humans (assumptions can be made based on other PBT chemicals)
  - ✗ No fraction of chemical stored in fat for rats
  - ✓ Partition coefficients from PBPK models for rats and humans
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
  - ✓ Peak or average concentrations from maternal or infant dose metrics
  - ✗ The critical window could not be defined
- ? What PBPK or other simpler biokinetic models are available for HCB?
  - ✓ Data-validated rat PBPK model and a non-validated human PBPK model are available that include lactation
  - ✓ Simple first order models are available
  - ✓ Simple biotransfer methods are available
- ? What is the best method for estimating the human equivalent dose using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

## Attachment B.3-1. Concentrations of HCB in Human Milk

**Table Att. B.3-1. Mean Levels of HCB in Breast Milk as Reported in IPCS ([1997](#)).**

Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk <sup>b</sup>	Reference
Australia	1970	39	0.042 (rural)	Newton and Greene ( <a href="#">1972</a> )
		28	0.063 (urban)	
Australia	1979-1980	137	0.007 (0.002-0.019) (rural)	Stacey et al. ( <a href="#">1985</a> )
		130	0.008 (0.002-0.017) (urban)	
Australia	-	60	0.017 (0.0007-0.32)	Quinsey et al. ( <a href="#">1995</a> )
Australia	1990-1991	128	0.0036 <sup>a</sup> (<0.01-0.216)	Stevens et al. ( <a href="#">1993</a> )
Brazil	1987-1988	30	0.00048 (0.00024-0.0036) <sup>b</sup>	Beretta & Dick ( <a href="#">1994</a> )
Canada	1986	412	0.0008 (max = 0.014)	Mes et al. ( <a href="#">1993</a> )
Canada	1982	210	0.002 (max = 0.009)	Mes et al. ( <a href="#">1986</a> )
Canada	1975	100	0.002 (max = 0.021)	Mes and Davies ( <a href="#">1979</a> )
Canada	1989-1990	536	0.0013 <sup>b</sup>	Dewailly et al. ( <a href="#">1991</a> ) as cited in IPCS ( <a href="#">1997</a> )
Canada	1978	127	0.00051	Frank et al. ( <a href="#">1988</a> )
	1979	15	0.0004	
	1980-1981	12	0.00028	
	1983-1984	13	0.00052	
	1985	18	0.00026	
Finland	1984-1985	143 <sup>b</sup>	0.002 <sup>b</sup>	Mussalo-Rauhamaa et al. ( <a href="#">1988</a> )
Finland	1982	50	0.0023 (0.0007-0.006)	Wickström et al. ( <a href="#">1983</a> )
France	1990-1991	20	0.002 (0.00004-0.008) <sup>b</sup>	Bordet et al. ( <a href="#">1993</a> )
Federal Republic of Germany	1984	144	0.021 <sup>b</sup>	Fürst et al. ( <a href="#">1994</a> )
	1985	220	0.019 <sup>b</sup>	
	1986	157	0.015 <sup>b</sup>	

Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk <sup>b</sup>	Reference
	1987	144	0.015 <sup>b</sup>	
	1988	196	0.013 <sup>b</sup>	
	1989	145	0.01 <sup>b</sup>	
	1990	286	0.0095 <sup>b</sup>	
	1991	113	0.0074 <sup>b</sup>	
Federal Republic of Germany	1985-1987	167	0.0126 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Federal Republic of Germany	1979-1981	2709	0.048 <sup>b</sup>	BUA ( <a href="#">1993</a> )
	1986	3778	0.013 <sup>b</sup>	
	1987	1897	0.014 <sup>b</sup>	
	1988	2994	0.011 <sup>b</sup>	
	1989	3256	0.01 <sup>b</sup>	
	1990	5340	0.009 <sup>b</sup>	
Former German Democratic Republic	1990-1991	483	0.007 <sup>b</sup>	BUA ( <a href="#">1993</a> )
India	1987	16	0.042 (0-0.25) <sup>d</sup>	Nair and Pillai ( <a href="#">1989</a> )
Israel	-	100	0.00256	Weisenberg et al. ( <a href="#">1985</a> )
Italy	-	56	0.058 <sup>c</sup>	Franchi & Focardi ( <a href="#">1991</a> )
Italy	1987	64	0.006 (0.004-0.009)	Larsen et al. ( <a href="#">1994</a> )
Netherlands	1972-1973	202	0.036 <sup>a,b</sup>	Greve and Van Zoonen ( <a href="#">1990</a> )
	1983	278	0.008 <sup>a,b</sup>	
New Zealand	1988	38	0.0011	Bates et al. ( <a href="#">1994</a> )
Norway	1991	28	0.0007	Johansen et al. ( <a href="#">1994</a> )
Spain	1984-1987	240	0.089(0.039-0.21) <sup>b</sup>	Conde et al. ( <a href="#">1993</a> )
	1990-1991	358	0.048(0.037-0.073) <sup>b</sup>	
Sweden	1978	20	0.0042 (0.002-0.009) <sup>b</sup>	Norén ( <a href="#">1983</a> )
Sweden	-	2	0.0007-0.004 <sup>b</sup>	Norén ( <a href="#">1983</a> )



Location (country)	Year	Participants/ Sample size	Mean tissue concentration (range), mg/kg whole milk <sup>b</sup>	Reference
Sweden	1972	227	0.003 (0.002-0.004) <sup>b</sup>	Norén ( <a href="#">1988</a> )
	1976	245	0.003 (0.003-0.004) <sup>b</sup>	
	1980	340	0.003 (0.003-0.004) <sup>b</sup>	
	1984-1985	102	0.001 (0.0008-0.001) <sup>b</sup>	
Sweden	1989	140	0.0012 <sup>b</sup>	Norén ( <a href="#">1993</a> )
Sweden	1986-1987	40	0.0017 <sup>b</sup>	Vaz et al. ( <a href="#">1993</a> )
Thailand	1985-1987	3	0.0003 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Turkey	1988	51	0.0035 <sup>b</sup>	Üstünbas et al. ( <a href="#">1994</a> )
Turkey	20-30 years post-exposure during 1955-1959	56	0.021 <sup>b</sup>	Gocmen et al. ( <a href="#">1989</a> )
United Kingdom	1989-1991	193	0.001 (<0.001-0.005)	WPPR ( <a href="#">1992</a> )
USA	1979	40	0.00052	Bush et al. ( <a href="#">1985</a> )
USA	1985-1987	8	0.0007-0.0008 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Vietnam	1985-1987	12	<0.00017 <sup>b</sup>	Schechter et al. ( <a href="#">1989</a> )
Yugoslavia	1978	10	0.006 (0.002-0.017)	Kodric-Smit et al. ( <a href="#">1980</a> )

<sup>a</sup> Median value.

<sup>b</sup> Originally expressed as mg/kg milk fat and subsequently converted to a wet weight basis using either the % fat reported, or if not given, using 4.2% fat ([DNHW, 1987](#)).

<sup>c</sup> Number of positive samples.

<sup>d</sup> Originally expressed on a dry weight basis and subsequently converted to wet weight using 88% moisture for conversion ([DNHW, 1987](#)).

**Table Att. B.3-2. Mean Levels of HCB in Breast Milk as Reported by ATSDR ([2002b](#))**

Location of study	N/n	Concentration <sup>a</sup>	Reference
New Zealand			
Auckland, urban	11/n/a	0.020	Bates et al. ( <a href="#">1994</a> )
Northland, rural	10/n/a	0.021	
Christchurch, urban	9/n/a	0.030	
Canterbury, rural	8/n/a	0.063	
Australia			
Victoria, Australia	60/59	0.41	Quinsey et al. ( <a href="#">1995</a> )
Victoria, Australia (1985/1986)	158/153	0.005 <sup>b</sup>	Monheit and Luke ( <a href="#">1990</a> )
Italy			
Total	27/NR	--	Larsen et al. ( <a href="#">1994</a> )
Rome	NR	0.25	
Pavia	NR	0.12	
Milan	NR	0.20	
Florence	NR	0.14	
Czech Republic			
Prague	17/NR	0.639	Schoula et al. ( <a href="#">1996</a> )
Kladno	12/NR	0.570	
Uherske Hradiste	7/NR	0.482	
Other European locations			
France (multiple locations)	20/19	0.147	Bordet et al. ( <a href="#">1993</a> )
Madrid, Spain	63/52	1.0	Conde et al. ( <a href="#">1993</a> )
Madrid, Spain: Industrialized area	NR	1.74	
Sweden (1972–80)	227–1,500/NR	0.110–220	Norén and Meironyté ( <a href="#">2000</a> )
Bratislava, Slovak Republic	26/26	0.339	Prachar et al. ( <a href="#">1993</a> )
Canada			
Canada (1967–1992)	NR	0.002–0.00044 <sup>b</sup>	Craan and Haines ( <a href="#">1998</a> )
Quebec	NR	0.002–0.00040 <sup>b</sup>	
Ontario	NR	0.002–0.00048 <sup>b</sup>	
Arctic Quebec Inuit women	107/107	0.136	Dewailly et al. ( <a href="#">1993</a> )

Location of study	N/n	Concentration <sup>a</sup>	Reference
Arctic Quebec Caucasian women	50/48	0.028	
Canada	412/no data	0.026	Mes et al. ( <a href="#">1993</a> )
Canada (multiple locations, in 1992)	497/497	0.0044 <sup>b</sup> 0.015 <sup>c</sup>	Newsome et al. ( <a href="#">1995</a> )
<b>Other North America</b>			
Arkansas, USA	942/57	0.03	Mattison et al. ( <a href="#">1992</a> )
Hawaii, USA (1979–1980)	54/54	0.046 ± 0.049	Takei et al. ( <a href="#">1983</a> )
Veracruz, Mexico	43/43	0.047	Waliszewski et al. ( <a href="#">1996</a> )
<b>Other</b>			
Akumadan, Ghana	20/19	0.04	Ntow ( <a href="#">2001</a> )
Northern Thailand	25/9	0.0051 <sup>b</sup>	Stuetz et al. ( <a href="#">2001</a> )
Porto Alegre, Brazil	30/19	0.02	Beretta and Dick ( <a href="#">1994</a> )

<sup>a</sup> µg/g on lipid basis unless otherwise noted<sup>b</sup> whole milk sample<sup>c</sup> milk fat sample

N = number of samples; n = number of samples with measurable levels; NR = not reported

Source: Reproduced from Pohl and Tylenda ([2000](#)) by ATSDR ([2002b](#)).

### B.3.7. References

- Abraham, K; Hille, A; Ende, M; Helge, H. (1994). Intake and fecal excretion of PCDDs, PCDFs, HCB and PCBs (138, 153, 180) in a breast-fed and a formula-fed infant. *Chemosphere* 29: 2279-2286. [http://dx.doi.org/10.1016/0045-6535\(94\)90395-6](http://dx.doi.org/10.1016/0045-6535(94)90395-6)
- Andrews, JE; Courtney, KD. (1986). Hexachlorobenzene-induced renal maldevelopment in CD-1 mice and CD rats. In CR Morris; JRP Cabral (Eds.), *IARC Scientific Publications* (pp. 381-391). Lyon, France: International Agency for Research on Cancer. <http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=73&codcch=77>
- ATSDR (Agency for Toxic Substances and Disease Registry). (2002b). Toxicological profile for hexachlorobenzene [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
- Ayotte, P; Dewailly, E; Bruneau, S; Careau, H; Vézina, A. (1995). Arctic air pollution and human health: what effects should be expected [Review]. *Sci Total Environ* 160-161: 529-537. [http://dx.doi.org/10.1016/0048-9697\(95\)04387-G](http://dx.doi.org/10.1016/0048-9697(95)04387-G)
- Bailey, J; Knauf, V; Mueller, W; Hobson, W. (1980). Transfer of hexachlorobenzene and polychlorinated biphenyls to nursing infant rhesus monkeys: enhanced toxicity. *Environ Res* 21: 190-196.
- Barnett, JB; Barfield, L; Walls, R; Joyner, R; Owens, R; Soderberg, LSF. (1987). The effect of in utero exposure to hexachlorobenzene on the developing immune response of BALB/c mice. *Toxicol Lett* 39: 263-274.
- Basri Ustünbas, H; Adnan Oztürk, MA; Hasanoğlu, E; Doğan, M. (1994). Organochlorine pesticide residues in human milk in Kayseri. *Hum Exp Toxicol* 13: 299-302. <http://dx.doi.org/10.1177/096032719401300501>
- Bates, MN; Hannah, DJ; Buckland, SJ; Taucher, JA; van Maanen, T. (1994). Chlorinated organic contaminants in breast milk of New Zealand women. *Environ Health Perspect* 102: 211-217.
- Beretta, M; Dick, T. (1994). Organochlorine compounds in human milk, Porto Alegre, Brazil. *Bull Environ Contam Toxicol* 53: 357-360. <http://dx.doi.org/10.1007/BF00197226>
- Bordet, F; Mallet, J; Maurice, L; Borrel, S; Venant, A. (1993). ORGANOCHLORINE PESTICIDE AND PCB CONGENER CONTENT OF FRENCH HUMAN MILK. *Bull Environ Contam Toxicol* 50: 425-432.
- BUA (GDCh Advisory Committee on Existing Chemicals). (1993). Hexachlorobenzene. (BUA Report 119). Stuttgart, Germany: S. Hirzel Verlag. <http://www.hirzel.de/fachbuch/naturwissenschaften/chemie-und-biologie/bua-reports/view/titel/1287.html>
- Bush, B; Snow, J; Connor, S; Koblintz, R. (1985). Polychlorinated biphenyl congeners (PCBs), p,p'-DDE and hexachlorobenzene in human milk in three areas of upstate New York. *Arch Environ Contam Toxicol* 14: 443-450. <http://dx.doi.org/10.1007/BF01055530>
- CDC (Centers for Disease Control and Prevention). (2009). Fourth national report on human exposure to environmental chemicals. Atlanta, GA. <http://www.cdc.gov/exposurereport/>
- Conde, C; Maluenda, C; Arrabal, C. (1993). Hexachlorobenzene (HCB) in human milk in Spain from 1984 to 1991. *Bull Environ Contam Toxicol* 51: 827-831. <http://dx.doi.org/10.1007/BF00198277>
- Courtney, KD; Andrews, JE. (1985). Neonatal and maternal body burdens of hexachlorobenzene (HCB) in mice: gestational exposure and lactational transfer. *Toxicol Sci* 5: 265-277. [http://dx.doi.org/10.1016/0272-0590\(85\)90074-0](http://dx.doi.org/10.1016/0272-0590(85)90074-0)
- Courtney, KD; Andrews, JE; Grady, MA; Ebron, MT. (1984). Postnatal effects of hexachlorobenzene (HCB) on cardiac lactic dehydrogenase (LDH) and creatine kinase (CK) isozymes in CD-1 mice. *Toxicol Lett* 22: 223-228.

- [Courtney, KD; Andrews, JE; Svendsgaard, DJ.](#) (1979). Hexachlorobenzene (HCB) deposition in maternal and fetal tissue of rat and mouse: I. Chemical quantification of HCB in tissues. *Environ Res* 19: 1-13.
- [Courtney, KD; Copeland, MF; Robbins, A.](#) (1976). The effects of pentachloronitrobenzene, hexachlorobenzene, and related compounds on fetal development. *Toxicol Appl Pharmacol* 35: 239-256.
- [Craan, AG; Haines, DA.](#) (1998). Twenty-Five Years of Surveillance for Contaminants in Human Breast Milk. *Arch Environ Contam Toxicol* 35: 702-710. <http://dx.doi.org/10.1007/s002449900434>
- [Cripps, DJ.](#) (1990). Transplacental and mammary absorption of hexachlorobenzene: experimental pembe yara porphyria in neonates. *Mol Aspects Med* 11: 81-82.
- [Cripps, DJ; Peters, HA; Gocmen, A; Dogramici, I.](#) (1984). Porphyria turcica due to hexachlorobenzene: a 20 to 30 year follow-up study on 204 patients. *Br J Dermatol* 111: 413-422.
- [Dewailly, E; Ayotte, P; Bruneau, S; Laliberte, C; Muir, DC; Norstrom, RJ.](#) (1993). Inuit exposure to organochlorines through the aquatic food chain in arctic quebec. *Environ Health Perspect* 101: 618-620.
- [DNHW](#) (Department of National Health and Welfare (Canada)). (1987). Nutrient value of some common foods. Ottawa, Canada: Department of National Health and Welfare.
- [Ennaceur, S; Gandoura, N; Driss, MR.](#) (2007). Organochlorine pesticide residues in human milk of mothers living in northern Tunisia. *Bull Environ Contam Toxicol* 78: 325-329. <http://dx.doi.org/10.1007/s00128-007-9185-8>
- [Falcó, G; Llobet, JM; Bocio, A; Domingo, JL.](#) (2008). Exposure to hexachlorobenzene through fish and seafood consumption in Catalonia, Spain. *Sci Total Environ* 389: 289-295. <http://dx.doi.org/10.1016/j.scitotenv.2007.09.025>
- [FDA](#) (U.S. Food and Drug Administration). (1989). Food and Drug Administration pesticide program: Residues in foods-1988 [Review]. *J Assoc Offic Anal Chem* 72: 133A-152A.
- [FDA](#) (U.S. Food and Drug Administration). (1990). Food and Drug Administration pesticide program: Residues in foods 1989. *J Assoc Offic Anal Chem* 73: 127A-146A.
- [FDA](#) (U.S. Food and Drug Administration). (1994). Pesticide program: Residue monitoring 1993. *J AOAC Int* 77: 161A-185A.
- [Fisher, JW; Whittaker, TA; Taylor, DH; Clewell, HJ, III; Andersen, ME.](#) (1990). Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol* 102: 497-513.
- [Franchi, E; Focardi, S.](#) (1991). Polychlorinated biphenyl congeners, hexachlorobenzene and DDTs in human milk in central Italy. *Sci Total Environ* 102: 223-228.
- [Frank, R; Rasper, J; Smout, MS; Braun, HE.](#) (1988). Organochlorine residues in adipose tissues blood and milk from Ontario, Canada residents 1976-1985. *Can J Public Health* 79: 150-158.
- [Fürst, P; Fürst, C; Wilmers, K.](#) (1994). Human milk as a bioindicator for body burden of PCDDs, PCDFs, organochlorine pesticides, and PCBs. *Environ Health Perspect* 102: 187-193.
- [Geyer, H; Scheunert, I; Korte, F.](#) (1986). Bioconcentration potential of organic environmental chemicals in humans. *Regul Toxicol Pharmacol* 6: 313-347. [http://dx.doi.org/10.1016/0273-2300\(86\)90002-4](http://dx.doi.org/10.1016/0273-2300(86)90002-4)
- [Gocmen, A; Peters, HA; Cripps, DJ; Bryan, GT; Morris, CR.](#) (1989). Hexachlorobenzene episode in Turkey. *Biomed Environ Sci* 2: 36-43.
- [Goldey, ES; Fisher, JW; Taylor, DH.](#) (1990). Maternal transfer of hexachlorobenzene in the rat [Abstract]. *Neurotoxicol Teratol* 12: 562-563. [http://dx.doi.org/10.1016/0892-0362\(90\)90029-C](http://dx.doi.org/10.1016/0892-0362(90)90029-C)
- [Goldey, ES; Taylor, DH.](#) (1992). Developmental neurotoxicity following premating maternal exposure to hexachlorobenzene in rats. *Neurotoxicol Teratol* 14: 15-21.

- [Greve, PA](#). (1986). Environmental and human exposure to hexachlorobenzene in the Netherlands. In Hexachlorobenzene: Proceedings of an international symposium, 24-25 June 1985. Lyon, France: International Agency for Research on Cancer.
- [Greve, PA; Van Zoonen, P](#). (1990). Organochlorine pesticides and PCBs in tissues from Dutch citizens (1968–1986). *Int J Environ Anal Chem* 38: 265-277. <http://dx.doi.org/10.1080/03067319008026932>
- [Gunderson, EL](#). (1988). FDA Total Diet Study, April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals. *J Assoc Offic Anal Chem* 71: 1200-1209.
- [Hansch, C; Leo, A; Hoekman, D](#). (1995a). Exploring QSAR: Hydrophobic, electronic, and steric constants. In ACS Professional Reference Book. Washington, DC: American Chemical Society.
- [Hardell, E; Carlberg, M; Nordstrom, M; Van Bavel, B](#). (2010). Time trends of persistent organic pollutants in Sweden during 1993-2007 and relation to age, gender, body mass index, breast-feeding and parity. *Sci Total Environ* 408: 4412-4419. <http://dx.doi.org/10.1016/j.scitotenv.2010.06.029>
- [Iatropoulos, MJ; Bailey, J; Adams, HP; Coulston, F; Hobson, W](#). (1978). Response of nursing infant rhesus to clophen A-30 or hexachlorobenzene given to their lactating mothers. *Environ Res* 16: 38-47.
- [Ingebrigtsen, K; Nafstad, I](#). (1983). Distribution and elimination of <sup>14</sup>C-hexachlorobenzene after single oral exposure in the male rat. *Acta Pharmacol Toxicol* 52: 254-260. <http://dx.doi.org/10.1111/j.1600-0773.1983.tb01096.x>
- [Ingebrigtsen, K; Skaare, JU; Nafstad, I; Grave, K; Forde, M](#). (1986). Metabolism of hexachlorobenzene (HCB) in the isolated perfused rat liver. *Vascul Pharmacol* 17: 19-24.
- [IPCS](#). (1997). Hexachlorobenzene. In Environmental Health Criteria (pp. 82). Geneva, Switzerland: World Health Organization. <http://www.inchem.org/documents/ehc/ehc/ehc195.htm>
- [Johansen, HR; Becher, G; Polder, A; Skaare, JU](#). (1994). Congener-specific determination of polychlorinated biphenyls and organochlorine pesticides in human milk from Norwegian mothers living in Oslo. *J Toxicol Environ Health A* 42: 157-171. <http://dx.doi.org/10.1080/15287399409531870>
- [Khera, KS](#). (1974). Teratogenicity and dominant lethal studies on hexachlorobenzene in rats. *Food Chem Toxicol* 12: 471-477.
- [Kitchin, KT; Linder, RE; Scotti, TM; Walsh, D; Curley, AO; Svendsgaard, D](#). (1982). Offspring mortality and maternal lung pathology in female rats fed hexachlorobenzene. *Toxicology* 23: 33-39.
- [Knight, CH; Docherty, AH; Peaker, M](#). (1984). Milk yield in rats in relation to activity and size of the mammary secretory cell population. *J Dairy Res* 51: 29-35.
- [Kodric-Smit, M; Smit, Z; Olie, K](#). (1980). Organochlorine contaminants in human milk from Slavonia Province, Yugoslavia, 1978. *Pestic Monit J* 14: 1-2.
- [Koss, G; Koransky, W](#). (1975). Studies on the toxicology of hexachlorobenzene: I pharmacokinetics. *Arch Toxicol* 34: 203-212. <http://dx.doi.org/10.1007/BF00353283>
- [Koss, G; Reuter, A; Koransky, W](#). (1986). Excretion of metabolites of hexachlorobenzene in the rat and in man. In CR Morris; JRP Cabral (Eds.), (pp. 261-266). Lyon, France: International Agency for Research on Cancer.
- [Koss, G; Seubert, S; Seubert, A; Koransky, W; Ippen, H](#). (1978). Studies on the toxicology of hexachlorobenzene: III observations in a long-term experiment. *Arch Toxicol* 40: 285-294. <http://dx.doi.org/10.1007/BF00310334>
- [Kunisue, T; Muraoka, M; Ohtake, M; Sudaryanto, A; Minh, NH; Ueno, D; Higaki, Y; Ochi, M; Tsydenova, O; Kamikawa, S; Tonegi, T; Nakamura, Y; Shimomura, H; Nagayama, J; Tanabe, S](#). (2006). Contamination status of persistent organochlorines in human breast milk from Japan: recent levels and temporal trend. *Chemosphere* 64: 1601-1608. <http://dx.doi.org/10.1016/j.chemosphere.2005.11.010>

- [Kunisue, T; Someya, M; Kayama, F; Jin, Y; Tanabe, S.](#) (2004). Persistent organochlorines in human breast milk collected from primiparae in Dalian and Shenyang, China. *Environ Pollut* 131: 381-392.  
<http://dx.doi.org/10.1016/j.envpol.2004.03.008>
- [Lackmann, GM.](#) (2002). Polychlorinated biphenyls and hexachlorobenzene in full-term neonates. Reference values updated. *Biol Neonate* 81: 82-85.
- [Lackmann, GM; Angerer, J; Salzberger, U; Tollner, U.](#) (1999). Influence of maternal age and duration of pregnancy on serum concentrations of polychlorinated biphenyls and hexachlorobenzene in full-term neonates. *Biol Neonate* 76: 214-219. <http://dx.doi.org/10.1159/000014161>
- [Larsen, BR; Turrio-Baldassarri, L; Nilsson, T; Iacovella, N; Di Domenico, A; Montagna, M; Facchetti, S.](#) (1994). Toxic PCB congeners and organochlorine pesticides in Italian human milk. *Ecotoxicol Environ Saf* 28: 1-13.
- [Lilienthal, H; Benthe, C; Heinzow, B; Winneke, G.](#) (1996). Impairment of Schedule-Controlled Behavior by Preand Postnatal Exposure to Hexachlorobenzene in Rats. *Arch Toxicol* 70: 174-181.
- [Lovelady, CA; Whitehead, RA; Mccrory, MA; Nommsen-Rivers, LA; Mabury, S; Dewey, KG.](#) (1999). Weight change during lactation does not alter the concentrations of chlorinated organic contaminants in breast milk of women with low exposure. *J Hum Lact* 15: 307-315.
- [Lu, Y; Lohitnavy, M; Reddy, MB; Lohitnavy, O; Ashley, A; Yang, RS.](#) (2006). An updated physiologically based pharmacokinetic model for hexachlorobenzene: incorporation of pathophysiological states following partial hepatectomy and hexachlorobenzene treatment. *Toxicol Sci* 91: 29-41.  
<http://dx.doi.org/10.1093/toxsci/kfj133>
- [Mattison, DR; Wohlleb, J; To, T; Lamb, Y; Faitak, S; Brewster, MA; Walls, RC; Selevan, SG.](#) (1992). Pesticide concentrations in Arkansas breast milk. *J Ark Med Soc* 88: 553-557.
- [Mendoza, CE; Collins, B; Shields, JB; Laver, GW.](#) (1977). Hexachlorobenzene residues and effects on esterase activities in pre-weanling rats after a reciprocal transfer between HCB-treated and control dams. *Arch Toxicol* 38: 191-199.
- [Mendoza, CE; Collins, BT; Shields, JB; Laver, GW.](#) (1978). Effects of hexachlorobenzene or hexabromobenzene on body and organ weights of preweanling rats after a reciprocal transfer between the treated and control dams. *J Agric Food Chem* 26: 941-945.
- [Mes, J; Davies, DJ.](#) (1979). Presence of polychlorinated biphenyl and organochlorine pesticide residues and the absence of polychlorinated terphenyls in Canadian human milk samples. *Bull Environ Contam Toxicol* 21: 381-387. <http://dx.doi.org/10.1007/BF01685440>
- [Mes, J; Davies, DJ; Doucet, J; Weber, D; McMullen, E.](#) (1993). Levels of chlorinated hydrocarbon residues in Canadian human breast milk and their relationship to some characteristics of the donors. *Food Addit Contam* 10: 429-441. <http://dx.doi.org/10.1080/02652039309374166>
- [Mes, J; Davies, DJ; Turton, D; Sun, WF.](#) (1986). Levels and trends of chlorinated hydrocarbon contaminants in the breast milk of Canadian women. *Food Addit Contam* 3: 313-322.  
<http://dx.doi.org/10.1080/02652038609373598>
- [Mes, J; Doyle, JA; Adams, BR; Davies, DJ; Turton, D.](#) (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. *Arch Environ Contam Toxicol* 13: 217-223.  
<http://dx.doi.org/10.1007/BF01055879>
- [Monheit, BM; Luke, BG.](#) (1990). Pesticides in breast milk--a public health perspective. *Community Health Stud* 14: 269-273.
- [Mussalo-Rauhamaa, H; Pyysalo, H; Antervo, K.](#) (1988). Relation between the content of organochlorine compounds in Finnish human milk and characteristics of the mothers. *J Toxicol Environ Health* 25: 1-19.  
<http://dx.doi.org/10.1080/15287398809531185>

- Nair, A; Pillai, MK. (1989). Monitoring of hexachlorobenzene residues in Delhi and Faridabad, India. *Bull Environ Contam Toxicol* 42: 682-686. <http://dx.doi.org/10.1007/BF01700388>
- Nakashima, Y; Ohsawa, S; Umegaki, K; Ikegami, S. (1997). Hexachlorobenzene accumulated by dams during pregnancy is transferred to suckling rats during early lactation. *J Nutr* 127: 648-654.
- Nakata, H; Kawazoe, M; Arizono, K; Abe, S; Kitano, T; Shimada, H; Li, W; Ding, X. (2002). Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from china: status of contamination, historical trend, and human dietary exposure. *Arch Environ Contam Toxicol* 43: 473-480. <http://dx.doi.org/10.1007/s00244-002-1254-8>
- Newsome, WH; Davies, D; Doucet, J. (1995). PCB and organochlorine pesticides in Canadian human milk-1992. *Chemosphere* 30: 2143-2153. [http://dx.doi.org/10.1016/0045-6535\(95\)00086-N](http://dx.doi.org/10.1016/0045-6535(95)00086-N)
- Newton, KG; Greene, NC. (1972). Organochlorine pesticide residue levels in human milk--Victoria, Australia--1970. *Pestic Monit J* 6: 4-8.
- Noren, K. (1983). Levels of organochlorine contaminants in human milk in relation to the dietary habits of the mothers. *Acta Paediatr Scand* 72: 811-816. <http://dx.doi.org/10.1111/j.1651-2227.1983.tb09821.x>
- Noren, K. (1993). Contemporary and retrospective investigations of human milk in the trend studies of organochlorine contaminants in Sweden. *Sci Total Environ* 139-140: 347-355.
- Norén, K. (1983). Some aspects of the determination of organochlorine contaminants in human milk. *Arch Environ Contam Toxicol* 12: 277-283. <http://dx.doi.org/10.1007/BF01059403>
- Norén, K. (1988). Changes in the levels of organochlorine pesticides, polychlorinated biphenyls, dibenzo--dioxins and dibenzofurans in human milk from Stockholm, 1972--1985. *Chemosphere* 17: 39-49. [http://dx.doi.org/10.1016/0045-6535\(88\)90042-2](http://dx.doi.org/10.1016/0045-6535(88)90042-2)
- Norén, K; Meironyté, D. (2000). Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere* 40: 1111-1123. [http://dx.doi.org/10.1016/S0045-6535\(99\)00360-4](http://dx.doi.org/10.1016/S0045-6535(99)00360-4)
- Ntow, WJ. (2001). Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. *Arch Environ Contam Toxicol* 40: 557-563.
- Peters, HA; Cripps, DJ; Gocmen, A; Bryan, GT; Erturk, E; Dogramaci, I; Morris, CR. (1982a). An Epidemiologic Study of Populations Previously Exposed to Hexachlorobenzene. In *Proceedings of the second NCI/EPA/NIOSH Collaborative Workshop, Progress on Joint Environmental and Occupational Cancer Studies* (pp. 194-199). (Niosh/00187945). Bethesda, MD: National Cancer Institute.
- Peters, HA; Gocmen, A; Cripps, DJ; Bryan, GT; Dogramaci, I. (1982b). Epidemiology of hexachlorobenzene-induced porphyria in Turkey: clinical and laboratory follow-up after 25 years. *Arch Neurol* 39: 744-749.
- Pohl, HR; Tylanda, CA. (2000). Breast-feeding exposure of infants to selected pesticides: a public health viewpoint [Review]. *Toxicol Ind Health* 16: 65-77. <http://dx.doi.org/10.1177/074823370001600203>
- Poulin, P; Krishnan, K. (1996). A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. *Toxicol Appl Pharmacol* 136: 126-130. <http://dx.doi.org/10.1006/taap.1996.0015>
- Prachar, V; Veningerova, M; Uhnak, J. (1993). Levels of polychlorinated biphenyls and some other organochlorine compounds in breast milk samples in Bratislava. *Sci Total Environ* 0: 237-242.
- Price, K; Haddad, S; Krishnan, K. (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol Environ Health A* 66: 417-433. <http://dx.doi.org/10.1080/15287390306450>
- Quinsey, PM; Donohue, DC; Ahokas, JT. (1995). Persistence of organochlorines in breast milk of women in Victoria, Australia. *Food Chem Toxicol* 33: 49-56.



- [Renner, G.](#) (1988). Hexachlorobenzene and its metabolism. *Toxicol Environ Chem* 18: 51-78.
- [Ribas-Fitó, N; Grimalt, JO; Marco, E; Sala, M; Mazón, C; Sunyer, J.](#) (2005). Breastfeeding and concentrations of HCB and p,p'-DDE at the age of 1 year. *Environ Res* 98: 8-13.  
<http://dx.doi.org/10.1016/j.envres.2004.05.017>
- [Rush, GF; Smith, JH; Maita, K; Bleavins, M; Aulerich, RJ; Ringer, RK; Hook, JB.](#) (1983). Perinatal hexachlorobenzene toxicity in the mink. *Environ Res* 31: 116-124.
- [Schechter, A; Fürst, P; Krüger, C; Meemken, HA; Groebel, W; Constable, JD.](#) (1989). Levels of polychlorinated dibenzofurans, dibenzodioxins, PCBS, DDT and DDE, hexachlorobenzene, dieldrin, hexachlorocyclohexanes and oxychlordane in human breast milk from the United States, Thailand, Vietnam, and Germany. *Chemosphere* 18: 445-454. [http://dx.doi.org/10.1016/0045-6535\(89\)90154-9](http://dx.doi.org/10.1016/0045-6535(89)90154-9)
- [Schlummer, M; Moser, GA; Mclachlan, MS.](#) (1998). Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. *Toxicol Appl Pharmacol* 152: 128-137.  
<http://dx.doi.org/10.1006/taap.1998.8487>
- [Schoula, R; Hajslova, J; Bencko, V; Poustka, J; Holadova, K; Vizek, V.](#) (1996). Occurrence of persistent organochlorine contaminants in human milk collected in several regions of Czech Republic. *Chemosphere* 33: 1485-1494. [http://dx.doi.org/10.1016/0045-6535\(96\)00287-1](http://dx.doi.org/10.1016/0045-6535(96)00287-1)
- [Skaare, JU; Polder, A.](#) (1990). Polychlorinated biphenyls and organochlorine pesticides in milk of Norwegian women during lactation. *Arch Environ Contam Toxicol* 19: 640-645. <http://dx.doi.org/10.1007/BF01183978>
- [Stacey, CI; Perriman, WS; Whitney, S.](#) (1985). Organochlorine pesticide residue levels in human milk: Western Australia, 1979-1980. *Arch Environ Health* 40: 102-108.
- [Stevens, MF; Ebell, GF; Psaila-Savona, P.](#) (1993). Organochlorine pesticides in Western Australian nursing mothers. *Med J Aust* 158: 238-241.
- [Stuetz, W; Prapamontol, T; Erhardt, JG; Classen, HG.](#) (2001). Organochlorine pesticide residues in human milk of a Hmong hill tribe living in Northern Thailand. *Sci Total Environ* 273: 53-60.
- [Takei, GH; Kauahikaua, SM; Leong, GH.](#) (1983). Analysis of human milk samples collected in Hawaii for residues of organochlorine pesticides and polychlorobiphenyls. *Bull Environ Contam Toxicol* 30: 606-613.  
<http://dx.doi.org/10.1007/BF01610182>
- [Taylor, DH; Goldey, E.](#) (1992). Assessment of the behavioral and neurotoxic effects of hexachlorobenzene (HCB) in the developing rat. (AAMRL-TR-90-076). Oxford, OH: Miami University, Department of Zoology.
- [Taylor, DH; Goldey, ES; Fisher, JW.](#) (1991). Lactational transfer of hexachlorobenzene. *Toxicologist* 11: 236.
- [Timson, BF; Coffman, JL.](#) (1984). Body composition by hydrostatic weighing at total lung capacity and residual volume. *Med Sci Sports Exerc* 16: 411-414.
- [Travis, CC; Arms, AD.](#) (1988). Bioconcentration of organics in beef, milk, and vegetation. *Environ Sci Technol* 22: 271-274. <http://dx.doi.org/10.1021/es00168a005>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1988). Recommendations for and documentation of biological values for use in risk assessment [EPA Report]. (EPA/600/6-87/008). Cincinnati, OH.  
<http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=34855>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1998). Methodology for assessing health risks associated with multiple pathways of exposure to combustor emissions [EPA Report]. (EPA 600/R-98/137). Washington, DC. <http://cfpub.epa.gov/ncea/CFM/recorddisplay.cfm?deid=55525>
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2011a). Exposure factors handbook 2011 edition (final) [EPA Report]. (EPA/600/R-09/052F). <http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=236252>

- [U.S. EPA](#). (2012b). EPA's reanalysis of key issues related to dioxin toxicity and response to NAS comments (CAS No. 1746-01-6) [EPA Report]. (EPA/600/R-10/038F). Washington, DC.
- [Vaz, R; Slorach, SA; Hofvander, Y](#). (1993). Organochlorine contaminants in Swedish human milk: Studies conducted at the national food administration 1981-1990. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 10: 407-418. <http://dx.doi.org/10.1080/02652039309374164>
- [Verner, MA; Ayotte, P; Muckle, G; Charbonneau, M; Haddad, S](#). (2009). A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. Environ Health Perspect 117: 481-487.
- [Verner, MA; Charbonneau, M; López-Carrillo, L; Haddad, S](#). (2008). Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: A new tool in breast cancer epidemiologic studies. Environ Health Perspect 116: 886-892. <http://dx.doi.org/10.1289/ehp.10917>
- [Waliszewski, SM; Pardio Sedas, VT; Chantiri-P, JN; Infanzon-R, RM; Rivera, J](#). (1996). Organochlorine pesticide residues in human breast milk from tropical areas in Mexico. Bull Environ Contam Toxicol 57: 22-28. <http://dx.doi.org/10.1007/s001289900150>
- [Weisenberg, E; Arad, I; Grauer, F; Sahm, Z](#). (1985). Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch Environ Contam Toxicol 14: 517-522. <http://dx.doi.org/10.1007/BF01055379>
- [Welch, RM; Findlay, JW](#). (1981). Excretion of drugs in human breast milk [Review]. Drug Metab Rev 12: 261-277. <http://dx.doi.org/10.3109/03602538108994032>
- [Wickstrom, K; Pyysalo, H; Siimes, MA](#). (1983). Levels of chlordane, hexachlorobenzene, PCB and DDT compounds in Finnish human milk in 1982. Bull Environ Contam Toxicol 31: 251-256. <http://dx.doi.org/10.1007/BF01608694>
- [Wolfe, GW; Pepperl, SG](#). (2005). Hexachlorobenzene: Reproductive Assessment by Continuous Breeding When Administered to Sprague-Dawley Rats by Oral Gavage, Volumes 1 and 2. (TherImmune No. 7244-206). Gaithersburg, MD: TherImmune Research Corporation.
- [WPPR](#). (1992). Report of the Working Party on Pesticide Residues, 1988-1990: The thirty-fourth report of the Steering Group on Chemical Aspects of Food Surveillance. In Food Surveillance Paper No 34. London, UK: Her Majesty's Stationery Office.
- [Yang, RSH; Coulston, F; Golberg, L](#). (1975). Binding of hexachlorobenzene to erythrocytes: Species variation. Life Sci 17: 545-549. [http://dx.doi.org/10.1016/0024-3205\(75\)90088-0](http://dx.doi.org/10.1016/0024-3205(75)90088-0)
- [Yang, RSH; Pittman, KA; Rourke, DR; Stein, VB](#). (1978). Pharmacokinetics and metabolism of hexachlorobenzene in the rat and the rhesus monkey. J Agric Food Chem 26: 1076-1083. <http://dx.doi.org/10.1021/jf60219a008>
- [Yesair, DW; Feder, PI; Chin, AE; Naber, SJ; Kuiper-Goodman, T; Siegel Scott, C; Robinson, PE](#). (1986). Development, evaluation and use of a pharmacokinetic model for hexachlorobenzene. In CR Morris; JRP Cabral (Eds.), IARC scientific publications no 77 (pp. 297-318). Lyon, France: International Agency for Research on Cancer.
- [Yess, NJ](#). (1991). FDA monitoring program. Residues in foods--1990. J Assoc Offic Anal Chem 74: 121A-141A.
- [Yess, NJ](#). (1992). US Food and Drug Administration pesticide program residues in foods 1991. J AOAC Int 75: 135A-157A.

## B.4. Mirex Briefing Packet



# Improving the Risk Assessment of Persistent, Bioaccumulative, and Toxic Chemicals in Breast Milk

## Workshop Briefing Packet: Mirex

### Prepared for

National Center for Environmental Assessment,  
U.S. EPA  
109 T.W. Alexander Drive  
Durham, NC 27711

### Prepared by

ICF International  
2222 NC 54 Highway  
Suite 480  
Durham, NC 27713

### Notice

Although the research described in this briefing packet has been supported by the U. S. Environmental Protection Agency through EP-C-09-009 to ICF International, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.



## Contents B.4

<b>Appendix B. Original Briefing Packets, as Distributed to Workshop Attendees .....</b>	<b>B-1</b>
B.4. Mirex Briefing Packet .....	B-206
B.4.1. Introduction .....	B-210
B.4.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans .....	B-210
B.4.2.1. Mothers' Exposure to Mirex .....	B-210
B.4.2.2. Is Mirex Sequestered in Breast Milk or Other Human Tissues? .....	B-212
B.4.2.3. How Much Mirex Passes from Mother to Infant via Breast Milk? .....	B-220
B.4.3. Maternal Dose vs. Nursing Offspring Dose in Animals .....	B-221
B.4.3.1. Maternal Exposure and Elimination in Milk .....	B-221
B.4.4. Developmental Effects of Mirex .....	B-222
B.4.4.1. Human Studies .....	B-222
B.4.4.2. Animal Studies .....	B-222
B.4.4.3. Developmental Effects Tables .....	B-224
B.4.4.4. Exposure-Response Arrays .....	B-234
B.4.5. Human Equivalent Dose (HED) Estimation .....	B-238
B.4.5.1. Available Pharmacokinetic Data for Mirex .....	B-240
B.4.5.2. Appropriate Dose Metric for Selected Point of Departure (POD) .....	B-243
B.4.5.3. Available Models for Estimating Mirex Internal Dose .....	B-244
B.4.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation .....	B-261
B.4.7. References .....	B-273

## List of Figures

Figure B-21. Compiled Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to Mirex .....	B-234
Figure B-22. Exposure-Response Array Showing Developmental Effects in Animals after Gestational Exposure to Mirex .....	B-235
Figure B-23. Exposure-Response Array Showing Developmental Effects in Animals after Combined Gestational and Lactational Exposure to Mirex .....	B-236
Figure B-24. Exposure-Response Array Showing Developmental Effects in Animals after Lactational Exposure to Mirex .....	B-237
Figure B-25. Process Diagram for Performing Cross-Species Extrapolation .....	B-239
Figure B-26. Maternal and Infant POP Model (Verner et al., 2009) .....	B-246
Figure B-27. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day .....	B-255
Figure B-28. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study (Gaines and Kimbrough, 1970) and an Administered Dose of 0.4 mg/kg-day (POD) .....	B-258

## List of Tables

Table B-44. Summary of Breast Milk Concentration Studies .....	B-214
Table B-45. Mirex in Maternal Blood in the Circumpolar North ( $\mu\text{g/kg}$ lipid) .....	B-215
Table B-46. Daily Intake of n-3 and n-6 Fatty Acids (FAs) and Mirex Compared by Two-tailed, Independent Samples T-test .....	B-216

Table B-47. Plasma n-3 Fatty Acids (% of total lipids) and Plasma Levels of Mirex ( $\mu\text{g/kg}$ plasma lipid) in Women from Greenland <sup>a</sup> .....	B-217
Table B-48. Mirex in Blood Plasma of Female Fish Eaters and Non-Fish Eaters <sup>a</sup> .....	B-218
Table B-49. Concentration of Mirex in Serum and Milk (1991-1993), from Greizerstein et. al. (1999) .....	B-219
Table B-50. Concentration of Mirex in Adipose Tissue and Blood Serum <sup>a</sup> .....	B-220
Table B-51. Developmental Effects from Gestational and/or Lactational Exposure to Mirex <sup>a</sup> .....	B-224
Table B-52. Available Pharmacokinetic Parameters for Mirex in Humans and Rats .....	B-241
Table B-53. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat and Monkey .....	B-248
Table B-54. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model .....	B-254
Table B-55. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model .....	B-255
Table B-56. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat .....	B-257
Table B-57. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model .....	B-258
Table B-58. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age .....	B-261
Table B-59. Estimated Infant-to-maternal Dose Ratio Based on Biotransfer Method .....	B-261
Table B-60. Potential Dose Metrics and Methods for HED Estimation .....	B-262
Table Att. B.4-1. Concentration of Mirex in Human Milk (whole milk) .....	B-265
Table Att. B.4-2. Concentration of Mirex in Human Milk (milk fat) .....	B-269

---

### B.4.1. Introduction

Mirex is a synthetic organochlorine insecticide that exists as an odorless, white crystalline solid. In the 1960s and 1970s mirex was used as a pesticide for fire ants, and from 1959 to 1972 it was used as a flame retardant additive called Dechlorane® in plastics, rubber, paint, paper, and electrical goods. Mirex is no longer produced or used in the United States, as all uses of the compound were cancelled in 1978 ([ATSDR, 1995](#)).

In this briefing packet, the available literature on gestational and lactational exposure to mirex was reviewed for key findings and data to help estimate the maternal dose and breastfeeding infant dose of mirex in humans. Selected references are summarized and evaluated in this briefing packet in five sections: maternal dose and breastfeeding infant dose of mirex in humans; maternal dose and nursing offspring dose in animals; developmental effects of mirex in humans and animals; human and animal kinetic models of exposure; and a final section, which discusses the application of kinetic models to derive an example human equivalent dose (HED).

These briefing packets are intended to stimulate ideas and provide material for discussion regarding innovative approaches to the assessment of risk to breastfeeding infants from mirex and other PBT chemicals in breast milk. The information in this and other briefing packets is provided to help workshop participants discuss and formulate answers to the workshop charge questions. Areas where there exists uncertainty, data gaps, or where there is a lack of understanding in the current literature are highlighted to foster further discussion.

---

### B.4.2. Maternal Dose vs. Breastfeeding Infant Dose in Humans

This section discusses how women of child-bearing age may be exposed to mirex; how their bodies sequester mirex in various tissues, and the extent and manner that mirex is eliminated through breast milk, leading to infant exposure. Mirex exposure levels vary widely and are dependent on social factors such as dietary choices and environmental factors such as the amount of residual mirex in the surrounding geographic location. Concentrations of mirex have been reported in various human tissues, including breast milk, blood plasma, and adipose tissue. Attempts have been made to correlate measured concentrations of mirex in the diet or environmental media with measured body burdens in mothers; and to correlate the body burden in mothers with intake by breastfeeding infants. However, the relationship between maternal exposure, intake, body burden, and infant dose in humans is not currently well understood.

---

#### B.4.2.1. Mothers' Exposure to Mirex

All uses of mirex in the United States were banned in 1978. Thus, the potential for direct exposure to the general population is limited and continues to decrease ([ATSDR, 1995](#)). Though dietary intakes for the

general public are thought to be low, some U.S. populations may still be exposed to mirex through residual contamination of food and soil. As discussed in ATSDR (1995), ingestion of mirex residues in the diet is the major route of exposure for the general population, but total exposures are reportedly low (ATSDR, 1995). Current FDA dietary intake estimates are not available due to the fact that mirex was detected in less than 2% of food samples evaluated in the Total Diet Study in 2004 and 2005. Mirex was not detected in food samples in the Total Diet Study in 2006 (FDA, 2011). Mirex has been detected in food samples in other recent studies from various regions around the globe (Deutch et al., 2006). Drinking water does not appear to be a significant source of human exposure since mirex is relatively insoluble in water and adsorbs rapidly to sediment. Both inhalation of mirex and exposure through drinking water are considered to be minor exposure pathways compared to dietary exposure (ATSDR, 1995).

Exposure to mirex via the diet is possible in areas where contamination of waterways from industrial discharge has occurred. Fish and shellfish from Lake Ontario, the St. Lawrence River, Spring Creek in Pennsylvania, and other areas in the Northeastern U.S. and parts of Canada have been found to contain measurable levels of mirex attributed to contamination from industrial discharge (Fitzgerald et al., 2001; Kosatsky et al., 1999; Kostyniak et al., 1999; Madden and Makarewicz, 1996; ATSDR, 1995; Newsome et al., 1995; Schantz et al., 1994; Oliver et al., 1993; Bush et al., 1983). For example, Madden and Makarewicz (1996) cited multiple studies that measured mirex concentrations in several species of Lake Ontario fish. Mirex was detected in smallmouth bass at 0.11 mg/kg, yellow perch at 0.06 mg/kg (photomirex also detected at 0.03 mg/kg), pumpkinseed sunfish at 0.02 mg/kg, brown bullhead at 0.04 mg/kg, brown trout at 0.30 mg/kg (photomirex also detected at 0.17 mg/kg). The authors provide two measurements for concentrations of mirex in salmon: 0.19 mg/kg in 1986 (photomirex also detected at 0.08 mg/kg), and at 0.24 mg/kg in 1992 (photomirex also detected at 0.10 mg/kg).

In a study conducted in 1992 by Kearney et al. (1999), mirex levels were reported in blood plasma of 232 Ontario sports fishing license holders (146 male, 86 female), and self-reported fish and waterfowl intakes were assessed as predictors of personal contaminant levels. The authors found that average mirex levels were twice as high in females who consumed fish than females who did not consume fish. Regression analysis illustrated that consumption of fish as well as consumption of waterfowl from the Great Lakes area resulted in higher mirex blood plasma concentrations. Fish consumption was not the sole source of mirex exposure, however, as 71% of females that did not consume any fish still had mirex concentrations above the minimum detection level (Kearney et al., 1999).

Aside from the Great Lakes region of the United States, limited information is available on human exposure to mirex in the United States. People living in areas of the southern United States (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Texas) where mirex was extensively used for the control of fire ants may be exposed to mirex by touching contaminated soil, eating with unwashed hands, or consuming locally grown food, since mirex binds to soil particles, and has an estimated soil half-life of 10 years (ATSDR, 1995). There are no quantitative data on exposure published for individuals in this region.



Studies that directly measure the dietary intake of mirex in humans are very limited, but many studies illustrate that the pathway is likely. Researchers conducted a dietary study in Uummannaq, West Greenland that compared levels of mirex in 177 traditional meals, analyzed in 1976, to levels in 90 traditional meals analyzed in 2004. The daily intake of mirex, based on contaminant levels in the traditional meals, significantly increased from 0.002 µg/kg of body weight per day in 1976 to 0.0044 µg/kg body weight per day in 2004 ([Deutch et al., 2006](#)). Study authors used the same sample preparation procedures and analytical methods in 1976 and 2004. Meal and blood samples from 1976 and 2004 were analyzed for lipid content and fatty acid composition using the same gas chromatography methods. The results of these studies are further discussed in [Section B.4.2.2](#). Study authors found that intake of local foods such as seal and polar bear (which contain high amounts of blubber), but not fish, were significantly correlated with mirex contaminant concentrations ([Deutch et al., 2006](#)). In another study in the same region, authors showed that mirex concentration in blood plasma was positively associated with self-reported monthly intake of traditional Inuit meals (such as seal, whale, and fish) ([Deutch et al., 2007](#)).

---

#### **B.4.2.2. Is Mirex Sequestered in Breast Milk or Other Human Tissues?**

##### ***Mirex in Human Breast Milk***

Nineteen studies were identified that measured mirex concentrations in human milk. All reviewed studies were performed between 1977 and 2009, though most studies were performed before 2000. We have not analyzed the relationship between the observed levels from the older studies as compared to more current reports of mirex in breast milk. The timeframe of the studies reviewed is consistent with that of the available data on exposure presented in the previous section. In this way, these studies serve the purpose of illustrating the relationship between maternal dose and infant dose. The data compiled from these studies are summarized in [Table B-44](#). More specific results from each study are summarized in [Attachment B.4-1](#); each study is represented in a row in [Table Att. B.4-1](#) (whole milk) and/or [Table Att. B.4-2](#) (milk fat), depending on how the study reported mirex levels in the milk. Milk levels were reported across studies in a variety of units such as ppm, ppb, mg/kg, or ng/g in whole milk or milk fat (lipids), but were standardized to units of ppb for the tables in this document.

The values for mirex concentrations in human breast milk presented in the tables in [Attachment B.4-1](#) have a wide range, depicting highly varied concentrations from one study to another. One potential reason for the apparent variability could be that the reported breast milk concentrations represent averages across study populations ranging in size from 4 individuals ([Bush et al., 1983](#)) to 1,436 ([Savage et al., 1982](#); [Savage et al., 1981](#)). Additionally, studies were conducted in a variety of international locations including the United States, Canada, Finland, Kazakhstan, Brazil, Japan, and China. The studies also vary by the size of the geographical areas from which study participants were solicited. Some studies represented a subpopulation chosen based on country-wide population distribution data ([Mes et al., 1993](#)), whereas others included participants from a small, specific geographic area ([Fitzgerald et al., 2001](#)) or city ([Bush et al., 1985](#); [Bush et al., 1983](#)). Additionally, many study authors noted that the contaminant of concern was not detected in 100% of samples and provided the percent occurrence.

However, other studies simply provided a mean or median along with a range that included a lower concentration of “not detected” or “detected at trace levels”. It is unclear how the occurrence of mirex in varying percentages of each population affects the ability to compare average concentrations measured among studies. While variability among studies from different population groups is expected due to variations in dietary choices and the historic patterns of mirex use in different locations, the wide ranges of values seen here highlights the difficulty that exists when attempting to link specific observed breast milk concentrations with specific maternal intake or dose.

Mirex concentrations in whole milk were reported from 34 populations across 13 studies; representing women from four countries. Reported values in individuals ranged from below detection level to 250 ppb ([Takei et al., 1983](#)). The occurrence of mirex in the breast milk of study populations varied, ranging from about 1% to 96%. For 13 populations, the most frequently observed values were below the level of detection; in instances where average/mean values above detection level were reported, the highest mean was 0.25 ppb ([Madden and Makarewicz, 1996](#)) and the majority of values were below 0.15 ppb. All but one study reported median values that were less than or equal to 0.1 ppb.

Many studies reported mirex concentrations in milk fat (i.e., the lipid-adjusted concentration in breast milk). The concentrations in milk fat were generally higher than the concentrations in whole milk, due to the lower volume and the fact that mirex is lipophilic. In addition, the conservative assumption for breast milk lipid content is approximately 4% ([U.S. EPA, 2011a](#)). Measured concentrations of mirex in breast milk fat from individuals ranged from below detection level to 124.5 ppb ([Mes et al., 1993](#)). With the exception of one outlying high value [30 ppb ([Beretta and Dick, 1994](#))], reported average/mean values ranged from 0.74 ppb ([Nakai et al., 2009](#)) to 6.8 ppb ([Madden and Makarewicz, 1996](#)).

**Table B-44. Summary of Breast Milk Concentration Studies**

Location	Number of studies	Number of distinct populations	Date Range	Population size range	Lowest reported average	Highest reported average	Frequency of reported values
<b>Measured in whole milk</b>							
All locations (Canada, Finland, Kazakhstan, United States)	13	34	1977 – 1999 <sup>a</sup>	4 – 1436	Below Detection Level	0.25 ppb	21 / 28 values ≤ 0.07 ppb
United States (Including NY)	7	20	1977 – 1999 <sup>a</sup>	4 – 1436	Below Detection Level	0.25 ppb	14 / 17 values ≤ 0.14 ppb
United States (Excluding NY)	3	8	1979 – 1983	54 – 1436	Below Detection Level	Below Detection Level	All 8 values below detection level
New York State, various regions	4	12	1977 – 1999 <sup>a</sup>	4 – 213	0.04 ppb	0.25 ppb	6 / 9 values ≤ 0.14 ppb
Canada, various regions	3	11	1978 <sup>a</sup> – 1992	14 – 497	Below Detection Level	0.14 ppb	9 / 10 values ≤ 0.07 ppb
Finland	1	1	1984 – 1985	165	Below Detection Level	Below Detection Level	The only reported value was below detection level
Kazakhstan	2	2	1997 <sup>a</sup> – 1998 <sup>a</sup>	76 – 92	NR	NR	Above detection level in ~1%
<b>Measured in milk fat</b>							
All locations (Brazil, Canada, China, Japan, United States)	11	23	1978 <sup>a</sup> – 2009 <sup>a</sup>	6 – 1237	0.74 ppb	30 ppb	17 / 22 values ≤ 3.7 ppm
New York State, various regions <sup>b</sup>	4	16	1986 – 1993 or 1999 <sup>a</sup>	6 – 213	0.8 ppb	6.8 ppb	12 / 16 values ≤ 3.7 ppm
Canada, various regions	4	4	1978 – 1997	12 – 497	1.89 ppb	4.2 ppb	2 / 3 values ≤ 2.3 ppm
Brazil, Porto Alegre	1	1	1987 – 1988	30	30 ppb	30 ppb	--
China, 12 provinces	1	1	2007	1237	1.0 ppb	2.4 ppb	--
Japan	1	1	2009 <sup>a</sup>	466	0.74 ppb	0.74 ppb	--

<sup>a</sup> A study was published in the year listed but did not report when data were collected.

<sup>b</sup> No data were available for other locations in the United States.

Note: See [Attachment B.4-1, Table Att. B.4-1](#), and [Table Att. B.4-2](#) for more detailed information and sources.

### Mirex in Other Tissues

Several studies were identified that measured mirex in the blood of mothers or women of child-bearing age ([Van Oostdam et al., 2004](#); [Greizerstein et al., 1999](#); [Kearney et al., 1999](#); [Bush et al., 1984](#)). In a study conducted by Van Oostdam et al. (2004), venous blood samples were collected during pregnancy or at childbirth from mothers in eight circumpolar countries between 1994 and 1997. [Table B-45](#) presents the results for average mirex concentration levels by country from that study, which ranged from 1.1 µg/kg lipid in Sweden to 8 µg/kg lipid in Greenland. The results illustrate significant differences in blood levels among the study populations. The study authors stated that maternal age and contaminant levels were not correlated for any persistent organic pollutant studied ([Van Oostdam et al., 2004](#)).

**Table B-45. Mirex in Maternal Blood in the Circumpolar North (µg/kg lipid)**

Country (Region)	Sample Size	Mean Maternal Age (years)	Sampling Year	Geometric Mean (95% CI, %D) <sup>a</sup>	Significant Differences <sup>b</sup>
<b>Inuit Populations</b>					
Greenland (Disko Bay)	30	26.6 ± 5.3	1994-1995	8 (5.5-12, 87%)	A
Canada (Nunavik)	30	24 ± 4.2	1995-1996	7.8 (5.7-11, 97%)	A
Canada (Kitikmeot)	63	24.2 ± 5.2	1994-1995	4.7 (3.7-5.8, 86%)	B
United States (Alaska, North Slope)	20	25.6 ± 6.5	1997	1.5 (1.2-1.9, 15%)	CD
<b>Non-Inuit Populations</b>					
Norway (Hammerfest, Kirkenes)	60	28.6 ± 4.9	1995-1996	1.4 (1.3-1.5, 15%)	CD
Sweden (Kiruna, Lapland)	40	28.6 ± 4.9	1995-1996	1.1 (1.0-1.2, 8%)	D
Iceland (Reykjavik)	40	29.6 ± 5.3	1996	1.9 (1.6-2.2, 35%)	C
Russia (Nikel, Kola Peninsula)	51	23.0 ± 3.8	1996	1.4 (1.3-1.5, 6%)	CD
Finland (Lapland)	143	29.5 ± 3.3	1997	NA (NA, 0%)	NA

<sup>a</sup> CI=Confidence interval; D = Detected; NA = Not applicable

<sup>b</sup> Population groups sharing a letter are not significantly different; population groups not sharing a letter are significantly different.

Source: Van Oostdam et al. (2004)

Deutch et al. ([Deutch et al., 2007](#); [Deutch et al., 2006](#)) conducted studies throughout the six districts of Greenland from 1976 to 2007. Deutch et al. (2006) focused on an Uummannaq municipality, where fishing, whaling, and seal-hunting remained prominent food sources until after 1976. Concentrations of organic pollutants were measured in blood plasma and meal composition in 1976 and 2004. The authors analyzed 177 duplicate meals (solids only) and blood plasma for 16 middle-aged married Inuit couples and one single woman in 1976 and 90 duplicate meals and blood plasma for 15 married couples in 2004.

Food samples from 1976 were freeze-dried after analysis and used for mirex determinations. Blood samples taken in 1976 were no longer available, thus contaminant levels could not be measured for this group. Food samples and blood samples were also taken in 2004. In 1976, close to 60% of the solid weight and 40% of the energy in meals came from local sources, whereas only 25% of the weight and around 20% of the energy came from local sources in 2004. The consumption of seal meat decreased from about 400 g/day in 1976 to 60 g/day in 2004, an 80% decrease. Local fish and bird intake also decreased significantly during this time period.

Fatty acid (FA) profiles also demonstrated these dietary changes. From 1976 to 2004, daily consumption of n-3 FAs, which were strongly correlated with the consumption of traditional local foods ( $p < 0.0001$ ), decreased from 8.5 to 3.3 g and consumption of linoleic acid (an n-6 FA) accounted for a greater percentage of FAs consumed and in plasma phospholipids. The n-3/n-6 FA ratio in food decreased from 3.3 in 1976 to 0.87 in 2004, and this ratio in plasma decreased from 1.7 to 0.60 (see [Table B-46](#)). Deutch et al. (2006) calculated ratios for daily intake of contaminants compared to daily intake of n-3 FAs in order to adjust for marine food content. The ratio of daily intake of mirex in micrograms and daily intake of n-3 FAs in grams significantly increased from 0.022 in 1976 to 0.082 in 2004 ( $p < 0.001$ ) (see [Table B-46](#)), indicating that mirex content was significantly higher in local foods in 2004. Study authors found that intake of local foods such as seal and polar bear (which contain high amounts of blubber), but not fish, were significantly correlated with mirex contaminant concentrations (Deutch et al., 2006).

**Table B-46. Daily Intake of n-3 and n-6 Fatty Acids (FAs) and Mirex Compared by Two-tailed, Independent Samples T-test**

Daily Intake or Ratio	1976	2004
Sum of n-3 FAs (g/day) <sup>a</sup>	8.5 (0.88)	3.8 (0.62)*
Mirex (µg/day) <sup>a</sup>	0.184 (0.02)	0.31 (0.03)
Mirex (µg/kg bodyweight-day) <sup>a</sup>	0.002 (0.0003)	0.0044 (0.0004)
Ratio of Mirex (µg/day)/n-3 FAs (g/day) <sup>b</sup>	0.022	0.082*

<sup>a</sup> Values presented as mean (standard error)

<sup>b</sup> Values presented as ratios

\*  $p < 0.001$  between years, with two-tailed, independent t-test

Source: Deutch et al. (2006)

In the 2007 study, Deutch et al. (2007) surveyed Inuit citizens from all six districts of Greenland as part of the Arctic Monitoring and Assessment Programme (AMAP). Average blood plasma concentration of mirex in women (approximately half of the 510 study participants) across the districts was  $31.65 \pm 40.54$  (Deutch et al., 2007). Concentrations of contaminants in blood correlated with marine food intake (indicated by n-3/n-6 FA ratios). Increased marine food intake and significantly increased mirex concentrations were found in Uummannaq ( $p < 0.001$ ) and Qaanaaq ( $p < 0.01$ ) compared to districts on the east coast. In Uummannaq, the mean plasma level for mirex was  $106.9 \mu\text{g/kg}$  plasma lipid for men (data for women were not provided) and in Qaanaaq, mean mirex levels were  $51.69 \mu\text{g/kg}$  plasma lipid

for men and 24.46 µg/kg plasma lipid for women (see [Table B-47](#)). The authors showed that mirex concentration in blood plasma was positively associated with self-reported monthly intake of traditional Inuit meals (such as seal, whale, and fish) ([Deutch et al., 2007](#)).

**Table B-47. Plasma n-3 Fatty Acids (% of total lipids) and Plasma Levels of Mirex (µg/kg plasma lipid) in Women from Greenland<sup>a</sup>**

District	Plasma sum n-3 Fatty Acids	Mirex
Ittoqqortoormiit	11.02 (3.40)	70.82 (53.3)
Tassilaq	11.20 (3.88)	44.84 (42.03)
Nuuk <sup>b</sup>	8.14 (2.95)	8.21 (10.27)
Sisimiut	8.13 (2.51)	7.76 (6.5)
Uummannaq	Not reported	Not reported
Qaanaaq	9.69 (3.88)	24.46 (21.33)
Total	9.69 (3.61)	31.65 (40.54)

<sup>a</sup> Values presented as mean (standard deviation)

<sup>b</sup> The men from Nuuk were on the average 20 years older than the women from Nuuk and the men from the other districts.

Source: Deutch et al. ([2007](#))

Kearney et al. ([1999](#)) further investigated the correlation between fish consumption and mirex concentrations. The study measured mirex in blood plasma samples from sport fishing license holders in Cornwall and Mississauga, Ontario, Canada. These areas are home to active fishing communities with fish consumption advisories due to levels of PCBs, mercury, or mirex in fish. Fish consumption was also recorded for the study population, and results were determined separately for men and women as well as fish consumers and non-fish consumers (See [Table B-48](#)). The study results suggest that a correlation exists between fish consumption and blood plasma mirex levels; however, mirex was detected in both fish consumers and non-consumers and the difference in mirex concentration between the groups was not statistically significant. Average blood plasma concentrations in women, grouped based on community and fish consumption, ranged from 0.03 to 0.07 µg/L wet weight; mirex was detected in 86% of fish consuming women and 71% of non-fish consuming women from the two communities.

**Table B-48. Mirex in Blood Plasma of Female Fish Eaters and Non-Fish Eaters<sup>a</sup>**

Population	Mirex <sup>a</sup>	Fish meal size (g)	Total Fish Consumed in 12 Months (kg)
Cornwall fish eaters	0.07 (<0.02-0.51), 89%	130.9	5.0
Cornwall non-fish eaters	0.03 (<0.02-0.14), 67%	NA	NA
Mississauga fish eaters	0.04 (<0.02-0.24), 80%	373.2	3.2
Mississauga non-fish eaters	0.04 (0.02-0.49), 76%	NA	NA
Both communities fish eaters	0.06 (<0.02-0.51), 86%	ND	ND
Both communities non-fish eaters	0.03 (<0.02-0.49), 71%	NA	NA

<sup>a</sup> Values reported as median µg/L, (range), % samples greater than detection limit.

Note: NA = Not available, ND = No data.

Source: Kearney et al. (1999).

Two additional studies were identified that provided information on maternal dose; however, both of these articles, with the exception of the abstract, were written in Japanese. In the first study, (Nakai et al., 2009), mirex levels were measured in maternal blood, cord blood, and breast milk in Japanese mothers. The abstract states that mirex was detected in all samples despite the fact that it has never been used in Japan (Nakai et al., 2009). Additional translated information is not available for this study.

One study was identified that compared maternal blood levels of mirex to that of breast milk concentrations (Greizerstein et al., 1999). In the study, one blood sample and one milk sample were obtained from each of 7 lactating women participating in the New York State Angler study from 1991 to 1993. Samples were collected at various times; blood serum samples were collected at times ranging from 5 months pregnant to 2 months post-partum, and milk samples were collected from 1 month to 11 months post-partum. Serum and milk samples from each participant were not collected on the same day. Milk samples were taken at times ranging from 33 days before, to 318 days after blood sampling occurred. Study authors hypothesized that at steady state, the concentration of mirex in blood and milk samples collected at similar gestation times would be similar when normalized for lipid content. However, results showed considerable differences among lipid adjusted concentrations of mirex in serum and milk samples from each individual (see Table B-49). For all individuals, the lipid-normalized concentration of mirex in blood serum was higher than that observed in milk. Mirex concentrations in blood serum ranged from 2.6 ng/g lipid to 28.8 ng/g lipid whereas concentrations in breast milk ranged from 1.0 ng/g lipid to 7.8 ng/g lipid.

**Table B-49. Concentration of Mirex in Serum and Milk (1991-1993), from Greizerstein et. al. (1999)**

Subject Number	Mirex in Serum (ng/g lipid)	Serum Sampling Time (months post-partum)	Mirex in Milk (ng/g lipid)	Milk Sampling Time (months post-partum)
1	2.6	2	1.0	1
2	18.6	2	10.2	2
3	21.3	2	1.4	2
4	28.8	7	5.5	11
5	3.1	6	2.4	2
6	8.5	5	1.9	2
7	27.9	7	7.8, 4.9 <sup>a</sup>	1, 8

<sup>a</sup> Subject number 7 supplied two milk samples, taken on different days.

Source: Greizerstein et al. (1999).

One toxicokinetic study reported levels of mirex in the serum and adipose tissue of residents living near a dump site in Memphis and South Memphis, Tennessee (Burse et al., 1989). Partway through analyzing a cohort of 297 adipose and 370 serum samples for presence of PCBs, the study authors noticed mirex contamination in an adipose tissue sample. The authors decided to further analyze 19 adipose tissue samples that appeared to be contaminated with mirex, and also analyzed the blood serum samples from matching participants. They found that the 19 adipose tissue samples (which included samples from 5 males and 14 females, 18-75 years old) contained concentrations of mirex ranging from 0.030 ppm to 4.68 ppm. Mirex was detected above the minimum detection level of 0.5 ppb in 13 of the matching participants' blood serum samples; concentrations ranged from 1.56 ppb to 16.8 ppb in those 13 samples. Mirex concentration levels in adipose tissue were, on average, 364 times higher than those in serum (see Table B-50)(Burse et al., 1989).



**Table B-50. Concentration of Mirex in Adipose Tissue and Blood Serum<sup>a</sup>**

Age (years)	Mirex in Adipose (ppm)	Mirex in Blood (ppb)	Concentration Ratio for Adipose to Serum <sup>b</sup>
18	0.086	<0.33	ND <sup>c</sup>
20	0.16	<0.33	ND <sup>c</sup>
21	0.064	<0.28	ND <sup>c</sup>
26	0.201	<0.66	ND <sup>c</sup>
29	0.431	1.56	276
29	0.03	<0.33	ND <sup>c</sup>
33	4.68	16.8	279
39	1.44	5.87	245
40	0.828	2.75	301
41	0.866	1.56	555
45	0.408	2.62	156
47	0.598	1.9	315
58	0.107	5.65	19
75	1.11	2.04	544

<sup>a</sup> Male data not reported.

<sup>b</sup> Authors noted that the mean concentration ratio for mirex in adipose tissue (lipid basis) to mirex in serum (whole weight basis) was 364 with a SE of 57.

<sup>c</sup> Authors did not determine a ratio because blood serum mirex was below the level of detection.

Source: Burse et al. (1989).

#### **B.4.2.3. How Much Mirex Passes from Mother to Infant via Breast Milk?**

Limited human data exist that inform the comparison of maternal dose and breastfeeding infant dose of mirex in humans. Based on the results of the Greizerstein et al. (1999) study, the times at which serum and milk samples are taken with respect to each other and age post-partum may play an important role in this comparison. Although the Greizerstein et al. (1999) study generally showed higher concentrations of mirex in maternal blood than in breast milk for the study participants, the sampling time (during pregnancy vs. various durations post-partum) may affect the relative measurements of mirex concentrations in the blood compared to breast milk. A human toxicokinetic study indicated that mirex partitions more into adipose tissue than serum lipids (Burse et al., 1989) (See Table B-50). Additionally, animal data indicate that elimination through milk is a major route of excretion of persistent lipophilic chemicals (Gallenberg and Vodcink, 1987).

---

### B.4.3. Maternal Dose vs. Nursing Offspring Dose in Animals

---

#### B.4.3.1. Maternal Exposure and Elimination in Milk

A number of studies have examined the fate of mirex following oral administration in animals and have found that mirex remains unmetabolized following exposure and is preferentially sequestered in fat; females generally accumulate greater amounts than males ([Chambers et al., 1982](#); [Wiener et al., 1976](#); [Gibson et al., 1972](#); [Mehendale et al., 1972](#)). In maternal animals, secretion of mirex in milk has been shown as a major route of excretion ([Kavlock et al., 1980](#); [Smrek et al., 1977](#); [Dorough and Ivie, 1974](#)).

##### *Cows and Goats*

Radio-labeled mirex was excreted in the milk of a single lactating cow with 13% cumulative excretion of the administered dose in the milk after 28 days of exposure via the diet ([Dorough and Ivie, 1974](#)). After 1 week of daily exposure to 0.011 mg/kg of mirex in the feed (corresponding to approximately 0.2 ppm mirex in the feed), a state of equilibration was reached; the concentration of mirex excreted in whole milk was 0.058 ppm. One week after exposure had ended, the concentration in milk declined to 0.006 ppm and further declined to 0.002 ppm by 28 days after the final exposure.

Contrarily, in a study of 3 lactating cows exposed to either 0, 0.01, or 1.00 ppm mirex in the diet daily for 31 weeks, excretion of mirex in the milk was limited ([Bond et al., 1975](#)). The cow exposed to the low dietary dosage of 0.01 ppm mirex showed a maximum concentration of mirex in milk of 0.02 ppm. The maximum concentration measured in the milk of the high dosage cow was 0.08 ppm. An increase in concentration over the 31 weeks, indicative of accumulation in milk, was not observed at the low dose. While the concentration in milk of the high dose cow did increase over time, the authors noted that “excessive residues” did not accumulate ([Bond et al., 1975](#)). In both of these studies ([Bond et al., 1975](#)), lactation was monitored as the exposure occurred. This exposure scenario would likely prevent accumulation in the tissues due to continuous lactation, which would reduce the body burden. This exposure and elimination scenario can be contrasted with human exposures to mirex and other bioaccumulative compounds. For humans, the exposure and accumulation may occur for many years before elimination from the breast milk happens, as there is no outlet for breast milk until mothers begin breastfeeding.

Smrek and colleagues ([Smrek et al., 1978](#); [Liddle et al., 1977](#)) administered 1 mg/kg mirex daily to groups of 5 female goats for 18 or 61 weeks (one or two gestation and lactation periods) followed by 10 mg/kg-day for an additional 4 weeks. The authors reported that mirex levels in milk were highest immediately post-partum, then decreased over the duration of lactation. The concentration of mirex in milk fat decreased by more than 50% in the 8 weeks following birth of kids ([Smrek et al., 1977](#)).

##### *Rats*

Kavlock et al. ([1980](#)) dosed CD rat dams via gavage with 1 or 10 mg/kg-day mirex on lactation days (LDs) 2-5, and pups were sacrificed at intervals up to 12 days after dosing (i.e., by LD 17) to determine the

occurrence of elimination through lactation. Milk samples from the dams were also collected on LDs 5, 9, and 15. The study authors reported that they detected mirex in maternal milk at levels directly proportional to the administered dose and that mirex levels in the milk declined rapidly after cessation of dosing. For dams in the 10 mg/kg-day dose group, concentrations of mirex in milk were approximately 100 ppm on LD 5, 25 ppm on LD 9, and 15 ppm on LD 15. The concentrations in pups' stomach milk was approximately 125 ppm at LD 3, 100 ppm on LD 5, 50 ppm on LD 7, and under 30 ppm for LDs 9, 11, and 15. For dams in the 1 mg/kg-day dose group, both maternal milk concentrations and pups' stomach milk concentrations were less than 15 ppm throughout the study ([Kavlock et al., 1980](#)).

---

#### **B.4.4. Developmental Effects of Mirex**

---

##### **B.4.4.1. Human Studies**

No studies were available that discussed the developmental effects of mirex in humans.

---

##### **B.4.4.2. Animal Studies**

The animal toxicology literature on controlled exposures to mirex was reviewed for studies presenting data specifically related to exposure occurring during gestation and/or lactation. To provide a comprehensive understanding of the hazard resulting from these specific types of exposures, all relevant studies that could be retrieved were reviewed and are summarized in [Table B-51](#). The endpoints examined and effects observed are noted, as are potential points of departure (PODs) for each study. For endpoints in the low-dose range, benchmark dose modeling using U.S. EPA's Benchmark Dose Software (BMDS) was considered. However, appropriate litter-specific data were not available for critical effects in the low-dose range, making BMD modeling inappropriate for this application. PODs are arrayed in [Figure B-21](#), [Figure B-22](#), [Figure B-23](#), and [Figure B-24](#) to facilitate comparisons across studies. [Table B-51](#) serves as a reference key for [Figure B-21](#), [Figure B-22](#), [Figure B-23](#), and [Figure B-24](#).

Studies were classified according to the timing of exposure for the pups. Gestational exposure studies were those in which maternal animals were exposed during gestation and (1) pups were sacrificed prenatally or at parturition, or (2) pups were nursed by unexposed dams. Combined lactational and gestational exposure studies were those in which maternal animals were exposed during gestation or during gestation and lactation and pups were nursed prior to sacrifice. Finally, lactational exposure studies were those in which dams were not dosed until lactation began or pups unexposed during gestation were cross-fostered to exposed mothers.

Of note are significant effects in rats and mice that include: decreased litter weight, heart arrhythmias, edema, decreased pup viability, and ocular effects including cataracts, cell degeneration, and microscopic lens changes. Cardiac arrhythmias were observed in Long-Evans rats following gestational and lactational exposure (GD 15.5 - 21.5) to  $\geq 0.1$  mg/kg-day mirex ([Grabowski, 1983](#)). However, no

studies investigating cardiac arrhythmias from lactation-only exposure were found. Ocular abnormalities were the most consistent effects reported and were observed following gestational and/or lactational exposure. Importantly, studies investigating ocular effects following lactation-only exposure demonstrated effects similar to the effects reported following combined gestational and lactational exposure. In a cross-fostering experiment, Gaines and Kimbrough ([1970](#)) reported adverse effects from lactation-only exposure, including significant increases in pup mortality and incidence of cataracts, at levels that did not reach significance in the group exposed during gestation only. The results of this experiment indicate that lactation may be the more sensitive exposure pathway for mirex.

Gaines and Kimbrough ([1970](#)) provide data indicating the presence of cataracts and increased pup mortality following exposure during lactation from maternal animals exposed to mirex at 0.40 mg/kg-day in the diet on postnatal days 1-21. Because this study only included a single mirex dose group, BMD modeling was precluded. Therefore, the LOAEL of 0.40 mg/kg-day is used as an example POD to assess the impact that accumulated exposure to a mother may have on lactational exposure and postnatal development of her offspring. The POD is used in [Section B.1.6](#), along with results from PBPK models, to estimate a HED. The choice of the POD and HED presented in this packet are intended only as examples to stimulate discussion for the workshop participants.

#### B.4.4.3. Developmental Effects Tables

**Table B-51. Developmental Effects from Gestational and/or Lactational Exposure to Mirex<sup>a</sup>**

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<b>Gestational Exposure</b>						
Rat, Sprague-Dawley, n=21–24 dams per dose, gavage, GD 5–14 or GD 6–15, sacrificed GD 20	0, 10	<p><b>Measured:</b> maternal survival, body weight, and uterine weight; implantation sites; viable fetuses; resorptions; fetal deaths and edema</p> <p><b>Observed GD 5–14:</b> increased maternal mortality, decreased weight gain, and decreased gravid uterine weight; gravid uterine weights &lt; those in animals dosed GD 6–15; <b>increased prenatal deaths</b>, % mortality &gt; those animals dosed GD 6–15; increased fetal edema, reduced fetal weight (dry weight), and late resorptions</p> <p><b>Observed GD 6–15:</b> increased maternal mortality, decreased maternal weight gain and gravid uterine weight; <b>increased prenatal deaths</b>; increased fetal edema, reduced fetal weight (dry weight), and late resorptions</p>	<p>Maternal: NA</p> <p>Develop GD 5–14: NA</p> <p>Develop GD 6–15: NA</p>	<p>Maternal: 10 (FEL)</p> <p>Develop GD 5–14: 10</p> <p>Develop GD 6–15: 10</p>	(Byrd et al., 1981) (Authors noted that previous studies indicated edema was associated with skeletal and visceral abnormalities in fetuses exposed to mirex.)	<p>Maternal: [Am]</p> <p>Develop GD 5–14: [A1]</p> <p>Develop GD 6–15: [A2]</p>

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, CD, n=10–37 dams per dose, gavage, GD 7-16, sacrificed GD 21	0, 5, 7, 9.5, 19, 38	<p><b>Measured:</b> maternal weight gain, food consumption and water intake; fetal ECG; resorptions; live and dead fetuses; fetal weight; visceral and skeletal malformations</p> <p><b>Observed:</b> increased maternal mortality, decreased maternal weight gain at <math>\geq 9.5</math> mg/kg-day, <b>increased maternal relative liver weight at <math>\geq 7</math> mg/kg-day</b>, increased fetal mortality and decreased fetal weight at <math>\geq 19</math> mg/kg-day, <b>increased number of sternal ossification centers and edematous live fetuses at <math>\geq 7</math> mg/kg-day</b></p>	Maternal: 5  Develop: 5	Maternal: 7  Develop: 7	( <a href="#">Chernoff et al., 1979a</a> )	Maternal: [Bm]  Develop: [B1]
Rat, Long-Evans, n= 3-11 dams per dose, gavage, GD 6.5 or 8.5-15.5, sacrificed GD 18.5	0, 5, 6, 7, 10	<p><b>Measured:</b> uterine weight; maternal weight gain; fetal resorptions, live fetuses, fetal weight and ECG</p> <p><b>Observed:</b> <b>increased maternal mortality at <math>\geq 7</math> mg/kg-day</b>, increased fetal resorptions at <math>\geq 7</math> mg/kg-day, possible increase in microphthalmos, tachycardia, <b>heart block at <math>\geq 5</math> mg/kg-day</b>, edema and swelling, increased PR intervals, cardiac arrhythmias</p>	Maternal: 6  Develop: NA	Maternal: 7 (FEL)  Develop: 5	( <a href="#">Grabowski and Payne, 1980</a> )	Maternal: [Cm]  Develop: [C1]
Rat, Long-Evans, n unclear, gavage, GD 8.5–15.5, sacrificed GD 18.5 or 20.5	0, 6	<p><b>Measured:</b> fetal mortality, fetal hematology for edema evaluation</p> <p><b>Observed:</b> decreased protein concentration, decreased colloid osmotic pressure, <b>edema and swelling</b></p>	Maternal: ND  Develop: NA	Maternal: ND  Develop: 6	( <a href="#">Grabowski, 1981</a> ) (Limited maternal effects were reported.)	Develop: [E]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Long-Evans, n=78 control and 136 fetuses examined per dose, n of treated dams unclear, gavage, GD 8.5–15.5, sacrificed 1 day prior to parturition	0, 6	<b>Measured:</b> fetal edema and ECG  <b>Observed:</b> heart block, edema, decreased and feeble heart rate	Maternal: ND  Develop: NA	Maternal: ND  Develop: 6	( <a href="#">Grabowski and Payne, 1983a</a> )  (Limited maternal effects were reported.)	Develop: [F1]
Rat, Sherman, n=9–14 dams per dose, diet, 49 days prior to mating through parturition  Pups cross fostered at birth and nursed with control dam not exposed to mirex to control for lactational exposure. Authors reported a range of exposures (0.31–0.49); the average of the range (0.4) is reported here.	0, 0.40 <sup>b</sup>	<b>Measured:</b> pup survival, % offspring with cataracts  <b>Observed:</b> no significant effects observed; non – significant increase in cataracts (1.6%), non-significant decrease in survival	Maternal: ND  Develop: 0.40	Maternal: ND  Develop: NA	( <a href="#">Gaines and Kimbrough, 1970</a> )  (An increase in cataracts was seen in pups exposed during lactation but not gestation.)	Develop: [G1]
Rat, CD, n=17–21 litters per dose, diet, GD 4 through parturition, pups examined on PND 2, 8, 10, 18, 24, 30, 34, sacrifice schedule not reported.  Pups were cross-fostered at birth and nursed with control dam not exposed to mirex to control for lactational exposure	0, 2.5 <sup>b</sup>	<b>Measured:</b> litter weight, litter viability and ophthalmological examination  <b>Observed:</b> decreased litter weight on PND 8, 24, and 30; decreased litter viability beginning at PND 8	Maternal: ND  Develop: 0	Maternal: ND  Develop: 2.5	( <a href="#">Chernoff et al., 1979b</a> )	Develop: [H1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, long-evans, n=5–10 litters per dose, gavage, GD 8-15, dams sacrificed on GD 18–21	0, 6	<p><b>Measured:</b> maternal mortality, fetal viability and ophthalmological examination,</p> <p><b>Observed:</b> decreased fetal viability on GD 20 (4%) and 21 (14%), cataracts in 49.6% of fetuses (126/254) on GD 20, decreased lens and eye weight GD 18–21, cataracts associated with ruptured lens fibers</p>	Maternal: ND  Develop: NA	Maternal: ND  Develop: 6	(Rogers and Grabowski, 1983)	Develop: [I1]
Rat, Wistar, n=20 females per dose, GD 6–15, sacrificed GD 22	0, 1.5, 3.0, 6.0, 12.5	<p><b>Measured:</b> maternal weight, organ weights, visceral and uterine pathological changes, fetal weight, viability, visceral and skeletal malformations</p> <p><b>Observed:</b> reduced incidence of pregnancy and increased maternal mortality at ≥3.0 mg/kg-day, increased visceral malformations in offspring and decreased pup viability at ≥6.0 mg/kg-day, decreased fetal weight at 12.5 mg/kg-day,</p>	Maternal: 1.5  Develop: 3.0	Maternal: 3.0 (FEL)  Develop: 6.0	(Khera et al., 1976)	Maternal: [Jm]  Develop: [J1]
<b>Gestational and Lactational Exposure</b>						
Rat, Long-Evans, n unclear, gavage, GD 8.5–15.5 and GD 15.5–21.5, sacrificed “newborn” and PND 5, respectively.	GD 8.5–15.5: 0, 1.5, 3, 6	<p><b>Measured:</b> fetal mortality and ECG (PND 1, 2, 5), pup mortality</p> <p><b>Observed GD 8.5–15.5:</b> increased perinatal mortality at ≥3 mg/kg-day; tachycardia, edema, and cardiac arrhythmias at 1.5 mg/kg-day</p>	Maternal: ND  Develop GD 8.5–15.5: NA	Maternal: ND  Develop GD 8.5–15.5: 1.5	(Grabowski, 1983)	Develop GD 8.5–15.5: [D1]
Treatment groups appeared to be treated sequentially. No raw data were presented. Due to postnatal sacrifice date, lactational exposure cannot be ruled out; study is included with gestational and lactational exposure studies.	GD 15.5–21.5: 0, 0.1, 0.25, 0.5, 1	<p><b>Observed GD 15.5–21.5:</b> increased perinatal mortality at ≥0.5 mg/kg-day, cardiac arrhythmias at ≥0.1 mg/kg-day</p> <p>Authors note that arrhythmias tend to occur several days after birth.</p>	Develop GD 15.5–21.5: NA	Develop GD 15.5–21.5: 0.1		Develop GD 15.5–21.5: [D2]



Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Sprague-Dawley, n=15–20 adults (M and F) per dose, diet, 13 weeks before mating through PND 21, sacrifice on PND 21	0, 0.5, 1.0, 2.0, 4.0 <sup>b</sup>	<p><b>Measured:</b> clinical signs of toxicity, maternal mortality, weight gain, hematological parameters, organ weights, and histopathological examination; pup survival and histopathological examination; litter body weight</p> <p><b>Observed:</b> maternal hypoactivity, irritability, and muscle tremor at the highest dose; decreased maternal weight gain and increased maternal relative liver weight at 4 mg/kg-day; <b>maternal liver and thyroid lesions at ≥0.5 mg/kg-day with dose-dependent increase in severity</b>; decreased litter size at ≥0.5 mg/kg-day with no dose-response trend; decreased pup survival at 2 mg/kg-day; <b>increased severity of cataracts at ≥0.5 mg/kg-day (pup-specific data)</b></p>	Maternal: NA  Develop: NA	Maternal: 0.5  Develop: 0.5	( <a href="#">Chu et al., 1981</a> )	Maternal: [Lm]  Develop: [L1]
Rat, Long-Evans, n = 8–17, gavage, GD 8–15, sacrificed PND 5  Limited maternal effects were reported. Due to day of sacrifice, lactational exposure cannot be ruled out, therefore this study is included with gestational and lactational exposure studies	0, 6	<p><b>Measured:</b> fetal mortality, ECG at birth</p> <p><b>Observed:</b> <b>perinatal mortality</b>, heart block, edema, respiratory problems, increased PND 5 weight, decreased heart rate, postnatal hydrocephaly</p>	Maternal: ND  Develop: NA	Maternal: ND  Develop: 6	( <a href="#">Grabowski and Payne, 1983b</a> )	Develop: [M1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<p>Rat, Sherman, n=10 adults (M and F) per dose, diet, 45 days or 102 days prior to mating through lactation.</p> <p>Authors reported a range of exposures (1.8–2.8); the average of the range (2.3) is reported here.</p>	0, 2.3	<p><b>Measured:</b> pup survival, % offspring with cataracts</p> <p><b>Observed 45 days:</b> decreased litter size at birth and weaning, increased offspring with cataracts (33.3%)</p> <p><b>Observed 102 days:</b> decreased litter size at weaning, increased offspring with cataracts (46.2%)</p>	<p>Maternal: ND</p> <p>Develop 45 days: NA</p> <p>Develop 102 days: NA</p>	<p>Maternal: ND</p> <p>Develop 45 days: 2.3</p> <p>Develop 102 days: 2.3</p>	<p>(<a href="#">Gaines and Kimbrough, 1970</a>)</p>	<p>Develop 45 days: [G2]</p> <p>Develop 102 days: [G3]</p>
<p>Rat, CD, n=17–21 litters, diet, pups examined on PND 2, 8, 10, 18, 24, 30, 34, sacrifice methods not reported.</p> <p>Authors noted that exposure was during gestation, lactation, and postlactation through PND 34. Authors did not specify on which gestational days exposure occurred.</p>	0, 2.5 <sup>b</sup>	<p><b>Measured:</b> litter weight and viability, fetal ophthalmological examination</p> <p><b>Observed:</b> decreased litter weight on PND 10 returned to normal by PND 18; <b>decreased litter viability beginning at PND 8; increased incidence of cataracts</b>, outlined lenses, and total lens changes</p>	<p>Develop: 0</p>	<p>Develop: 2.5</p>	<p>(<a href="#">Chernoff et al., 1979b</a>)</p>	<p>Develop: [H2]</p>

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
<p>Mouse, CD-1, n=24-25 dams per group, gavage, GD 8–12, sacrificed PND 3</p> <p>Due to postnatal sacrifice, lactational exposure cannot be ruled out, therefore this study is included with gestational and lactational exposure studies.</p>	0, 7.5	<p><b>Measured:</b> maternal mortality, conception, maternal weight gain, pup weight, litter size and viability</p> <p><b>Observed:</b> decreased litter survival and weight</p>	Develop: NA	Develop: 7.5	<a href="#">(Chernoff and Kavlock, 1982)</a>	Develop: [K1]
<b>Lactational Exposure</b>						
Rat, Long-Evans, n=8–20 litters per dose group, gavage, PND 1	0, 5, 10, 15	<p><b>Measured:</b> fetal ophthalmological examination</p> <p><b>Observed:</b> increased cataracts at ≥10 mg/kg-day</p>	Develop: 5	Develop: 10	<a href="#">(Chernoff et al., 1979a)</a>	Develop: [B2]
Rat, Sherman, 10–19 litters per dose group, gavage, PND 1–4	0, 1, 2.5, 5.0, 10	<p><b>Measured:</b> fetal ophthalmological examination, pup survival, pup litter body weight</p> <p><b>Observed:</b> decreased pup survival at highest dose (data for lower doses not reported), <b>increased cataracts and total lens changes (cataracts and outlined lenses) at ≥2.5 mg/kg-day</b></p>	Develop: 1	Develop: 2.5	<a href="#">(Chernoff et al., 1979a)</a>	Develop: [B3]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Long-Evans, n unclear, gavage, PND 1–4	0, 1, 2.5, 5.0, 10	<p><b>Measured:</b> fetal ophthalmological examination, pup survival, pup litter body weight</p> <p><b>Observed:</b> decreased pup survival at highest dose beginning on PND 8, <b>decreased pup litter weight beginning on PND 8</b> (not significant at highest dose on PND 29 and 37), <b>increased cataracts, and increased total lens changes (includes cataracts and outlined lenses) in offspring at <math>\geq 2.5</math> mg/kg-day</b></p>	Develop: 1	Develop: 2.5	( <a href="#">Chernoff et al., 1979a</a> )	Develop: [B4]
<p>Rat, Sherman, n=9–14 dams per dose, diet, PND 1–21.</p> <p>Pups were raised by a foster mother exposed to Mirex for 73 days prior to transferral and throughout lactation. Increase in cataracts was seen in pups exposed during lactation but not gestation. Authors reported a range of exposures (0.31–0.49); the average of the range (0.4) is reported here.</p>	0, 0.40 <sup>b</sup>	<p><b>Measured:</b> pup survival, % offspring with cataracts</p> <p><b>Observed:</b> significantly decreased pup survival (53.9%), increased offspring with cataracts (37.5%)</p>	Develop: NA	Develop: 0.40	( <a href="#">Gaines and Kimbrough, 1970</a> )	Develop: [G4]
<p>Rat, Long-Evans, n=10 offspring per group, gavage, PND 1–4</p> <p>Although increases were significant when compared to controls, they did not appear to be dose dependent.</p>	0, 2.5, 10	<p><b>Measured:</b> locomotor activity (measured as maze activity)</p> <p><b>Observed:</b> increased activity (<b>hyperactivity</b>) at PND 41</p>	Develop: NA	Develop: 2.5	( <a href="#">Reiter, 1977</a> )	Develop: [M1]

Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Rat, Long Evans, n=4–12 litters, 10 offspring per group, gavage, PND 1–4, pups sacrificed at PND 6–14	0, 10	<b>Measured:</b> fetal ophthalmological examination (lens cation balance, lens growth, histology)  <b>Observed:</b> cataracts, anterior and posterior cortical vacuoles, swollen lens fibers, ocular cell degeneration	Develop: NA	Develop: 10	( <a href="#">Rogers and Grabowski, 1984</a> )	Develop: [N1]
Rat, Sherman, n=8 control pups, 20–32 pups per examination day (PND 3, 5, 7, 9, 11, 13), gavage, PND 1–5	0, 5	<b>Measured:</b> fetal ophthalmological examination  <b>Observed:</b> increased incidence and severity of microscopic lens changes in a time-dependent fashion (swelling of individual lens fibers PND 7, more pronounced swelling of lens fibers PND 9, cortical degeneration and necrosis PND 11)	Develop: NA	Develop: 5	( <a href="#">Scotti et al., 1981</a> )	Develop: [O1]
Rat, CD, n=17–21 litters, diet, pups examined on PND 2, 8, 10, 18, 24, 30, 34, sacrifice methods not reported  Pups were nursed by exposed foster mothers. The study does not list specific PNDs of exposure, only that exposure occurred “during lactation”.	0, 2.5 <sup>b</sup>	<b>Measured:</b> litter weight and viability, fetal ophthalmological examination  <b>Observed:</b> increased incidence of total lens changes (including cataracts and outlined lenses)	Develop: 0	Develop: 2.5	( <a href="#">Chernoff et al., 1979b</a> )	Develop: [H3]

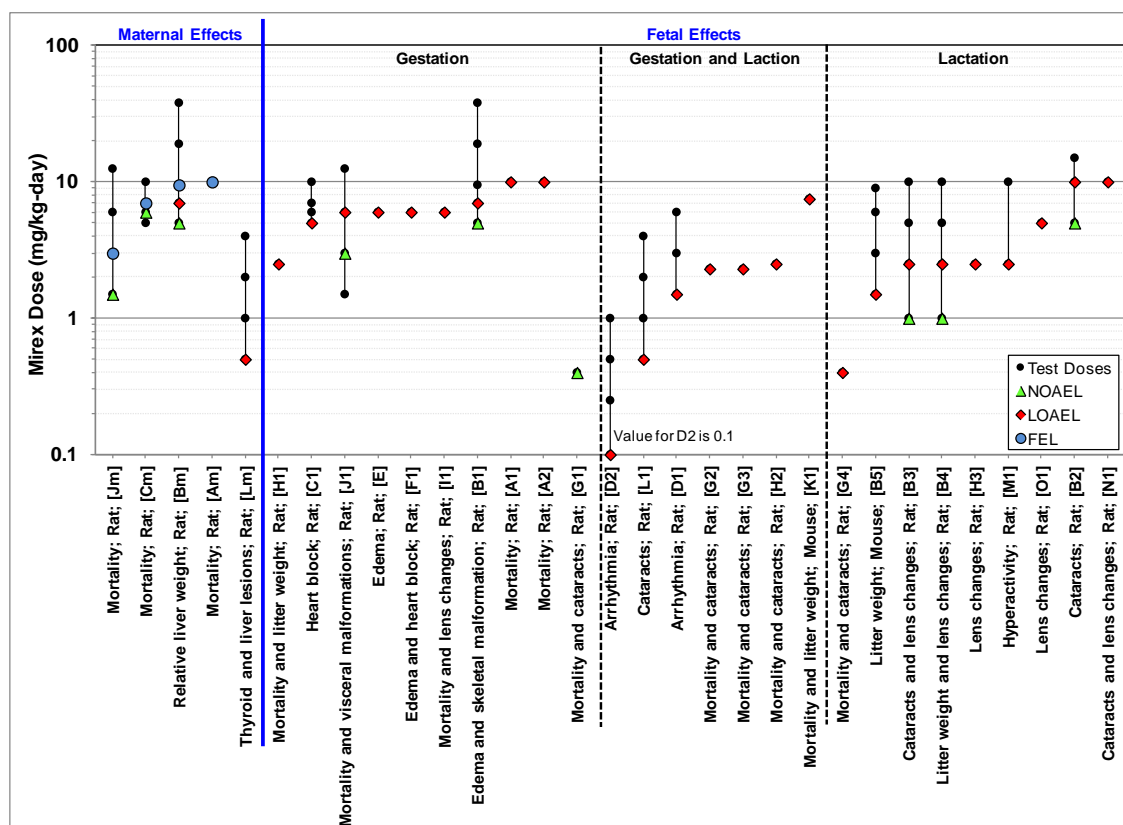
Study Design	Dosimetry (mg/kg-d)	Toxicological Endpoints (Bold text identifies the endpoint on which the LOAEL is based)	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Reference (Comments)	E-R Array Reference Key
Mouse, CD1, n=10–25 maternal animals per dose group, gavage, PND 1–4	0, 1.5, 3.0, 6.0, 9.0	<p><b>Measured:</b> fetal ophthalmological examination, pup survival, pup litter body weight, pup relative liver weight</p> <p><b>Observed:</b> decreased litter weight on PND 8, 15, and 21 at <math>\geq 1.5</math> mg/kg-day; decreased pup litter weight on PND 26 and 37 at <math>\geq 6.0</math> mg/kg-day; increased pup mortality throughout study at <math>\geq 6.0</math> mg/kg-day; dose-dependent increased pup relative liver weight on PND 37 (data not reported); increased cataracts and lens changes at <math>\geq 3.0</math> mg/kg-day</p>	Develop: NA	Develop: 1.5	( <a href="#">Chernoff et al., 1979a</a> )	Develop: [B5]

<sup>a</sup> No data were suitable for BMD modeling.

<sup>b</sup> Doses estimated using Default body weight and food intake values U.S. EPA ([1998](#)).

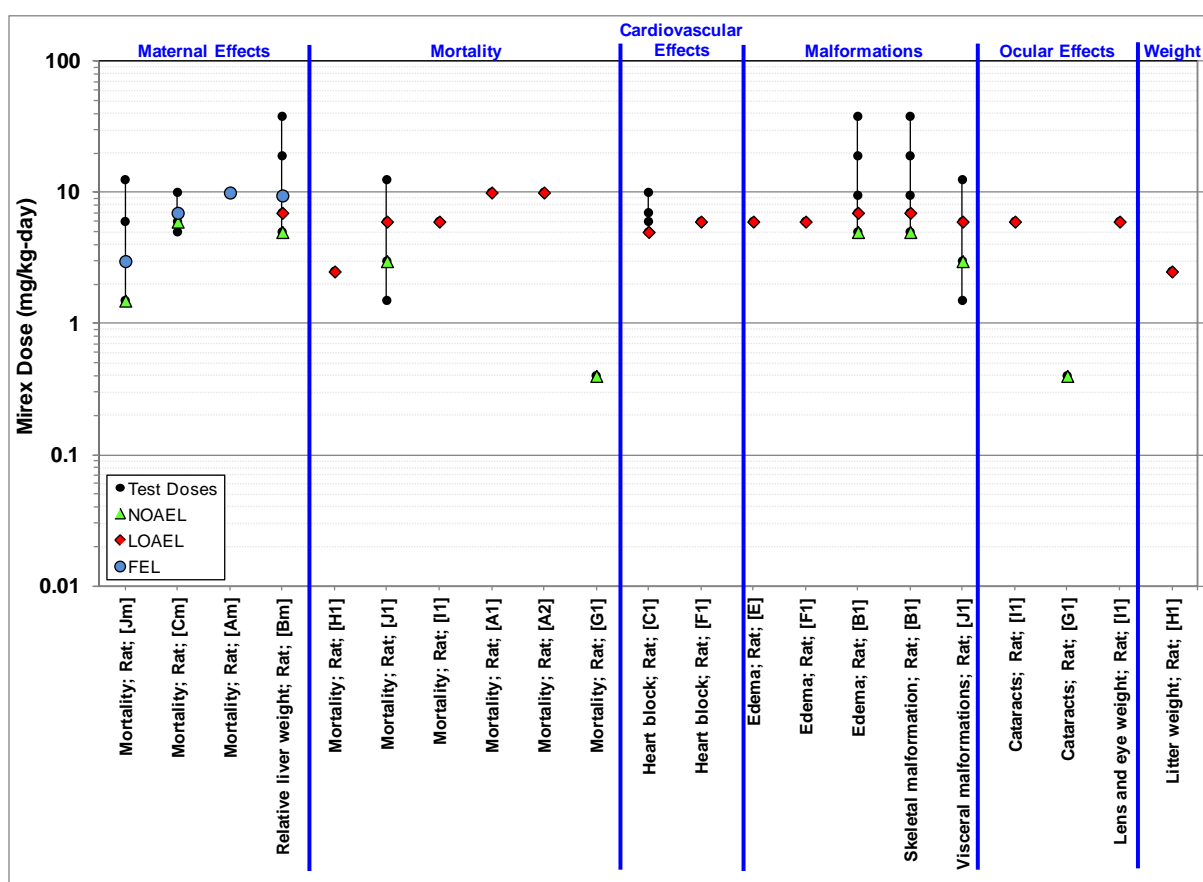
NA = Not applicable; ND = No data.

#### B.4.4.4. Exposure-Response Arrays



Note: Each array element represents the dose-response data for a unique study design. Elements are labeled with all of the adverse effects on which NOAEL and LOAEL decisions were based. The endpoint “Lens changes” includes one or more of the following effects: cataracts, lens and eye weight, ocular cell degeneration, cortical vacuoles, and/or swollen lens fibers. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. [Figure B-22](#), [Figure B-23](#), and [Figure B-24](#) detail the effects observed in the gestational, gestational/lactation, and lactation-only studies, respectively. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-51](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; FEL = frank effect level

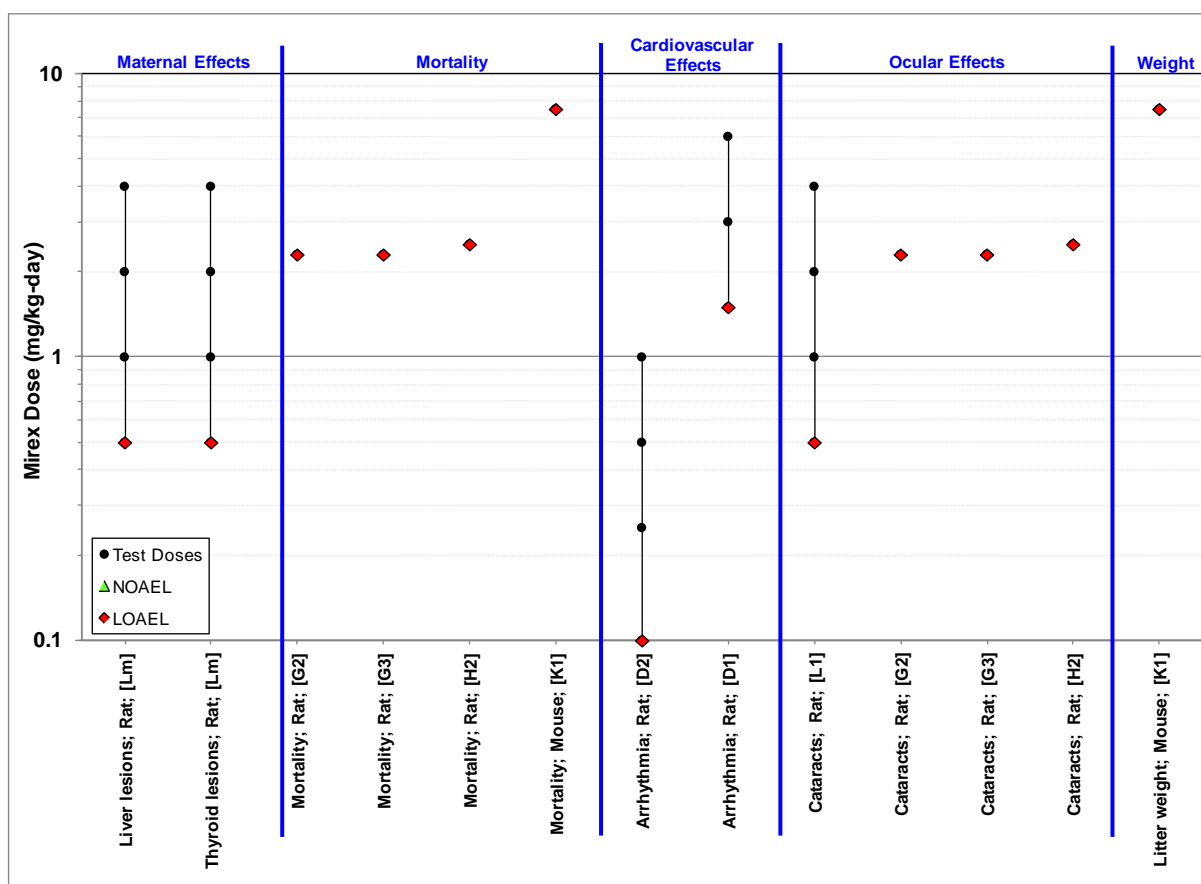
**Figure B-21. Compiled Exposure-Response Array Showing All Developmental Effects in Animals after Exposure to Mirex**



Note: Each array element represents the dose-response data for a unique study design and endpoint. Elements are labeled with the adverse effect on which NOAEL and LOAEL decisions were based. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-51](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; FEL = frank effect level

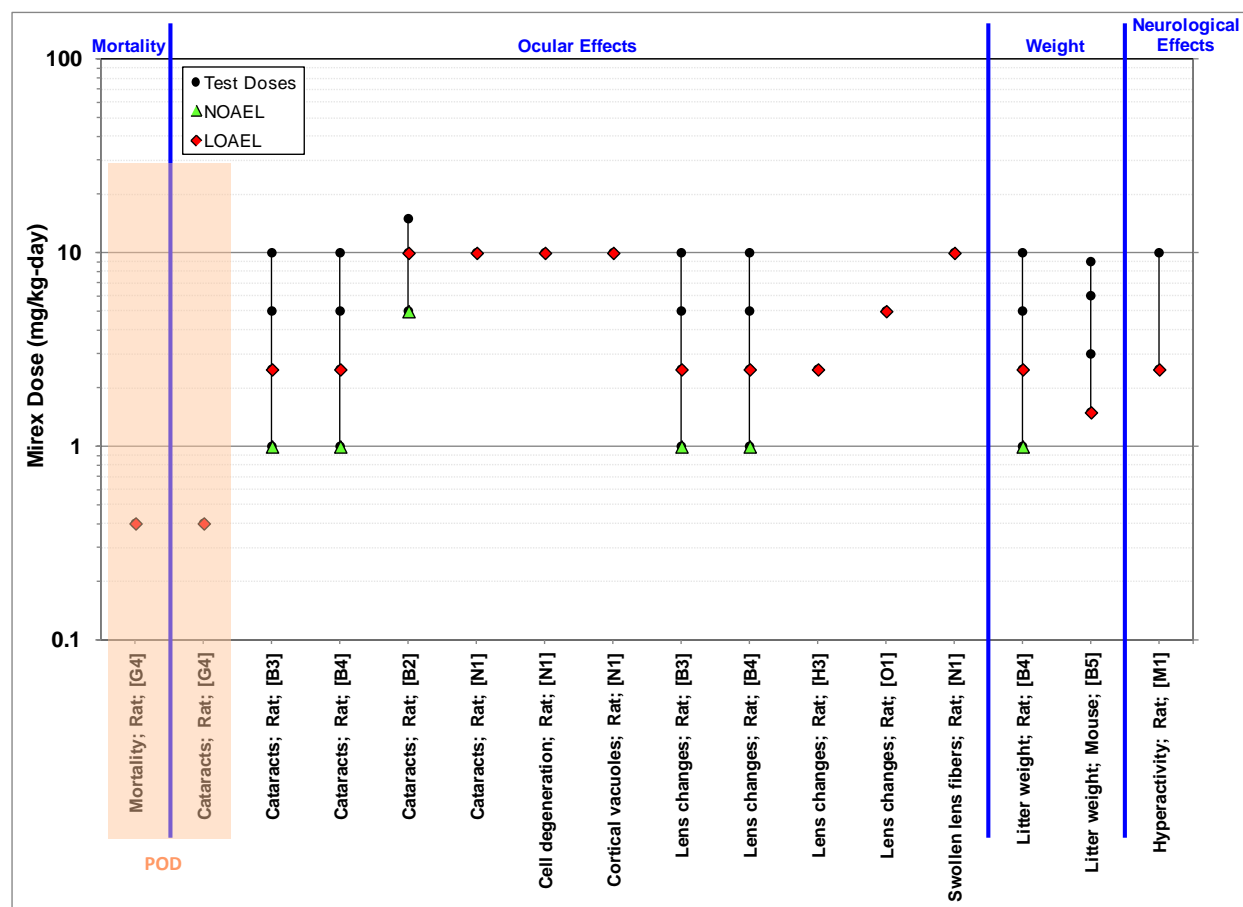
**Figure B-22. Exposure-Response Array Showing Developmental Effects in Animals after Gestational Exposure to Mirex**





Note: Each array element represents the dose-response data for a unique study design and endpoint. Elements are labeled with the adverse effect on which NOAEL and LOAEL decisions were based. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-51](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; FEL = frank effect level

**Figure B-23. Exposure-Response Array Showing Developmental Effects in Animals after Combined Gestational and Lactational Exposure to Mirex**



Note: Each array element represents the dose-response data for a unique study design and endpoint. Elements are labeled with the adverse effect on which NOAEL and LOAEL decisions were based. All endpoints pertaining to survival, viability, and mortality are labeled as “Mortality”. Element labels also include the test species and a Reference Key, which can be used to find detailed study information in [Table B-51](#). NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level; FEL = frank effect level

**Figure B-24. Exposure-Response Array Showing Developmental Effects in Animals after Lactational Exposure to Mirex**

---

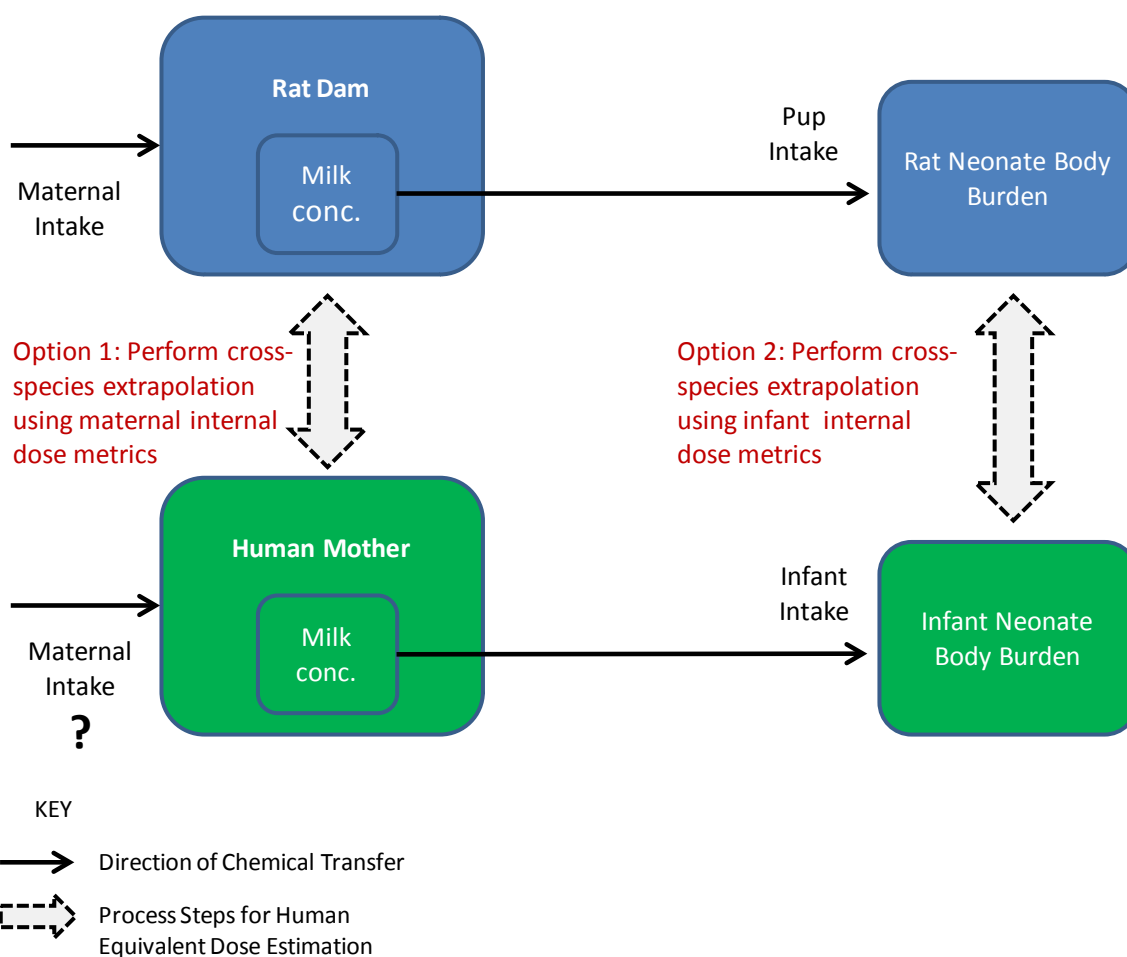
### B.4.5. Human Equivalent Dose (HED) Estimation

Once the POD has been selected, the next step in the reference dose determination is estimation of the HED. This HED is intended to quantify the maternal continuous daily exposure in humans that may result in an adverse developmental effect similar to that observed in animal offspring as described in [Section B.4.4](#).

The information provided in this section is designed to promote discussion amongst the workshop attendees as to how best to perform the HED estimation. In order to provide a concrete example to facilitate discussion, one method is chosen and carried through in this document to demonstrate an example calculation. This example is for illustrative purposes only and may not represent the conclusions of the workshop panel after discussion.

The HED can be estimated in a number of different ways. Generally speaking, an internal dose metric is selected for the human and the animal, and then these two metrics are assumed to be equal in magnitude. The decision must be made as to which internal dose metric is appropriate to serve as an indicator of the associated adverse effect.

For developmental effects resulting from lactational exposure, [Figure B-25](#) provides a diagram of the pathways that may be chosen to perform cross-species extrapolation to estimate the HED. The assumption is made that the maternal or infant animal intake is known, and the HED is the unknown human continuous daily exposure (depicted with a “?” in the figure). Because exposure occurs through the mother and then passes to the offspring by nursing, cross-species extrapolation may be performed by selecting either an internal maternal dose metric or an internal infant dose metric. Performing the extrapolation on internal maternal dose metrics will not account for species differences regarding infant feeding habits and infant kinetics, while performing the extrapolation on the infants requires more input data and more judgment about how to appropriately link very young members of both species. The overall choice will depend on judgment as to the reliability of the models available and the input data driving the models, balanced against the desire for increased biological accuracy.



**Figure B-25. Process Diagram for Performing Cross-Species Extrapolation**

The main questions considered in derivation of an HED from an animal-derived POD are shown in the text box below (“Discussion Points for Mirex HED Estimation”). This section discusses some of the chemical-specific properties that affect (1) the selection of the modeling method, (2) the kinetic information available for mirex, and (3) the types of models available for assessing mirex exposure in animals and humans. Summary statements for each of the discussion points are provided in the appropriate sections.

### Discussion Points for Mirex HED Estimation

- ? What pharmacokinetic data or relevant chemical-specific information are available for mirex in animals and humans to facilitate model implementation?
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ? What PBPK or other simpler biokinetic models are available for mirex?
- ? **What is the best method for estimating the HED using the proposed POD?**

#### B.4.5.1. Available Pharmacokinetic Data for Mirex

Before discussing the potential modeling techniques for mirex, the available database of pharmacokinetic data is summarized. This database should help to determine which modeling techniques may be most appropriate and subject to the least uncertainty.

#### Pharmacokinetic Data

- ? What pharmacokinetic data or relevant chemical-specific information are available for mirex in animals and humans to facilitate model implementation?
- ✓ **Octanol-water partition coefficient,  $K_{ow}$**
- ✓ **Monkey and rat half-lives in fat**
- ✓ **Absorption fractions and fraction of chemical stored in fat (assumptions can be made based on other PBTs)**
- × **No available half-life in humans**
- × **No partition coefficients in humans**

Potential parameters that may be useful in estimating HED by a variety of methods are presented in [Table B-52](#). A discussion of the parameters and their sources follows the table. The various applications and uses of these parameters are discussed in the context of different modeling techniques in [Section B.1.5.4](#).

**Table B-52. Available Pharmacokinetic Parameters for Mirex in Humans and Rats**

Parameter (units)	Variable	Value	Note/source
Chemical-Specific Only			
Octanol-water partition coefficient	log(K <sub>ow</sub> )	6.89	Veith et al. ( <a href="#">1979</a> )
Human Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	1	U.S. EPA ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9	U.S. EPA ( <a href="#">1998</a> ) (default)
Biological elimination constant for mirex (day <sup>-1</sup> )	k <sub>elim</sub>	7.6 × 10 <sup>-5</sup>	Estimated as ln(2) / t <sub>1/2</sub>
Half-life for mirex (days)	t <sub>1/2</sub>	9,125	Pittman et al. ( <a href="#">1976</a> ) (9,125 days = 25 years for estimated half-life in fat of rhesus monkey)
Partition coefficients in different human body compartments for mirex	None identified in the literature		
Animal Chemical Parameters			
Fraction of ingested contaminant that is absorbed by the rat pup (dimensionless)	f <sub>ai</sub>	0.64	Assumed
Fraction of ingested contaminant that is absorbed by the rat dam (dimensionless)	f <sub>am</sub>	0.64	Used midpoint value from ( <a href="#">Mehendale et al., 1972</a> ) and Gibson et al. ( <a href="#">1972</a> )
Fraction of contaminant that is stored in rat dam/maternal fat (dimensionless)	f <sub>f</sub>	0.5	Estimated from Belfiore et al. ( <a href="#">2007</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.0023	Estimated as ln(2) / t <sub>1/2</sub>
Half-life of contaminant in rats (days)	t <sub>1/2</sub>	300	Ivie et al. ( <a href="#">1974</a> )
Partition coefficients in different rat compartments for mirex	Tissue: blood	Fat: 400; Liver:17.5; Rapidly Perfused Tissue: 6.97; Slowly Perfused Tissue: 2.82	Belfiore et al. ( <a href="#">2007</a> )

The log octanol-water partition coefficient (**log K<sub>ow</sub>**) for mirex was calculated to be 6.89 by Veith et al. (1979). Veith and colleagues (1979) calculated the K<sub>ow</sub> using an experimentally-determined correlation equation between bioconcentration factor (BCF) and K<sub>ow</sub>. The BCF was empirically determined using the fathead minnow as the test system and exposing the fish to 1.2 µg/L mirex for 32 days in lake water at 25°C.

In the absence of specific information on mirex in humans, the input values presented here were patterned after those in the EPA's Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions (U.S. EPA, 1998). Chapter 9 of this methodology (hereafter "Combustor Assessment") presents a framework for evaluating exposure to persistent lipophilic contaminants in breast milk. Thus, the assumptions made in that document are applied to mirex when values specific to mirex could not be located. The **maternal absorption fraction** for mirex was not available in the reviewed literature. An absorption fraction value of 1 is used in the current calculations to serve as the most conservative estimate, assuming complete absorption of the chemical (U.S. EPA, 1998). Quantitative information about the **fraction of mirex in the body that is stored in fat** also could not be found in the literature. The reviewed literature generally described mirex as highly lipophilic. The Combustor Assessment states that for highly lipophilic compounds, >90% of the compound may be stored in the fat (U.S. EPA, 1998). In the absence of more specific information, this parameter was set to 0.9 in the example calculations.

No studies in the literature have identified the **half-life** of mirex in human fat or the whole body. However, a kinetic model in the female rhesus monkey written by Pittman et al. (1976) estimated that the biological half-life of mirex in the fat compartment "would probably exceed the lifetime of the individual monkey." Therefore, the half-life of mirex in human fat is estimated to be equivalent to the average lifespan of a rhesus monkey of approximately 25 years (9,125 days) (Bodkin et al., 2003).

Animal parameters were determined from values found in the literature where available. The search focused on rats, since rats are the species studied in the candidate POD study (Gaines and Kimbrough, 1970). Mirex-specific values for the maternal absorption fraction, the fraction of contaminant stored in the fat relative to the total body burden, and the half-life are presented below.

The gastrointestinal absorption of mirex in rats has been determined experimentally in several studies using varying dosing regimens (single dose or diet) and durations. As reported in the U.S. EPA Toxicological Review for mirex (U.S. EPA, 2003b), absorption of mirex was estimated to be 45% (Mehendale et al., 1972), 75.2% (Ivie et al., 1974), 71-79% (Chambers et al., 1982; Chambers and Yarbrough, 1979), and ~83% (Gibson et al., 1972). The midpoint between the lowest (0.45) and highest (0.83) values is used as the estimate of compound absorption in the lactating dam. Therefore, 0.64 is used as the **maternal absorption fraction**. No absorption fractions could be found in the literature for rat pups. Thus, a value equal to the maternal value (0.64) was assumed.

The **fraction of mirex stored in fat** could not be found in the literature. The fraction was estimated using the study in Belfiore et al. (2007). Rats with an average body weight of 359 g were given a single oral

dose of mirex of 44.48 mg/kg. After 14 days, the fat concentration peaked at an average of 142.4 µg/g. Assuming a fat volume fraction of 0.09 [midpoint of the range reported by Fisher et al. (1990)] an absorption fraction of 0.64 (see previous paragraph), and a long half-life leading to limited elimination during the 14 day time period, the fraction of the mirex absorbed dose stored in the fat compartment can be estimated as:

$$f_f = \frac{\text{mass of mirex in fat compartment}}{\text{mass of mirex in oral dose}} = \frac{C_{\text{FAT}} \times \text{BW} \times f_{\text{fm}}}{\text{DI} \times \text{BW} \times f_{\text{am}}}$$

Appendix Equation 32

Where:

$f_f$	=	the fraction of contaminant that is stored in maternal fat (dimensionless),
$C_{\text{FAT}}$	=	the measured concentration of contaminant in the fat (mg contaminant per kg fat),
BW	=	the animal body weight (kg),
$f_{\text{fm}}$	=	fraction of the mother's weight that is fat (kg fat/kg BW).
DI	=	the applied dose (mg contaminant/kg BW), and
$f_{\text{am}}$	=	the fraction of ingested contaminant absorbed by the mother (dimensionless).

Using values reported above in [Table B-52](#) and [Table B-56](#), [Appendix Equation 32](#) yields:

$$f_f = \frac{142.4 \frac{\text{mg}}{\text{kg}} \times 0.359 \text{ kg} \times 0.09}{44.48 \frac{\text{mg}}{\text{kg}} \times 0.359 \text{ kg} \times 0.64} = 0.5$$

**Half-life** data for absorbed mirex in male CD-1 rats was determined to be greater than 100 days following a single gavage dose ([Mehendale et al., 1972](#)). A half-life of greater than 10 months (~300 days) was reported by Ivie et al. (1974) in Sprague-Dawley rats dosed through the diet for 6 months. For the purposes of these calculations, 300 days was used.

#### B.4.5.2. Appropriate Dose Metric for Selected Point of Departure (POD)

Developmental effects may be associated with a critical exposure window during which exposure to mirex will result in the adverse effect. Metrics can be selected to look at either peak or average maternal or infant exposure, depending on the effect in question and the dosing protocol in the animal study. For developmental effects, if a critical window can be determined, then the model solutions during the critical window can be used for the cross-species extrapolation; otherwise, peak or average concentrations from different exposure metrics (see below) can be used based on the judgment of the



assessor as to what is most appropriate. For the proposed POD presented here, no critical window could be determined. The ocular effects were observed during a variety of different exposure periods in animal studies, but changes in effects associated with specific dosing periods were not studied systematically. While it is possible that there would be differences in effects for different dosing periods, sufficient data are not available to determine a critical window. Thus, possible dose metrics for specified lactation durations include:

- The average maternal body burden,
- The peak maternal body burden,
- The average maternal fat concentration,
- The peak maternal fat concentration,
- The average milk concentration,
- The peak milk concentration,
- The average infant body burden, and
- The peak infant body burden.

#### B.4.5.3. Available Models for Estimating Mirex Internal Dose

The following section discusses potential models that can be used to estimate internal dose metrics based on maternal intake. The section starts with the most biologically-robust class of models, physiologically-based pharmacokinetic (PBPK) models. The section then discusses simpler first-order kinetic modeling techniques and a biotransfer method for estimating maternal exposure. Thus, the section generally progresses from the most biologically robust to the more simple models. Where possible, the parameters needed for each technique have been collected and presented. The final choice in technique depends on weighing both the biological sophistication of the method and the uncertainty in the model parameters, as discussed in [Section B.4.6](#).

#### Dose Metric

- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
- ✓ **Peak or average concentrations from maternal or infant dose metrics**
- × **The critical window could not be defined**

#### Available Models

- ? What PBPK or other simpler biokinetic models are available for mirex?
- × **No mirex-specific human PBPK model available (existing models could be modified for mirex if appropriate model parameters are identified)**
- ✓ **Simple first order models are available**
- ✓ **Simple biotransfer methods are available**

### ***Multi-Compartment PBPK Models***

Generally speaking, the most biologically complete kinetic model for a given chemical is a multi-compartment, data-validated, PBPK model. However, no such model is available for mirex in rats or humans, either with or without a lactation component. Thus, this section discusses other models that could be applied to mirex.

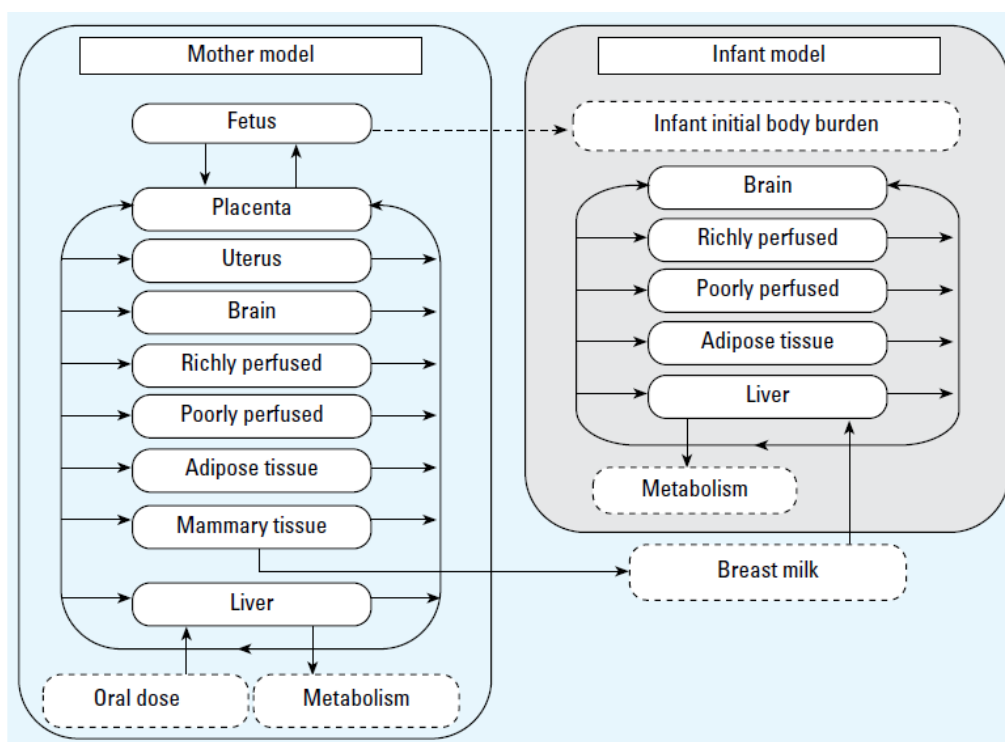
### ***Human Models***

Byczkowski and Lipscomb ([2001](#)) developed a human PBPK model to simulate neonatal exposure to methyl mercury; Ayotte et al. ([1996](#)), Gentry et al. ([2003](#)), and Kreuzer et al. ([1997](#)) developed lactational models for 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD); Redding et al. ([2008](#)) constructed a lactational model for PCB-153 and validated the predictions against human biomonitoring data, and Byczkowski et al. ([1994](#)) developed a lactational model for tetrachloroethylene.

These models could be adapted to mirex using mirex-specific kinetic parameters. However, as stated above, limited information is available on the absorption, distribution, metabolism, and excretion (ADME) of mirex in humans. There is no information about the partition coefficients in different compartments, the permeability area cross products in the gastric and mammary tissues, or the elimination rates that would be needed for these models.

One group in Canada has developed a generic maternal-infant multi-compartment model for persistent organic pollutants (POPs) ([Verner et al., 2009](#); [Verner et al., 2008](#)). The primary utility of this model for mirex is that the chemical-specific parameters can be estimated using only the half-life and octanol-water partition coefficient ( $K_{ow}$ ) of the compound.

The full maternal and infant model ([Verner et al., 2009](#)) is intended to simulate the entire life-cycle of a woman up to age 55 years and includes nine maternal tissue compartments and five infant tissue compartments, as shown in [Figure B-26](#). The oral dose is modeled as being directly absorbed into the liver and assumed to be fully bioavailable. First-order hepatic metabolism is included and is intended to represent all excretion in the absence of breastfeeding or childbirth. The elimination rate constant is determined for the chemical from the adult whole-body half-life. The concentrations in each compartment are determined using partition coefficients, and the chemical-specific partition coefficients are estimated using an equation based on  $K_{ow}$  ([Poulin and Krishnan, 1996](#)) and using the fraction of blood and tissue that are lipid and water ([Price et al., 2003](#)). Physiological parameters such as body weight and fat volume fraction are assumed to vary in time to capture changes over the life of the woman.



**Figure B-26. Maternal and Infant POP Model (Verner et al., 2009)**

Although mirex was not included, the model has been validated against human cord blood, breast milk, and infant blood concentrations for seven POPs ( $p,p'$ -DDE,  $p,p'$ -DDT, HDB,  $\beta$ -HCH, PCB-138, PCB-153, and PCB-180). The computer code was not provided in the publication although a number of details about the parameters and equations are included in the main papers and in supplemental information. Considerable time would be needed in order to further evaluate the potential for this model to be used for mirex, including model implementation and manipulation in modeling software.

### Animal Models

The candidate POD study by Gaines and Kimbrough (1970) investigates developmental effects in rats, so rat models are the focus of this section. Although no mirex-specific lactation PBPK model currently exists, there are several lactation models for rats for exposure to other chemicals. Fisher et al. (1990) constructed a lactation model for trichloroethylene while Clewell et al. (2003) constructed a lactation model for iodide and perchlorate. The lactational components of these models could potentially serve as a guide for developing a mirex animal model, although the chemicals modeled in these models are not lipophilic and the model might have to be adapted to account for fat storage or other chemical-specific differences. The necessary mirex-specific parameters would include mammary partition coefficients and permeability cross products, the clearance rate from the mother to the infant, and partition coefficients in the infant. It may be possible to determine partition coefficients in different compartments for rat

models. A paper by Poulin and Krishnan ([1995](#)) describes an algorithm for predicting rat tissue:blood partition coefficients based on  $K_{ow}$ . The algorithm accounts for chemical partitioning including phospholipids in tissues and blood, with erythrocytes and plasma considered separately. However, the estimates for tissue:blood partition coefficients were validated using hydrophilic organic compounds and may not be an appropriate representation of lipophilic compounds.

Other PBPK models for mirex without lactational components are discussed below.

#### ***Belfiore et al. (2007)***

Belfiore and colleagues ([2007](#)) developed a PBPK model in male Sprague-Dawley rats and rhesus monkeys for oral exposure to both mirex and chlordecone (a structurally similar chemical). Data from the study in rhesus monkeys by Wiener et al. ([1976](#)) as well as Belfiore et al.'s ([2007](#)) study data on Sprague-Dawley rats were used to construct the mirex model. The model represents flow-limited perfusion into liver, fat (monkey), slowly- and richly-perfused tissue, as well as diffusion-limited mirex uptake into fat (rat). Values for  $k_A$  (first-order rate constant for uptake of mirex from stomach into liver),  $k_{ELIM}$  (first-order clearance rate constant based on free concentration in liver), and  $K_D$  (dissociation constant) were optimized using ACSL Math or by iterative adjustment ([Belfiore et al., 2007](#)). Model parameters for mirex are presented in [Table B-53](#).

**Table B-53. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat and Monkey**

Parameter (units)	Value
<b>Pharmacokinetic constants</b>	
Uptake rate ( $\text{h}^{-1}$ ) <sup>a</sup>	0.0379
Elimination rate ( $\text{l/h}$ ) <sup>a</sup>	0.005
Fat permeation coefficient, visual optimization ( $\text{l/h}$ )	0.01 (Rat)
Blood binding (%) <sup>b</sup>	0.0001
Maximal liver binding ( $\text{nmol/g liver}$ ) <sup>c</sup>	0.00001
Binding constant to liver protein ( $\text{nM}$ ) <sup>a</sup>	0.0001
<b>Tissue:blood partition coefficients</b>	
Fat <sup>d</sup>	400
Liver <sup>e</sup>	17.5
Rapidly perfused tissues <sup>f</sup>	6.97
Slowly perfused tissues <sup>f</sup>	2.82
<b>Physiological constants</b>	
Cardiac output ( $\text{l/h/kg}$ ) <sup>g</sup>	14
Blood flow fractions <sup>g,h,i</sup>	
QRC - rapidly perfused	0.76 - QLC
QSC - slowly perfused	0.24 - QFC
QFC - fat blood flow	0.07
QLC - liver blood flow	0.18
Volume fractions <sup>g,h,i</sup>	
Blood	0.074
Fat <sup>j</sup>	0.05–0.099 (Rat) 0.15–0.25 (Monkey)
Liver	0.0375
Rapidly perfused (including liver)	0.09
Slowly perfused (including fat)	0.82
Body weight at dosing (kg)	Measured

<sup>a</sup> Value obtained by optimization.<sup>b</sup> Value derived from data of Soine et al. (1982).<sup>c</sup> Value derived from data of Soine et al. (1984).<sup>d</sup> Value derived from data of Lutz et al. (1977).<sup>e</sup> Calculated using equations of Fiserova-Bergerova and Diaz (1986).<sup>f</sup> Calculated using data of Wiener et al. (1976).<sup>g</sup> Reference values, Brown et al. (1997).<sup>h</sup> Reference values, Travis and Arms (1988).<sup>i</sup> Reference values, Sivarajan et al. (1975).<sup>j</sup> Reference values, Brown et al. (1997), based on age of animal.

The equations are provided in Appendix B of Belfiore et al. ([2007](#)), so straight-forward implementation of the model in the most common PBPK model application, ACSLXtreme®, is possible. The value for one variable (the fat blood volume, VBF) could not be found in the paper but could be found in the literature.

This approach allows the use of the existing full PBPK model but relies on a simplified approach to estimate the lactational exposure component.

#### ***Byrd et al. (1982)***

Byrd et al. ([1982](#)) developed an open-system pharmacokinetic model for intravenous (i.v.) or oral administration of mirex in rat that consisted of three compartments (blood, rapidly-equilibrating tissue, and slowly-equilibrating tissue) and parallel first-order elimination into urine and feces. The model for i.v. administration shows the blood compartment equilibrating with rapidly-equilibrating and slowly-equilibrating tissue, and excretion via urine and feces. In the model for oral administration, the gut and time lag compartments are added.

#### ***Pittman et al. (1976)***

Pittman et al. ([1976](#)) constructed a four-compartment, open-system model in female rhesus monkey that providing for the urinary excretion of mirex from a central compartment (containing plasma and rapid-distribution phase tissues) and for the fecal excretion from a fast tissue compartment. Study authors stipulate that the selection of a four-compartment model allowing for excretion into the feces from a peripheral compartment was arbitrary but best-modeled the long-term fate of mirex in the monkey.

#### ***First-Order Steady-State Single-Compartment Model (U.S. EPA, 1998)***

A first-order single-compartment model is an intermediate step between a full multi-compartment PBPK model and simpler dosimetric conversions or biotransfer models. The model presented here is patterned after the first-order models presented in the Combustor Assessment ([U.S. EPA, 1998](#)) and the dioxin reassessment ([U.S. EPA, 2012b](#)) and is adapted to fully incorporate time-dependence during lactation. The general model equations are presented first, and then the model is applied to both humans and rats in the next section. Rats are the focus here since that is the species in the candidate POD study. Because the equations are applied for rats and humans as an example in this briefing packet, they are presented in greater detail than the equations in the PBPK models discussed in [Section B.4.3.1](#). However, this is not meant to imply that the first-order models are superior for mirex.

The single body compartment represented by the model is generally defined as “body burden,” or the total average concentration of contaminant in the body. In a first-order model, the elimination from the body is represented as a rate constant multiplied by the total body burden. This rate constant can be estimated from the whole-body half-life, which is often readily available in the literature. The input to

the body is the dose, assumed to be normalized by the total body weight. Thus, the simple model can be represented by the differential equation<sup>10</sup>:

$$\frac{\partial BB(t)}{\partial t} = f_{am} DI_{mat}(t) - k_{elim} \times BB(t)$$

Appendix Equation 33

Where:

- $BB(t)$  = the time-dependent total body burden (ng/kg),
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{mat}(t)$  = the time-dependent maternal dose (ng/kg-day), and
- $f_{am}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless).

This equation can be converted to a difference equation and iterated in time:

$$BB_{t+\Delta t} = BB_t + \Delta t (f_{am} DI_{mat,t} - k_{elim} \times BB_t)$$

Appendix Equation 34

Where:

- $BB_{t+\Delta t}$  = the total body burden at the next time step (ng/kg),
- $BB_t$  = the total body burden at the current time step (ng/kg),
- $k_{elim}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{mat,t}$  = the maternal dose at the current time step (ng/kg-day),
- $f_{am}$  = the fraction of ingested contaminant absorbed by the mother (dimensionless), and
- $\Delta t$  = the time step (days).

This equation will approximate the solution to the differential equation. To help eliminate errors introduced by converting the differential equation to an algebraic equation, the time step ( $\Delta t$ ) should be much smaller than the elimination time scale.

This equation allows approximation of the maternal body burden; however, additional assumptions can be made to estimate the time dependent infant intake from breast milk. First, the concentration in the

<sup>10</sup> Strictly speaking, this equation is only correct if the maternal body weight is not changing in time. However, this approximation is sufficient given the overall uncertainty in using a first-order model.

maternal milk fat is assumed to be equal to the concentration in the maternal fat and can be estimated as:

$$C_{\text{milk fat},t} = \frac{BB_t \times f_f}{f_{fm}}$$

Appendix Equation 35

Where:

- $C_{\text{milk fat},t}$  = the concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_f$  = the fraction of contaminant that is stored in maternal fat (dimensionless), and
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW).

Although nonpolar compounds partition to some degree into the aqueous compartment, the most commonly analyzed organic contaminants, including mirex, are highly concentrated in the lipid phase; the concentration of mirex in breast milk is not expected to be significantly underestimated by ignoring the aqueous phase.

To account for the fact that lactation acts as an additional removal mechanism from the mother, the elimination rate during lactation is increased to equal:

$$k_{\text{elac}} = k_{\text{elim}} + \frac{CR_{\text{milk},t} \times f_f \times f_{\text{mbm}}}{f_{fm} \times BW_{\text{Mat}}}$$

Appendix Equation 36

Where:

- $k_{\text{elac}}$  = the maternal elimination rate during lactation ( $\text{days}^{-1}$ ),
- $k_{\text{elim}}$  = the first order elimination rate ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $CR_{\text{milk},t}$  = the time-dependent infant ingestion rate of milk (kg/day),
- $f_f$  = the fraction of contaminant that is stored in maternal fat (dimensionless)
- $f_{\text{mbm}}$  = the fraction of fat in milk (dimensionless),
- $f_{fm}$  = the fraction of the mother's weight that is fat (kg fat/kg BW), and
- $BW_{\text{Mat}}$  = the maternal body weight (kg).



Then, the time-dependent infant intake can be estimated as:

$$DI_{INF,t} = \frac{(C_{milk\ fat,t} \times f_{mbm}) \times CR_{milk,t}}{BW_{INF,t}}$$

Appendix Equation 37

Where:

- $DI_{INF,t}$  = the time-dependent infant daily ingestion (ng/kg-day),
- $C_{milk\ fat,t}$  = the time-dependent concentration of contaminant in the maternal milk fat at the current time step (ng/kg milk fat),
- $f_{mbm}$  = the fraction of fat in milk (dimensionless),
- $CR_{milk,t}$  = the time-dependent infant ingestion rate of milk (kg/day), and
- $BW_{INF,t}$  = the time-dependent infant's body weight (kg).

The time-dependent body weights and ingestion rates are incorporated in the models by using different point estimates over the course of the simulation (see the Sections on [Input Parameters and Application to Mirex in Humans](#) and [Input Parameters and Application to Mirex in Rats](#)). Finally, if the infant is modeled using a first-order model as well, the infant body burden can be estimated as:

$$BB_{INF,t+\Delta t} = BB_{INF,t} + \Delta t (f_{ai} DI_{INF,t} - k_{elim,INF} \times BB_{INF,t})$$

Appendix Equation 38

Where:

- $BB_{INF,t+\Delta t}$  = the infant total body burden at the next time step (ng/kg),
- $BB_{INF,t}$  = the infant total body burden at the current time step (ng/kg),
- $k_{elim,INF}$  = the first order elimination rate in the infant ( $\text{days}^{-1}$ ) =  $\ln(2)/\text{half-life (days)}$ ,
- $DI_{INF,t}$  = the infant dose at the current time step (ng/kg-day),
- $f_{ai}$  = the fraction of ingested contaminant absorbed by the infant (dimensionless), and
- $\Delta t$  = the time step (days).

The above equations approximate the maternal body burden and exposure to a lactating infant. The model does not, however, include any fetal exposure *in utero*.

This model is generic and not species specific. For purposes of illustration, the model was implemented in Excel and time integrated through the life of the mother. Then, the time-dependent solution can be used to estimate the internal dose metrics in [Section B.1.6](#).

The above model was parameterized so that the maternal dose, the infant body weight, and the infant ingestion rate all vary with time. In the case of the maternal dose, this allows the model to follow dosing protocols from animal studies. In the case of the body weight and ingestion rate, this allows the model to more accurately simulate effects on infants during very early exposure when body weight is changing rapidly and when critical windows for developmental effects may occur.

### *Input Parameters and Application to Mirex in Humans*

For humans, the model was implemented using a time step of 1 week. This represents a balance between a time step short enough to capture changes in the infant during lactation and long enough so that data are not applied beyond their measurement range. Since the Exposure Factors Handbook provides infant estimates on a monthly basis (see below), values across weeks within a single month were kept equal.

The input parameters used for mirex for humans are shown in [Table B-54](#). In addition to using mid-range (i.e., mean) values in the model, low and high-end estimates were also used for some parameters, where available, to determine the approximate range of human variability. Values for the parameters that are not chemical-specific were retained from the Combustor Assessment ([U.S. EPA, 1998](#)) or taken from the Exposure Factors Handbook ([U.S. EPA, 2011a](#)). Mirex-specific values are the same as those presented and discussed in [Section B.1.5.2](#).

**Table B-54. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in First-Order Single-Compartment Human Model**

Parameter (units)	Variable	Value			Note/source
Human Parameters (not chemical-specific)					
Maternal age at pregnancy (years)	Age	18	25	40	Assumptions
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 1 (kg)	BW <sub>INF,t</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, Month 2-3 (kg)		5.9			
Infant body weight, Month 4-6 (kg)		7.4			
Infant body weight, Month 7-12 (kg)		9.2			
Infant ingestion rate, Month 1 (kg milk/day)	CR <sub>milk,t</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, Month 2-3 (kg milk/day)		0.69			
Infant ingestion rate, Month 4-6 (kg milk/day)		0.77			
Infant ingestion rate, Month 7-12 (kg milk/day)		0.62			
Fraction of mother’s weight that is fat (kg maternal fat/kg BW) <sup>b</sup>	f <sub>fm</sub>	0.2	0.3	0.4	Timson and Coffman ( <a href="#">1984</a> ) ; U.S. EPA ( <a href="#">1998</a> )
Fraction of fat in breast milk (dimensionless) <sup>c</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Human Chemical-Specific Parameters					
Fraction of ingested contaminant that is absorbed by the infant (dimensionless)	f <sub>ai</sub>	1			U.S. EPA ( <a href="#">1998</a> )
Fraction of ingested contaminant that is absorbed by the mother (dimensionless)	f <sub>am</sub>	1			U.S. EPA ( <a href="#">1998</a> )
Fraction of contaminant that is stored in maternal fat (dimensionless)	f <sub>f</sub>	0.9			U.S. EPA ( <a href="#">1998</a> )
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	7.6 × 10 <sup>-5</sup>			Estimated as ln(2) / t <sub>1/2</sub>
Half-life in humans for mirex (days)	t <sub>1/2</sub>	9,125			Pittman et al. ( <a href="#">1976</a> ) (9,125 days = 25 years for estimated half-life in fat of rhesus monkey)

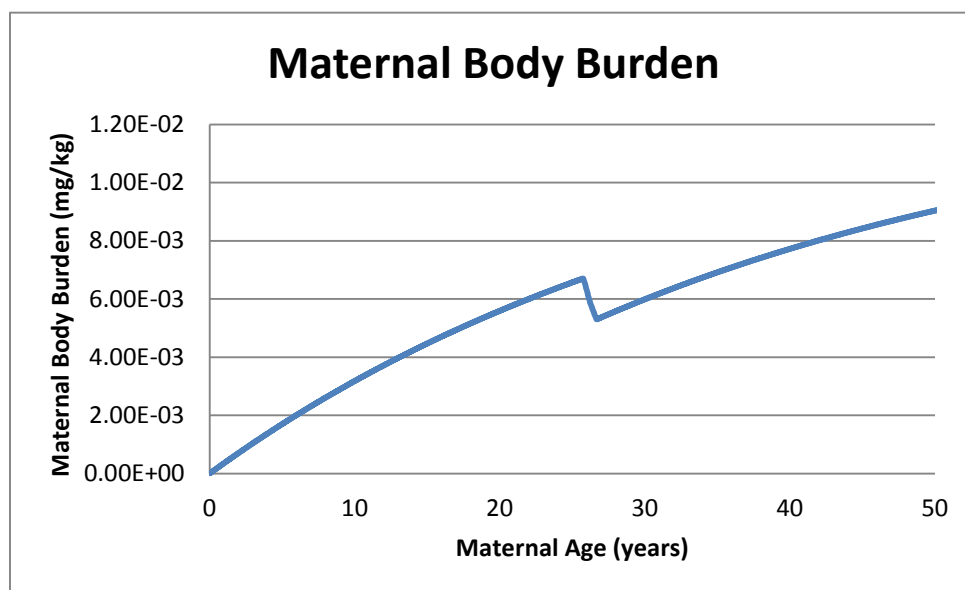
<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents mean ± standard deviation.

<sup>c</sup> Represents the typical range and midpoint of the range.

An example figure showing the maternal body burden assuming pregnancy at age 25 years and a daily intake of 1 ng/kg-day is shown in [Figure B-27](#). Because the model is linear with respect to dose, this intake level is chosen for illustrative purposes and the body burden would scale linearly with any change in intake. The dip at age 25 years and 9 months is due to the additional elimination during lactation.

One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, assuming the “mid” parameters and pregnancy at age 25 years, the average infant to maternal dose ratio was estimated to be 63.5 (1 month lactation duration) and 47.8 (12 month lactation duration). The overall range of the ratio, accounting for human variability and including the peak estimates, was from 20.2 to 179.1 (see [Table B-55](#)).



**Figure B-27. Human Maternal Body Burden Assuming Pregnancy at Age 25 and a Continuous Daily Exposure of 1 ng/kg-day**

**Table B-55. Estimated Infant-to-Maternal Dose Ratio Based on First-Order Model**

Infant Nursing Duration	Pregnancy at 18 years; Low parameters	Pregnancy at 25 years; Mid parameters	Pregnancy at 40 years; High parameters
1 month, Peak Infant Intake	25.4	63.9	168.8
1 month, Average Infant Intake	25.4	63.5	166.7
12 months, Peak Infant Intake	27.8	69.2	179.1
12 months, Average Infant Intake	20.2	47.8	112.9

### *Input Parameters and Application to Mirex in Rats*

For rats, the model was implemented using a time step of one half a day (one week used for humans). This was deemed sufficient to resolve changes during the typical three-week lactation period. The input parameters used for mirex are shown in [Table B-56](#). The age of the rats at the beginning of gestation was not reported in the candidate POD study, so the age at which rats become sexually mature

(5 weeks) was assumed for this analysis. In addition, a gestation time of 3 weeks and lactation duration of 3 weeks were assumed for the analysis as representative typical values. A litter size parameter was added so that the nursing rat fed 11 pups at a time, rather than the single infant assumed in the human model. This litter size increased the maternal lactational elimination rate and matches the average litter size at the POD dose in the POD study.

The physiological parameters needed for the model were not presented in the candidate POD study. Knight et al. ([1984](#)) reported the suckling rate in rat litters was assumed to be equal to the milk production in the dam. Experimentally determined milk production values of 0.0009, 0.0016, 0.0018, and 0.0016 liters/hr were measured on lactation days 3, 10, 17, and 23, respectively ([Knight et al., 1984](#)). The values for day 3, 10, and 17 were used for weeks 1, 2, and 3, respectively, assuming a milk density of 1 kg/liter and dividing by the total number of pups to get a per pup ingestion rate. Knight et al. ([1984](#)) also reported average pup body weights corresponding to these suckling rates of 8.5, 21, and 31 grams for weeks 1, 2, and 3, respectively.

Knight et al. ([1984](#)) reported the weight of lactating Wistar dams at day 2, 7, 14, and 21. The average across these days for the control group, 273 g, was used in the model. Fisher et al. ([1990](#)) reported fat as a percentage of body weight to be 6.0 to 12.0% in lactating F344 dams. A midpoint of 9.0% was used for this application. The fraction of fat in milk was reported to be 15.0% in Fisher et al. ([1990](#)), compared to a value of 4% in humans ([Welch and Findlay, 1981](#)).

The same chemical-specific values as presented in [Section B.1.5.2](#) were used: maternal absorption fraction, the fraction of contaminant stored in the fat relative to the total body burden, and the half-life.

**Table B-56. Model Parameters and Their Values Used in First-Order Steady State Single-Compartment Model in Rat**

Parameter (units)	Variable	Value	Note/source
<b>Rat Parameters (not chemical-specific)</b>			
Dam age at pregnancy (weeks)	Age	5	Assumption
Dam weight during lactation (kg)	BW <sub>Mat</sub>	0.273	Fisher et al. (1990)
Litter size (number of pups)	Litter	11	Gaines and Kimbrough (1970)
Pup body weight, Week 1 (kg)	BW <sub>INF,t</sub>	0.0085	Knight et al. (1984)
Pup body weight, Week 2 (kg)		0.021	
Pup body weight, Week 3 (kg)		0.031	
Pup ingestion rate, Week 1 (kg milk/day)	CR <sub>milk,t</sub>	0.0031	Knight et al. (1984), adjusted for litter size
Pup ingestion rate, Week 2 (kg milk/day)		0.0054	
Pup ingestion rate, Week 3 (kg milk/day)		0.0061	
Fraction of dam's weight that is fat (kg maternal fat/kg BW)	f <sub>fm</sub>	0.09	Fisher et al. (1990)
Fraction of fat in milk (dimensionless)	f <sub>mbm</sub>	0.15	Welch and Findlay (1981)
<b>Rat Chemical-Specific Parameters</b>			
Fraction of ingested contaminant that is absorbed by the rat pup (dimensionless)	f <sub>ai</sub>	0.64	Assumed
Fraction of ingested contaminant that is absorbed by the rat dam (dimensionless)	f <sub>am</sub>	0.64	Used midpoint value from (Mehendale et al., 1972) and Gibson et al. (1972)
Fraction of contaminant that is stored in rat dam/maternal fat (dimensionless)	f <sub>f</sub>	0.5	Estimated from Belfiore et al. (2007)
Biological elimination constant for contaminant (day <sup>-1</sup> )	k <sub>elim</sub>	0.0023	Estimated as ln(2) / t <sub>1/2</sub>
Half-life of contaminant in rats (days)	t <sub>1/2</sub>	300	Ivie et al. (1974)

The dosing protocol in the POD study (Gaines and Kimbrough, 1970) was applied to this rat model. In the study, the pups born to the control dams were cross-fostered with the dams that were exposed to mirex in diet during gestation as well as the 21 days of lactation. Thus, the model was applied by dosing the dams throughout gestation and lactation.

An example figure showing the maternal body burden using the candidate POD dose (0.4 mg/kg-day) is shown in Figure B-28. One useful metric from the model is the estimate of the ratio between the infant and maternal intake. For the model parameterized as discussed above, the average infant to maternal dose ratio was estimated to be 2.6 (1 week lactation duration) and 1.1 (3 week lactation duration, see Table B-57).

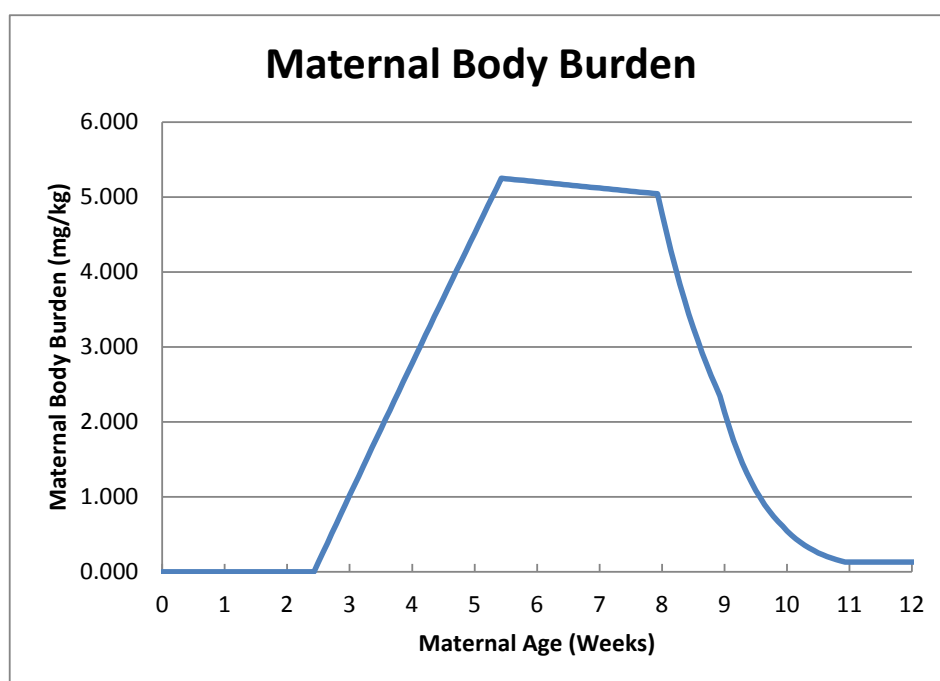


Figure B-28. Rat Maternal Body Burden Assuming the Dosing Protocol Used by the POD Study ([Gaines and Kimbrough, 1970](#)) and an Administered Dose of 0.4 mg/kg-day (POD)

Table B-57. Estimated Rat Pup-to-Maternal Dose Ratio Based on First-Order Model

Pup Nursing Duration	Ratio
1 week, Peak Pup Intake	3.6
1 week, Average Pup Intake	2.6
3 weeks, Peak Pup Intake	3.6
3 weeks, Average Pup Intake	1.1

### Biotransfer Method

The simplest technique for linking maternal and infant intake involves estimating the transfer of mirex to milk using a biotransfer factor. This method is discussed in the Combustor Assessment ([U.S. EPA, 1998](#)). The technique is based on a study by Travis and Arms ([1988](#)) and assumes that the milk fat contaminant concentration is proportional to maternal contaminant intake, with the proportionality constant represented by a biotransfer factor:

$$C_{\text{milk fat}} = DI_{\text{MAT}} \times BTF_m \times BW_{\text{Mat}}$$

Appendix Equation 39

Where:

- $C_{\text{milk fat}}$  = the concentration of contaminant in the maternal milk fat (ng/kg milk fat),
- $DI_{\text{MAT}}$  = daily maternal intake of contaminant (ng/kg BW-day),
- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $BW_{\text{MAT}}$  = the maternal body weight (kg).

The biotransfer factor is then estimated using a regression equation based on the octanol-water partition coefficient ( $K_{ow}$ ) using the expression:

$$BTF_m = 0.00062 \times K_{ow}$$

Appendix Equation 40

Where:

- $BTF_m$  = the biotransfer factor for milk fat (kg milk fat/day)<sup>-1</sup>, and
- $K_{ow}$  = the octanol-water partition coefficient (unitless).

The regression equation was estimated using six highly lipophilic compounds with  $\log(K_{ow})$  in the range of 5.16 to 6.5. According to the Combustor Assessment ([U.S. EPA, 1998](#)), the model tends to over-predict concentrations. For example, for dioxin, the equation predicts milk fat concentrations that are ten times higher than those actually measured in the United States when assuming background maternal intake values. Thus, the Combustor Assessment suggests the equation should only be used if parameters for more sophisticated kinetic models cannot be found in the literature and if the  $K_{ow}$  of the chemical in question is within the range of the  $K_{ow}$  used to fit the model. Because the pharmacokinetic data for mirex are generally sparse, this method is included for consideration, but the results likely have a high degree of uncertainty. In addition, because the  $\log(K_{ow})$  for mirex (6.89) is outside the range of the compounds used to generate the regression and was itself estimated from a regression, there is additional uncertainty in applying the model to mirex.



The daily infant contaminant dose can then be estimated for the biotransfer method using the equation:

$$DI_{INF} = \frac{(C_{milk\ fat} \times f_{mbm}) \times CR_{milk} \times ED_{INF}}{BW_{INF} \times AT}$$

Appendix Equation 41

Where:

$DI_{INF}$	=	the daily infant contaminant dose (ng/kg-day),
$C_{milk\ fat}$	=	the concentration of contaminant in the maternal milk fat (ng/kg milk fat),
$f_{mbm}$	=	the fraction of fat in milk (dimensionless),
$CR_{milk}$	=	the infant ingestion rate of milk (kg/day),
$ED_{INF}$	=	the infant exposure duration (year),
$BW_{INF}$	=	the infant's body weight (kg), and
$AT$	=	the averaging time (year).

Using the above equations, the ratio between the infant and maternal intake can be estimated as:

$$\frac{DI_{INF}}{DI_{MAT}} = \frac{0.00062 \times K_{OW} \times f_{mbm} \times CR_{milk} \times ED_{INF} \times BW_{Mat}}{BW_{INF} \times AT}$$

Appendix Equation 42

### *Input Parameters and Application to Mirex in Humans*

The input parameters used for mirex are shown in [Table B-58](#). In addition to using mid-range (e.g., mean) values in the model, low and high end estimates were also used for some parameters where available to determine the approximate range of human variability. The  $ED_{INF}$  and  $AT$  values are equal to each other (either 1 month or 12 months) and cancel from the equation.

**Table B-58. Model Parameters and Their Values (Low, Mid, and High End Estimates) Used in Biotransfer Method – 1 Month and 12 Months of Age**

Parameter (units)	Variable	Value			Note/source
Maternal weight during lactation (kg) <sup>a</sup>	BW <sub>Mat</sub>	75			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, 1 month (kg)	BW <sub>INF, 1</sub>	4.8			U.S. EPA ( <a href="#">2011a</a> )
Infant body weight, average over 12 months (kg)	BW <sub>INF, 12</sub>	7.8			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, 1 month (kg milk/day)	CR <sub>milk,1</sub>	0.51			U.S. EPA ( <a href="#">2011a</a> )
Infant ingestion rate, average over 12 months (kg milk/day)	CR <sub>milk,12</sub>	0.66			U.S. EPA ( <a href="#">2011a</a> )
Fraction of fat in milk (dimensionless) <sup>b</sup>	f <sub>mbm</sub>	0.02	0.03	0.04	U.S. EPA ( <a href="#">1998</a> )
Octanol-water partition coefficient	log(K <sub>ow</sub> )	6.89			Veith et al. ( <a href="#">1979</a> )

<sup>a</sup> Data were not available for body weight during lactation. Instead, the average body weight value is for women of child-bearing age (21 to <50 years) and does not account for weight gained during pregnancy but not yet lost post-partum.

<sup>b</sup> Represents the typical range and midpoint of the range.

Based on the K<sub>ow</sub> value and using [Appendix Equation 40](#), the biotransfer factor for mirex is 4,813. Using this BTF<sub>m</sub>, the average infant to maternal dose ratio was estimated for infants at 1 and 12 months of age and is presented in [Table B-59](#). For the model parameterization presented here, these ratios tend to be an order of magnitude larger than the estimates from the simple one-compartment kinetic model. It should also be noted that the estimated log(K<sub>ow</sub>) for mirex is higher than the range used in fitting the relationship between the BTF and K<sub>ow</sub>.

**Table B-59. Estimated Infant-to-maternal Dose Ratio Based on Biotransfer Method**

Age	Low	Mid	High
1 month	767.0	1150.5	1534.1
12 months	610.8	916.3	1221.7

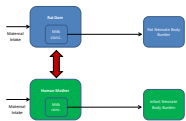

#### B.4.6. Overview of Human Equivalent Dose (HED) Methods and Example Calculation

The previous sections discuss the tools and input data available to estimate internal dose metrics for mirex in rats and humans. The next step is to select a method for performing cross-species extrapolation by weighing biological sophistication against methodology and data uncertainty.

[Table B-60](#) presents potential dose metrics and methods for performing the cross-species extrapolation. This list may not be exhaustive but is intended to foster discussion at the workshop. For illustrative

purposes in this briefing packet, one method for HED calculation was selected (shown in bold type), and sample calculations were performed.

**Table B-60. Potential Dose Metrics and Methods for HED Estimation**

Should the Cross-Species Extrapolation Be Performed on Maternal or Offspring Dose Metric?	What Technique is Used to Estimate the Dose Metrics?	What Dose Metric Should Be Used for Cross-Species Extrapolation?
<b>Maternal Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Maternal average or peak body burden during lactation</li> <li>• Maternal average or peak fat concentration during lactation</li> <li>• Maternal average or peak milk concentration during lactation</li> </ul>
<b>Offspring Concentration</b> 	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• <b>First order kinetic model with infant/pup body burden</b></li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Infant average or peak body burden during lactation</b></li> </ul>
Other?	<ul style="list-style-type: none"> <li>• PBPK model in rats and humans</li> <li>• First order kinetic maternal model in rats and humans</li> <li>• First order kinetic model with infant/pup body burden model</li> <li>• Biotransfer method</li> <li>• Other?</li> </ul>	<ul style="list-style-type: none"> <li>• Other?</li> </ul>

In selecting the dose metric to use for cross-species extrapolation and the technique to use to estimate the dose metrics, the discussion points listed in the text boxes throughout this section should be considered. This information is synthesized in the following text box.

As an example, the points were considered, and an example was provided to foster discussion. The chemical-specific information available should be considered to help guide the decision on whether a maternal or offspring dose metric should be utilized. Mirex is not metabolized, so the kinetics of linking maternal to infant intake are more easily captured in a simple model. Also, the POD study included dosing to the dams, but pups were only exposed during lactation. For these reasons, the pup body

burden portion of the model can be applied with greater confidence. Thus, the offspring dose metrics were selected.

Next, a model was selected to characterize the dose metrics. No full PBPK model was available for humans, and the pharmacokinetic data were not deemed sufficient to build or adapt a model. The human half-life value is relatively uncertain, but the first order method was deemed superior to the cruder biotransfer method since the biotransfer method itself uses a  $K_{ow}$  value outside the original regression range. Thus, the first-order kinetic model including the offspring body burden model was selected.

Finally, the dose metric was selected. No critical window could be determined for the developmental effect in the proposed POD. However, ocular effects were measured in a variety of studies that dosed the animals over different time points during lactation. Because the exact timing of dosing does not appear to be crucial for developing this particular effect, the average pup body burden during lactation (rather than the peak body burden) was selected.

The selections described in the preceding paragraphs were applied to the candidate POD study. The dose was administered during gestation and for three weeks during lactation. The models were applied assuming that the infant and pup half-lives and elimination rates for mirex were the same as for the mother and dam, respectively. The average pup body burden at the POD maternal dose (0.4 mg/kg-day) was predicted to be 4.6 mg/kg. The human first-order model was used to find the maternal continuous daily exposure that resulted in the same average infant body burden during lactation, assuming “mid” parameters for the human model (e.g., pregnancy at age 25). Continuous daily exposure was used because the reference dose should reflect a chronic maternal intake. The resulting maternal continuous daily exposure HED is  $4.9 \times 10^{-4}$  mg/kg-day in this example.

It should be noted that, in fact, the elimination rate in the pup and infant may be higher than the adult values. The sample calculation was made assuming these elimination rates were the same, but this variable represents a data gap and a potential discussion point.

### Discussion Points for Mirex HED Estimation

- ? What pharmacokinetic data or relevant chemical-specific information are available for the mirex constituents for both animals and humans to facilitate model implementation?
  - ✓  $K_{ow}$
  - ✓ Monkey and rat half-lives in fat
  - ✓ Absorption fractions and fraction of chemical stored in fat (assumptions can be made based on other PBTs)
  - ✗ No available half-life in humans
  - ✗ No available partition coefficients in humans
- ? What is the most appropriate dose metric to use for the effect associated with the selected POD?
  - ✓ Peak or average concentrations from maternal or infant dose metrics
  - ✗ The critical window could not be defined
- ? What PBPK or other simpler biokinetic models are available for mirex?
  - ✗ No mirex-specific human or rat PBPK model available (existing models could be modified for mirex if appropriate model parameters are identified)
  - ✓ Simple first order models are available
  - ✓ Simple biotransfer methods are available
- ? What is the best method for estimating the human equivalent dose using the proposed POD?
- ? Is there an alternative POD that may be more appropriate than the one used as an example in this briefing packet? If so, what is the best method for estimating the human equivalent dose using that alternative POD?

## Attachment B.4-1. Concentrations of Mirex in Human Milk

**Table Att. B.4-1. Concentration of Mirex in Human Milk (whole milk)**

Location	Date	Participants / Samples	Concentration (ppb)	Reference
Albany, NY	May–December 1977	24 samples of colostrum	16/25 samples < 0.01 (ng/g wet weight) (Occurrence: 64%)* Other samples contained the following amounts (ng/g wet weight): 0.03, 0.07, 0.09, 0.14, 0.21, 0.24, 0.29, 0.3	Bush et al. ( <a href="#">1983</a> )
Oswego, NY	May–December 1977	18 samples of colostrum	10/18 samples < 0.01 (ng/g wet weight) (Occurrence: 56%) Other samples contained the following amounts (ng/g wet weight): 0.01, 0.02, 0.06, 0.16, 0.32, 0.38, 2.18, 6.00	Bush et al. ( <a href="#">1983</a> )
Rochester, NY	May – December 1977	4 samples of colostrum	2/4 samples < 0.01 (ng/g wet weight) (Occurrence: 50%) Other samples contained the following amounts (ng/g wet weight): 0.06, 0.08	Bush et al. ( <a href="#">1983</a> )
Albany, NY	May–December 1977	6 samples; 3 to 4 weeks post-partum	Sample concentrations: 0.04, 0.05, 0.06, 0.06, 0.07, 0.14 Mean: 0.07 SD: 0.036 (51%)	Bush et al. ( <a href="#">1983</a> )

Location	Date	Participants / Samples	Concentration (ppb)	Reference
Oswego, NY	May–December 1977	16 samples, 3 to 4 weeks post-partum	Sample concentrations: 0.02, 0.05, 0.07, 0.08, 0.13, 0.14, 0.14, 0.14, 0.16, 0.17, 0.2, 0.2, 0.21, 0.21, <0.01, <0.01 Mean: 0.120 SD: 0.074 (62%)	Bush et al. ( <a href="#">1983</a> )
Rochester, NY	May–December 1977	6 samples, 3 to 4 weeks post-partum	Sample concentrations: 0.1, 0.12, 0.13, 0.16, 0.2, 0.26 Mean: 0.162 SD: 0.059 (36%)	Bush et al. ( <a href="#">1983</a> )
New York	NR, assumed to be before 1999	7 women, 8 milk samples, age range: 25-36 years, fish meals per year ranged from none to 84; 1 to 11 months post-partum. Serum levels of mirex reported in the same study ranged from 0.02 – 0.17 ng/g (ppb).	Mirex concentrations: 0.03, 0.03, 0.03, 0.07, 0.12, 0.18, 0.33, 0.45 ng/g Range: 0.03-0.45 ng/g Mean: 0.16 ng/g* Median: 0.10 ng/g*	Greizerstein et al. ( <a href="#">1999</a> )
Southern Kazakstan (sites: Almaty, Aralsk, Atyrau, Shymkent, Ozyr-Orda, and rural Kazakstan)	NR, assumed to be before 1997	76 women, age $22.5 \pm 3.8$ (16-34) years, $6.0 \pm 2.2$ (1.6-10.4) weeks post-partum	Occurrence: 1%	Hooper et al. ( <a href="#">1997</a> )
Kazakstan (sites: Almaty, Aralsk, Atyrau, Chimkent, Djetisay, Kirov, and Kyzyl Orda)	NR, assumed to be before 1998	92 samples, age $22.4 \pm 3.8$ (15-34) years	Occurrence: 1/92 (1.09%)	Lutter et al. ( <a href="#">1998</a> )
New York	1991–1993	213 total women, 100 samples from 98 women analyzed; 50 (of the 98 women) were primiparous, 48 were women of parity; samples were selected based on their potential contamination related to fish consumption reported in the 1991 questionnaire; NY State Angler Cohort; sample collected after the second morning feeding	Mean: $0.133 \pm 0.278$ ng/g Range: 0.001-2.56 ng/g Occurrence: 96%	Kostyniak et al. ( <a href="#">1999</a> )

Location	Date	Participants / Samples	Concentration (ppb)	Reference
Rochester, NY	September 1991–April 1993	15 samples from <b>non</b> -Lake Ontario fish eaters; 3 to 4 weeks post-partum; avg age 30 (range 25-28) years; avg weight 66.5 kg (range 52.3-81.8 kg)	Median: 0.06 Mean: 0.07 Range: ND-0.20	Madden and Makarewicz ( <a href="#">1996</a> )
Rochester, NY	September 1991–April 1993	15 samples from Lake Ontario fish eaters; 3 to 4 weeks post-partum; avg age 30.7 (range 26-37) years; avg weight 67.7 kg (range 47.3-89.5 kg) All consumed <1 fish meal per week; 33% consumed Lake Ontario fish >15 years, 25% for 5-10 years, 33% for 1-5 years, the rest for <1 year	Median: 0.09 Mean: 0.14 Range: ND-0.70	Madden and Makarewicz ( <a href="#">1996</a> )
Rochester, NY	September 1991–April 1993	7 Lake Ontario fish eaters consumed primarily salmonines; avg age 30.4 (range 26-35) years	Median: 0.21 Mean: 0.25 Range: 0.10-0.70	Madden and Makarewicz ( <a href="#">1996</a> )
Rochester, NY	September 1991–April 1993	6 Lake Ontario fish eaters consumed primarily panfish; avg age 31.5 (range 28-37) years	Median: 0.03 Mean: 0.04 Range: ND-0.10	Madden and Makarewicz ( <a href="#">1996</a> )
Canada	NR, before 1978	14 samples analyzed using chromatography, 3 samples measured for mirex concentration, collected during first 4-5 days of lactation	Range: 0.1-0.4	Mes et al. ( <a href="#">1978</a> )
Canada (all provinces)	1986	412 samples	Mean: 0.14 Median: 0.08 Maximum: 6.56 Occurrence: 62%	Mes et al. ( <a href="#">1993</a> )
Finland	1984–1985	165 women; mean age 26, SD 4, range 19-39 years; about 45% of the milk samples were taken after only one nursing, 18 of the mothers donated two samples, one before (foremilk) and one within 1 h after	ND	Mussalo-Rauhamaa et al. ( <a href="#">1988</a> )



Location	Date	Participants / Samples	Concentration (ppb)	Reference
Canada	1992	497 samples, median age 30 years, 54% primiparous	Mean: 0.06 Median: 0.05 Occurrence: 58%	Newsome et al. ( <a href="#">1995</a> )
Western region, Canada	1992	101 samples	ND	Newsome et al. ( <a href="#">1995</a> )
Central region, Canada	1992	80 samples	ND	Newsome et al. ( <a href="#">1995</a> )
Ontario region, Canada	1992	151 samples	Median: 0.07	Newsome et al. ( <a href="#">1995</a> )
Quebec region, Canada	1992	55 samples	Median: 0.05	Newsome et al. ( <a href="#">1995</a> )
Eastern region, Canada	1992	110 samples	Median: 0.04	Newsome et al. ( <a href="#">1995</a> )
Great Lakes Basin, Canada	1992	119 samples	Median: 0.07	Newsome et al. ( <a href="#">1995</a> )
Ontario excluding the Great Lakes Basin, Canada	1992	32 samples	Median: 0.06	Newsome et al. ( <a href="#">1995</a> )
Canada (excluding the Great Lakes Basin)	1992	378 samples	Median: 0.03	Newsome et al. ( <a href="#">1995</a> )
United States (all regions)	NR, assumed before 1981	1,436 women; 163 general care hospitals; ages: 75 (5%) were 15-19, 410 (28%) were 20-24, 606 (>42%) were 25-29, 265 (18%) were 30-34, 70 (5%) were ≥35, and 10 (1%) unknown years; occupation: 50 laborers, 378 professionals, 998 housewives, and 10 unknown; race: 84% white, 4% Mexican American, 4% black, 9% unknown or not reported.	ND	Savage et al. ( <a href="#">1982</a> ; <a href="#">1981</a> )
Northeast, United States	NR, assumed before 1981	233 women	ND	Savage et al. ( <a href="#">1982</a> ; <a href="#">1981</a> )
Southeast, United States	NR, assumed before 1981	288 women	ND	Savage et al. ( <a href="#">1982</a> ; <a href="#">1981</a> )
Midwest, United States	NR, assumed before 1981	378 women	ND	Savage et al. ( <a href="#">1982</a> ; <a href="#">1981</a> )

Location	Date	Participants / Samples	Concentration (ppb)	Reference
Southwest, United States	NR, assumed before 1981	388 women	ND	Savage et al. ( <a href="#">1982</a> ; <a href="#">1981</a> )
Northwest, United States	NR, assumed before 1981	149 women	ND	Savage et al. ( <a href="#">1982</a> ; <a href="#">1981</a> )
Hawaii (Oahu, Hawaii, and Kauai islands)	1979–1980	54 women; Hawaii residency ranged 1-37 years, up to lifetime, average residency 18 years; samples collected up to 1 month post-partum	ND	Takei et al. ( <a href="#">1983</a> )
Mainland U.S.	NR, assumed before 1983	National survey, up to 1 month post-partum	Mean: ND Range: ND-250 Occurrence: 0.98%	Takei et al. ( <a href="#">1983</a> )

Avg=Average; ND=Not detected; NR=Not reported; SD=Standard Deviation, \*=Calculations performed by ICFI using reported data

**Table Att. B.4-2. Concentration of Mirex in Human Milk (milk fat)**

Location	Year	Participants/Samples	Concentration (ppb)	Source
St. Lawrence River	1986-1989	19 Mohawk women, avg age 24.9 years, 1 month post-partum	Adjusted lifetime mean: 2.6	Fitzgerald et al. ( <a href="#">2001</a> )
Other rural New York state areas (control)	1986-1989	52 women, avg age 26.4 years, 1 month post-partum	Adjusted lifetime mean: 1.2	Fitzgerald et al. ( <a href="#">2001</a> )
St. Lawrence River	1990	38 Mohawk women, avg age 24.9 years, 1 month post-partum	Adjusted lifetime mean: 2.3	Fitzgerald et al. ( <a href="#">2001</a> )
Other rural New York state areas (control)	1990	57 women, avg age 26.4 years, 1 month post-partum	Adjusted lifetime mean: 1.0	Fitzgerald et al. ( <a href="#">2001</a> )
St. Lawrence River	1991-1992	40 Mohawk women, avg age 24.9 years, 1 month post-partum	Adjusted lifetime mean: 3.0	Fitzgerald et al. ( <a href="#">2001</a> )
Other rural New York state areas (control)	1991-1992	45 women, avg age 26.4 years, 1 month post-partum	Adjusted lifetime mean: 1.4	Fitzgerald et al. ( <a href="#">2001</a> )

Location	Year	Participants/Samples	Concentration (ppb)	Source
St. Lawrence River	1986-1992	17 Mohawk women, avg age 24.9 years, 1 month post-partum; no fish exposure	Adjusted lifetime mean: 2.4	Fitzgerald et al. ( <a href="#">2001</a> )
St. Lawrence River	1986-1992	40 Mohawk women, avg age 24.9 years, 1 month post-partum; low fish exposure	Adjusted lifetime mean: 2.3	Fitzgerald et al. ( <a href="#">2001</a> )
St. Lawrence River	1986-1992	40 Mohawk women, avg age 24.9 years, 1 month post-partum; high fish exposure	Adjusted lifetime mean: 3.1	Fitzgerald et al. ( <a href="#">2001</a> )
Other rural New York state areas (control)	1986-1992	154 women, avg age 26.4 years, 1 month post-partum	Adjusted lifetime mean: 1.2	Fitzgerald et al. ( <a href="#">2001</a> )
New York	NR	7 women, 8 milk samples, age range: 25-36 years, fish meals per year ranged from none to 84; 1 to 11 months post-partum	Mirex concentrations: 1.0, 1.4, 1.9, 2.4, 4.9, 5.5, 7.8, 10.2 Range: 0.03-0.45 Mean: 4.4* Median: 3.7*	Greizerstein et al. ( <a href="#">1999</a> )
New York	NR	213 total women, 100 samples from 98 women analyzed; 50 (of the 98 women) were primiparous, 48 were women of parity; samples were selected based on their potential contamination related to fish consumption reported in the 1991 questionnaire; NY State Angler Cohort; sample collected after the second morning feeding	Mean $\pm$ SD: 4.80 $\pm$ 11.3 Range: 0.54-106 Occurrence: 96%	Kostyniak et al. ( <a href="#">1999</a> )
Rochester, NY	September 1991–April 1993	15 samples from <b>non</b> -Lake Ontario fish eaters; 3 to 4 weeks post-partum; avg age 30, range 25-28 years; avg weight 66.5 kg, range 52.3-81.8 kg	Median: 1.2 Mean: 1.5 Range: ND-4.1	Madden and Makarewicz ( <a href="#">1996</a> )

Location	Year	Participants/Samples	Concentration (ppb)	Source
Rochester, NY	September 1991–April 1993	15 samples from Lake Ontario fish eaters; 3 to 4 weeks post-partum; avg age 30.7 (range 26-37) years; avg weight 67.7 kg (range 47.3-89.5 kg) All consumed <1 fish meal per week; 33% consumed Lake Ontario fish > 15 years, 25% for 5-10 years, 33% for 1-5 years, the rest for <1 year	Median: 2.1 Mean: 3.7 Range: ND-16.6	Madden and Makarewicz ( <a href="#">1996</a> )
Rochester, NY	September 1991–April 1993	7 Lake Ontario fish eaters consumed primarily salmonines; avg age 30.4 (range 26-35) years	Median: 5.8 Mean: 6.8 Range: 2.2-16.6	Madden and Makarewicz ( <a href="#">1996</a> )
Rochester, NY	September 1991–April 1993	6 Lake Ontario fish eaters consumed primarily panfish; avg age 31.5 (range 28-37) years	Median: 1.0 Mean: 0.8 Range: ND-2.1	Madden and Makarewicz ( <a href="#">1996</a> )
Canada	NR	14 samples analyzed using chromatography, 3 samples measured for mirex concentration, collected during first 4-5 days of lactation	Range: 2.3-11.1	Mes et al. ( <a href="#">1978</a> )
Porto Alegre, Brazil	1987–1988	30 women who lived in the Porto Alegre area for at least 5 years	Mean: 30 Median: <30 Range: <30-60 (original data reported in µg/g fat) Occurrence: 5/30 (16.6%)	Beretta and Dick. ( <a href="#">1994</a> )
Canada (all provinces)	1986	412 samples	Mean: 4.2 Median: 2.3 Maximum: 124.5 Occurrence: 62%	Mes et al. ( <a href="#">1993</a> )
Japan	NR	466 women, age 31.3 ± 4.4 years; average fish intake 391.8 (0-2779) g/kg body weight/year.	Mean: 0.74 Range: 0.17-1.9 Occurrence: 100%	Nakai et al. ( <a href="#">2009</a> ) <sup>c</sup>

Location	Year	Participants/Samples	Concentration (ppb)	Source
Canada (all regions)	1992	497 samples; median age 30 years, 54% primiparous	Mean: 1.89 Median: 1.55 Occurrence: 58%	Newsome and Ryan ( <a href="#">1995</a> )
Keewatin, Canada	July 1996–April 1997	12 women; median age 25 years, median children breastfed 2.0	Mean: 2.30 Median: 1.76	Newsome and Ryan ( <a href="#">1999</a> )
China (12 provinces: Heilongjiang, Liaoning, Hebei, Henan, Shanxi, Ningxia, Jiangxi, Fujian, Shanghai, Hubei, Sichuan and Guangxi)	August–November 2007	1,237 samples	Lower bound <sup>a</sup> mean $\pm$ SD: $1.0 \pm 2.3$ Lower bound median: ND Lower bound range: ND-8.1 Upper bound <sup>b</sup> mean $\pm$ SD: $2.4 \pm 1.6$ Upper bound median: ND Upper bound range: ND-8.1  Occurrence: 20.8%	Zhou et al. ( <a href="#">2011</a> )

<sup>a</sup> Lower bound: concentrations below LOD were treated as 0.0 for lower bound calculation.

<sup>b</sup> Upper bound: concentrations below LOD were treated as not detected for upper bound calculation.

<sup>c</sup> Reference published in Japanese; Information from abstract published in English presented.

Avg=Average; LOD = Level of detection; ND=Not detected; NR=Not reported; SD=Standard Deviation; \*=Calculations performed by ICFI using reported data

## B.4.7. References

- ATSDR (Agency for Toxic Substances and Disease Registry). (1995). Toxicological profile for mirex (CASRN 2385-85-5) and chlordecone (CASRN 143-50-0). <http://www.atsdr.cdc.gov/toxprofiles/tp66.pdf>
- Ayotte, P; Carrier, G; Dewailly, E. (1996). Health risk assessment for inuit newborns exposed to dioxin-like compounds through breast feeding. *Chemosphere* 32: 531-542.
- Belfiore, CJ; Yang, RS; Chubb, LS; Lohitnavy, M; Lohitnavy, OS; Andersen, ME. (2007). Hepatic sequestration of chlordecone and hexafluoroacetone evaluated by pharmacokinetic modeling. *Toxicology* 234: 59-72. <http://dx.doi.org/10.1016/j.tox.2007.02.002>
- Beretta, M; Dick, T. (1994). Organochlorine compounds in human milk, Porto Alegre, Brazil. *Bull Environ Contam Toxicol* 53: 357-360. <http://dx.doi.org/10.1007/BF00197226>
- Bodkin, NL; Alexander, TM; Ortmeier, HK; Johnson, E; Hansen, BC. (2003). Mortality and morbidity in laboratory-maintained Rhesus monkeys and effects of long-term dietary restriction. *J Gerontol A Biol Sci Med Sci* 58: B212-B219. <http://dx.doi.org/10.1093/gerona/58.3.B212>
- Bond, CA; Woodham, DW; Ahrens, EH; Medley, JG. (1975). The cumulation and disappearance of mirex residues. II. In milk and tissues of cows fed two concentrations of the insecticide in their diet. *Bull Environ Contam Toxicol* 14: 25-31. <http://dx.doi.org/10.1007/BF01685593>
- Brown, RP; Delp, MD; Lindstedt, SL; Rhomberg, LR; Beliles, RP. (1997). Physiological parameter values for physiologically based pharmacokinetic models [Review]. *Toxicol Ind Health* 13: 407-484.
- Burse, VW; Head, SL; McClure, PC; Korver, MP; Alley, CC; Phillips, DL; Needham, LL; Rowley, DL; Kahn, SE. (1989). Partitioning of mirex between adipose tissue and serum. *J Agric Food Chem* 37: 692-699. <http://dx.doi.org/10.1021/jf00087a025>
- Bush, B; Snow, J; Conner, S; Rueckert, L; Han, Y; Dymerski, P; Hilker, D. (1983). Mirex in human milk in upstate New York. *Arch Environ Contam Toxicol* 12: 739-746. <http://dx.doi.org/10.1007/BF01060759>
- Bush, B; Snow, J; Connor, S; Koblitz, R. (1985). Polychlorinated biphenyl congeners (PCBs), p,p'-DDE and hexachlorobenzene in human milk in three areas of upstate New York. *Arch Environ Contam Toxicol* 14: 443-450. <http://dx.doi.org/10.1007/BF01055530>
- Bush, B; Snow, J; Koblitz, R. (1984). Polychlorobiphenyl (PCB) congeners, p,p'-DDE, and hexachlorobenzene in maternal and fetal cord blood from mothers in Upstate New York. *Arch Environ Contam Toxicol* 13: 517-527. <http://dx.doi.org/10.1007/BF01056331>
- Byczkowski, JZ; Kinkead, ER; Leahy, HF; Randall, GM; Fisher, JW. (1994). Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically based model. *Toxicol Appl Pharmacol* 125: 228-236. <http://dx.doi.org/10.1006/taap.1994.1068>
- Byczkowski, JZ; Lipscomb, JC. (2001). Physiologically based pharmacokinetic modeling of the lactational transfer of methylmercury. *Risk Anal* 21: 869-882.
- Byrd, RA; Kimmel, CA; Morris, MD; Holson, JF; Young, JF. (1981). Altered pattern of prenatal toxicity in rats due to different treatment schedules with mirex. *Toxicol Appl Pharmacol* 60: 213-219. [http://dx.doi.org/10.1016/0041-008X\(91\)90225-4](http://dx.doi.org/10.1016/0041-008X(91)90225-4)
- Byrd, RA; Young, JF; Kimmel, CA; Morris, MD; Holson, JF. (1982). Computer simulation of mirex pharmacokinetics in the rat. *Toxicol Appl Pharmacol* 66: 182-192. [http://dx.doi.org/10.1016/0041-008X\(82\)90283-6](http://dx.doi.org/10.1016/0041-008X(82)90283-6)

- [Chambers, J; Yarbrough, J.](#) (1979). Disposition and excretion of mirex, 2, 8-dihydromirex, and 5, 10-dihydromirex by adult rats. *Fed Proc* 38: 266.
- [Chambers, JE; Case, RS; Alley, EG; Yarbrough, JD.](#) (1982). Short-term fate of mirex and 2,8-dihydromirex in rats. *J Agric Food Chem* 30: 878-882. <http://dx.doi.org/10.1021/jf00113a020>
- [Chernoff, N; Kavlock, RJ.](#) (1982). An in vivo teratology screen utilizing pregnant mice. *J Toxicol Environ Health* 10: 541-550. <http://dx.doi.org/10.1080/15287398209530275>
- [Chernoff, N; Linder, RE; Scotti, TM; Rogers, EH; Carver, BD; Kavlock, RJ.](#) (1979a). Fetotoxicity and cataractogenicity of mirex in rats and mice with notes on kepone. *Environ Res* 18: 257-269. [http://dx.doi.org/10.1016/0013-9351\(79\)90102-6](http://dx.doi.org/10.1016/0013-9351(79)90102-6)
- [Chernoff, N; Stevens, J; Rogers, E.](#) (1979b). Perinatal toxicology of mirex administered in the diet: I. viability, growth, cataractogenicity and tissue levels. *Toxicol Lett* 4: 263-268. [http://dx.doi.org/10.1016/0378-4274\(79\)90063-8](http://dx.doi.org/10.1016/0378-4274(79)90063-8)
- [Chu, I; Villeneuve, DC; Secours, VE; Valli, VE; Becking, GC.](#) (1981). Effects of photomirex and mirex on reproduction in the rat. *Toxicol Appl Pharmacol* 60: 549-556. [http://dx.doi.org/10.1016/0041-008X\(81\)90341-0](http://dx.doi.org/10.1016/0041-008X(81)90341-0)
- [Clewell, RA; Merrill, EA; Yu, KO; Mahle, DA; Sterner, TR; Fisher, JW; Gearhart, JM.](#) (2003). Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. *Toxicol Sci* 74: 416-436. <http://dx.doi.org/10.1093/toxsci/kfg147>
- [Deutch, B; Dyerberg, J; Pedersen, HS; Asmund, G; Moller, P; Hansen, JC.](#) (2006). Dietary composition and contaminants in north Greenland, in the 1970s and 2004. *Sci Total Environ* 370: 372-381. <http://dx.doi.org/10.1016/j.scitotenv.2006.07.015>
- [Deutch, B; Pedersen, HS; Asmund, G; Hansen, JC.](#) (2007). Contaminants, diet, plasma fatty acids and smoking in Greenland 1999-2005. *Sci Total Environ* 372: 486-496. <http://dx.doi.org/10.1016/j.scitotenv.2006.10.043>
- [Dorough, HW; Ivie, GW.](#) (1974). Fate of mirex-14C during and after a 28-day feeding period to a lactating cow. *J Environ Qual* 3: 65-67.
- [FDA, US.](#) (2011). Pesticide Residue Monitoring Program FY 2004-2006. U.S. FDA. <http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Pesticides/ResidueMonitoringReports/ucm125183.htm>
- [Fiserova-Bergerova, V; Diaz, ML.](#) (1986). Determination and prediction of tissue-gas partition coefficients. *Int Arch Occup Environ Health* 58: 75-87. <http://dx.doi.org/10.1007/BF00378543>
- [Fisher, JW; Whittaker, TA; Taylor, DH; Clewell, HJ, III; Andersen, ME.](#) (1990). Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol* 102: 497-513.
- [Fitzgerald, EF; Hwang, SA; Deres, DA; Bush, B; Cook, K; Worswick, P.](#) (2001). The association between local fish consumption and DDE, mirex, and HCB concentrations in the breast milk of Mohawk women at Akwesasne. *J Expo Anal Environ Epidemiol* 11: 381-388. <http://dx.doi.org/10.1038/sj.jea.7500180>
- [Gaines, TB; Kimbrough, RD.](#) (1970). Oral toxicity of mirex in adults and suckling rats: With notes on the ultrastructure of liver changes. *Arch Environ Health* 21: 7-14.
- [Gallenberg, LA; Vodcnik, MJ.](#) (1987). Potential mechanisms for redistribution of polychlorinated biphenyls during pregnancy and lactation. *Xenobiotica* 17: 299-310. <http://dx.doi.org/10.3109/00498258709043940>
- [Gentry, PR; Covington, TR; Clewell, HJ, III.](#) (2003). Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. *Regul Toxicol Pharmacol* 38: 1-16. [http://dx.doi.org/10.1016/S0273-2300\(03\)00047-3](http://dx.doi.org/10.1016/S0273-2300(03)00047-3)

- Gibson, JR; Ivie, GW; Dorrough, HW. (1972). Fate of mirex and its major photodecomposition product in rats. *J Agric Food Chem* 20: 1246-1248.
- Grabowski, CT. (1981). Plasma proteins and colloid osmotic pressure of blood of rat fetuses prenatally exposed to Mirex. *J Toxicol Environ Health* 7: 705-714. <http://dx.doi.org/10.1080/15287398109530013>
- Grabowski, CT. (1983). Persistent cardiovascular problems in newborn rats prenatally exposed to sub-teratogenic doses of the pesticide, mirex. In AW Hayes; RC Schnell; TS Miya (Eds.), *Developments in the science and practice of toxicology: Proceedings of the Third International Congress on Toxicology held in San Diego, California, USA, August 28-September 3, 1983* (pp. 537-540). New York, NY: Elsevier Science.
- Grabowski, CT; Payne, DB. (1980). An electrocardiographic study of cardiovascular problems in mirex-fed rat fetuses. *Teratology* 22: 167-177. <http://dx.doi.org/10.1002/tera.1420220205>
- Grabowski, CT; Payne, DB. (1983a). The causes of perinatal death induced by prenatal exposure of rats to the pesticide, mirex. Part I: Pre-parturition observations of the cardiovascular system. *Teratology* 27: 7-11. <http://dx.doi.org/10.1002/tera.1420270103>
- Grabowski, CT; Payne, DB. (1983b). The Causes Of Perinatal Death Induced By Prenatal Exposure Of Rats To The Pesticide, Mirex. Part II: Postnatal Observations. *J Toxicol Environ Health* 11: 301-316.
- Greizerstein, HB; Stinson, C; Mendola, P; Buck, GM; Kostyniak, PJ; Vena, JE. (1999). Comparison of PCB congeners and pesticide levels between serum and milk from lactating women. *Environ Res* 80: 280-286. <http://dx.doi.org/10.1006/enrs.1999.3956>
- Hooper, K; Hopper, K; Petreas, MX; She, J; Visita, P; Winkler, J; McKinney, M; Mok, M; Sy, F; Garcha, J; Gill, M; Stephens, RD; Semenova, G; Sharmanov, T; Chuvakova, T; Hopper, K. (1997). Analysis of breast milk to assess exposure to chlorinated contaminants in Kazakhstan: PCBs and organochlorine pesticides in southern Kazakhstan. *Environ Health Perspect* 105: 1250-1254.
- Ivie, GW; Gibson, JR; Bryant, HE; Begin, JJ; Barnett, JR; Dorrough, HW. (1974). Accumulation, distribution, and excretion of mirex-14C in animals exposed for long periods to the insecticide in the diet. *J Agric Food Chem* 22: 646-653. <http://dx.doi.org/10.1021/jf60194a054>
- Kavlock, R; Chernoff, N; Rogers, E; Whitehouse, D. (1980). Comparative tissue distribution of mirex and chlordecone in fetal and neonatal rats. *Pestic Biochem Physiol* 14: 227-235. [http://dx.doi.org/10.1016/0048-3575\(80\)90029-2](http://dx.doi.org/10.1016/0048-3575(80)90029-2)
- Kearney, JP; Cole, DC; Ferron, LA; Weber, JP. (1999). Blood PCB, p,p'-DDE, and mirex levels in Great Lakes fish and waterfowl consumers in two Ontario communities. *Environ Res* 80: S138-S149. <http://dx.doi.org/10.1006/enrs.1998.3927>
- Khera, K; Villeneuve, D; Terry, G; Panopio, L; Nash, L; Trivett, G. (1976). Mirex: A teratogenicity, dominant lethal and tissue distribution study in rats. *Food Cosmet Toxicol* 14: 25-29. [http://dx.doi.org/10.1016/S0015-6264\(76\)80359-8](http://dx.doi.org/10.1016/S0015-6264(76)80359-8)
- Knight, CH; Docherty, AH; Peaker, M. (1984). Milk yield in rats in relation to activity and size of the mammary secretory cell population. *J Dairy Res* 51: 29-35.
- Kosatsky, T; Przybysz, R; Shatenstein, B; Weber, JP; Armstrong, B. (1999). Contaminant exposure in Montrealers of Asian origin fishing the St. Lawrence River: Exploratory assessment. *Environ Res* 80: S159-S165. <http://dx.doi.org/10.1006/enrs.1998.3911>
- Kostyniak, PJ; Stinson, C; Greizerstein, HB; Vena, J; Buck, G; Mendola, P. (1999). Relation of Lake Ontario fish consumption, lifetime lactation, and parity to breast milk polychlorobiphenyl and pesticide concentrations. *Environ Res* 80: S166-S174. <http://dx.doi.org/10.1006/enrs.1998.3939>
- Kreuzer, PE; Csanády, GA; Baur, C; Kessler, W; Pöpke, O; Greim, H; Filser, JG. (1997). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and congeners in infants. A toxicokinetic model of human lifetime body burden by TCDD with special emphasis on its uptake by nutrition. *Arch Toxicol* 71: 383-400.



- [Liddle, JA; Smrek, AL; Adams, SR; Kimbrough, RD.](#) (1977). Pharmacodynamics of Mirex in Goats: Effect of Reproduction and Lactation. *Toxicol Appl Pharmacol* 41: 138.
- [Lutter, C; Iyengar, V; Barnes, R; Chuvakova, T; Kazbekova, G; Sharmanov, T.](#) (1998). Breast milk contamination in Kazakhstan: Implications for infant feeding. *Chemosphere* 37: 1761-1772.  
[http://dx.doi.org/10.1016/S0045-6535\(98\)00241-0](http://dx.doi.org/10.1016/S0045-6535(98)00241-0)
- [Lutz, RJ; Dedrick, RL; Matthews, HB; Eling, TE; Anderson, MW.](#) (1977). A preliminary pharmacokinetic model for several chlorinated biphenyls in the rat. *Drug Metab Dispos* 5: 386-396.
- [Madden, AB; Makarewicz, JC.](#) (1996). Salmonine consumption as a source of mirex in human breast milk near Rochester, New York. *J Great Lakes Res* 22: 810-817. [http://dx.doi.org/10.1016/S0380-1330\(96\)71002-X](http://dx.doi.org/10.1016/S0380-1330(96)71002-X)
- [Mehendale, HM; Fishbein, L; Fields, M; Matthews, HB.](#) (1972). Fate of mirex-14 C in the rat and plants. *Bull Environ Contam Toxicol* 8: 200-207. <http://dx.doi.org/10.1007/BF01839512>
- [Mes, J; Davies, DJ; Doucet, J; Weber, D; McMullen, E.](#) (1993). Levels of chlorinated hydrocarbon residues in Canadian human breast milk and their relationship to some characteristics of the donors. *Food Addit Contam* 10: 429-441. <http://dx.doi.org/10.1080/02652039309374166>
- [Mes, J; Davies, DJ; Miles, W.](#) (1978). Traces of mirex in some Canadian human milk samples. *Bull Environ Contam Toxicol* 19: 564-570. <http://dx.doi.org/10.1007/BF01685842>
- [Mussalo-Rauhamaa, H; Pyysalo, H; Antervo, K.](#) (1988). Relation between the content of organochlorine compounds in Finnish human milk and characteristics of the mothers. *J Toxicol Environ Health* 25: 1-19.  
<http://dx.doi.org/10.1080/15287398809531185>
- [Nakai, K; Nakamura, T; Murata, K; Satoh, H.](#) (2009). Tohoku study of child development and exposure assessment [Review]. *Nippon Eiseigaku Zasshi* 64: 749-758.
- [Newsome, WH; Davies, D; Doucet, J.](#) (1995). PCB and organochlorine pesticides in Canadian human milk-1992. *Chemosphere* 30: 2143-2153. [http://dx.doi.org/10.1016/0045-6535\(95\)00086-N](http://dx.doi.org/10.1016/0045-6535(95)00086-N)
- [Newsome, WH; Ryan, JJ.](#) (1999). Toxaphene and other chlorinated compounds in human milk from northern and southern Canada: A comparison. *Chemosphere* 39: 519-526. [http://dx.doi.org/10.1016/S0045-6535\(98\)00600-6](http://dx.doi.org/10.1016/S0045-6535(98)00600-6)
- [Oliver, BG; Cleland, GB; Sonstegard, RA.](#) (1993). PCB congeners and organochlorine pesticides in the blood of Lake Ontario fish consumers [Abstract]. In *Proceedings of the Nineteenth Annual Aquatic Toxicity Workshop* (pp. 77). Oliver, BG; Cleland, GB; Sonstegard, RA.
- [Pittman, KA; Wiener, M; Treble, DH.](#) (1976). Mirex kinetics in the Rhesus monkey. II. Pharmacokinetic model. *Drug Metab Dispos* 4: 288-295.
- [Poulin, P; Krishnan, K.](#) (1995). An algorithm for predicting tissue: Blood partition coefficients of organic chemicals from n-octanol: Water partition coefficient data. *J Toxicol Environ Health* 46: 117-129.  
<http://dx.doi.org/10.1080/15287399509532021>
- [Poulin, P; Krishnan, K.](#) (1996). A tissue composition-based algorithm for predicting tissue:air partition coefficients of organic chemicals. *Toxicol Appl Pharmacol* 136: 126-130.  
<http://dx.doi.org/10.1006/taap.1996.0015>
- [Price, K; Haddad, S; Krishnan, K.](#) (2003). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J Toxicol Environ Health A* 66: 417-433.  
<http://dx.doi.org/10.1080/15287390306450>
- [Redding, LE; Sohn, MD; Mckone, TE; Chen, JW; Wang, SL; Hsieh, DP; Yang, RS.](#) (2008). Population physiologically based pharmacokinetic modeling for the human lactational transfer of PCB-153 with consideration of worldwide human biomonitoring results. *Environ Health Perspect* 116: 1629-1635.  
<http://dx.doi.org/10.1289/ehp.11519>

- Reiter, L. (1977). Behavioral toxicology: effects of early postnatal exposure to neurotoxins on development of locomotor activity in the rat. *J Occup Med* 19: 201-204.
- Rogers, JM; Grabowski, CT. (1983). Mirex-induced fetal cataracts: Lens growth, histology and cation balance, and relationship to edema. *Teratology* 27: 343-349. <http://dx.doi.org/10.1002/tera.1420270308>
- Rogers, JM; Grabowski, CT. (1984). Postnatal mirex cataractogenesis in rats: Lens cation balance, growth and histology. *Exp Eye Res* 39: 563-573. [http://dx.doi.org/10.1016/0014-4835\(84\)90056-3](http://dx.doi.org/10.1016/0014-4835(84)90056-3)
- Savage, EP; Keefe, TJ; Tessari, JD. (1982). Pesticides in human breast milk. In Banbury report 11: Environmental factors in human growth and development (pp. 77-84). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Savage, EP; Keefe, TJ; Tessari, JD; Wheeler, HW; Applehans, FM; Goes, EA; Ford, SA. (1981). National study of chlorinated hydrocarbon insecticide residues in human milk, USA: I. Geographic distribution of dieldrin, heptachlor, heptachlor epoxide, chlordane, oxychlordane, and mirex. *Am J Epidemiol* 113: 413-422.
- Schantz, SL; Jacobson, JL; Humphrey, HE; Jacobson, SW; Welch, R; Gasior, D. (1994). Determinants of polychlorinated biphenyls (PCBs) in the sera of mothers and children from Michigan farms with PCB-contaminated silos. *Arch Environ Health* 49: 452-458. <http://dx.doi.org/10.1080/00039896.1994.9955000>
- Scotti, TM; Chernoff, N; Linder, R; McElroy, WK. (1981). Histopathologic lens changes in mirex-exposed rats. *Toxicol Lett* 9: 289-294. [http://dx.doi.org/10.1016/0378-4274\(81\)90163-6](http://dx.doi.org/10.1016/0378-4274(81)90163-6)
- Sivarajan, M; Amory, DW; Lindbloom, LE; Schwettmann, RS. (1975). Systemic and regional blood-flow changes during spinal anesthesia in the rhesus monkey. *Anesthesiology* 43: 78-88.
- Smrek, AL; Adams, SR; Liddle, JA; Kimbrough, RD. (1977). Pharmacokinetics of mirex in goats. 1. Effects on reproduction and lactation. *J Agric Food Chem* 25: 1321-1325. <http://dx.doi.org/10.1021/jf60214a011>
- Smrek, AL; Adams, SR; Liddle, JA; Kimbrough, RD. (1978). Pharmacokinetics of mirex in goats. 2. Residue tissue levels, trans-placental passage during recovery. *J Agric Food Chem* 26: 945-947. <http://dx.doi.org/10.1021/jf60218a006>
- Soine, PJ; Blanke, RV; Guzelian, PS; Schwartz, CC. (1982). Preferential binding of chlordecone to the protein and high density lipoprotein fractions of plasma from humans and other species. *J Toxicol Environ Health* 9: 107-118. <http://dx.doi.org/10.1080/15287398209530146>
- Soine, PJ; Blanke, RV; Schwartz, CC. (1984). Isolation of chlordecone binding proteins from pig liver cytosol. *J Toxicol Environ Health* 14: 305-317. <http://dx.doi.org/10.1080/15287398409530582>
- Takei, GH; Kauahikaua, SM; Leong, GH. (1983). Analysis of human milk samples collected in Hawaii for residues of organochlorine pesticides and polychlorobiphenyls. *Bull Environ Contam Toxicol* 30: 606-613. <http://dx.doi.org/10.1007/BF01610182>
- Timson, BF; Coffman, JL. (1984). Body composition by hydrostatic weighing at total lung capacity and residual volume. *Med Sci Sports Exerc* 16: 411-414.
- Travis, CC; Arms, AD. (1988). Bioconcentration of organics in beef, milk, and vegetation. *Environ Sci Technol* 22: 271-274. <http://dx.doi.org/10.1021/es00168a005>
- U.S. EPA (U.S. Environmental Protection Agency). (1998). Methodology for assessing health risks associated with multiple pathways of exposure to combustor emissions [EPA Report]. (EPA 600/R-98/137). Washington, DC. <http://cfpub.epa.gov/ncea/CFM/recordisplay.cfm?deid=55525>
- U.S. EPA (U.S. Environmental Protection Agency). (2003b). IRIS Toxicological Review of Mirex (External Review Draft). (NCEA-C-1343). US Environmental Protection Agency.
- U.S. EPA (U.S. Environmental Protection Agency). (2011a). Exposure factors handbook 2011 edition (final) [EPA Report]. (EPA/600/R-09/052F). <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252>

- [U.S. EPA](#). (2012). EPA's reanalysis of key issues related to dioxin toxicity and response to NAS comments (CAS No. 1746-01-6) [EPA Report]. (EPA/600/R-10/038F). Washington, DC.
- [Van Oostdam, JC; Dewailly, E; Gilman, A; Hansen, JC; Odland, JO; Chashchin, V; Berner, J; Butler-Walker, J; Lagerkvist, BJ; Olafsdottir, K; Soininen, L; Bjerregard, P; Klopov, V; Weber, JP](#). (2004). Circumpolar maternal blood contaminant survey, 1994-1997 organochlorine compounds. *Sci Total Environ* 330: 55-70. <http://dx.doi.org/10.1016/j.scitotenv.2004.02.028>
- [Veith, GD; Defoe, DL; Bergstedt, BV](#). (1979). Measuring and estimating the bioconcentration factor of chemicals in fish. *J Fish Res Board Can* 36: 1040-1048. <http://dx.doi.org/10.1139/f79-146>
- [Verner, MA; Ayotte, P; Muckle, G; Charbonneau, M; Haddad, S](#). (2009). A physiologically based pharmacokinetic model for the assessment of infant exposure to persistent organic pollutants in epidemiologic studies. *Environ Health Perspect* 117: 481-487.
- [Verner, MA; Charbonneau, M; López-Carrillo, L; Haddad, S](#). (2008). Physiologically based pharmacokinetic modeling of persistent organic pollutants for lifetime exposure assessment: A new tool in breast cancer epidemiologic studies. *Environ Health Perspect* 116: 886-892. <http://dx.doi.org/10.1289/ehp.10917>
- [Welch, RM; Findlay, JW](#). (1981). Excretion of drugs in human breast milk [Review]. *Drug Metab Rev* 12: 261-277. <http://dx.doi.org/10.3109/03602538108994032>
- [Wiener, M; Pittman, KA; Stein, V](#). (1976). Mirex kinetics in the rhesus monkey. I. Disposition and excretion. *Drug Metab Dispos* 4: 281-287.
- [Zhou, P; Wu, Y; Yin, S; Li, J; Zhao, Y; Zhang, L; Chen, H; Liu, Y; Yang, X; Li, X](#). (2011). National survey of the levels of persistent organochlorine pesticides in the breast milk of mothers in China. *Environ Pollut* 159: 524-531. <http://dx.doi.org/10.1016/j.envpol.2010.10.014>

---

## Appendix C. Workshop Charge and Instructions for Attendees

---

### C.1. Charge for Chemical Breakout Groups

---

#### Roles and Responsibilities

- Identify facilitator for group
- Identify recorder for notes and for presentation
  - ◆ Templates provided
  - ◆ Everyone contributes and reviews
- Group will present their approach for their chemical and draft text for workshop report
- ICF notetaker will capture detailed notes

#### Ground Rules

- Everyone should contribute
- Agree to disagree
  - ◆ Capture other opinions in report and presentation
- Focus on the approach to improving risk estimates

#### Identify Group Approach

- Each person presents their approach (or approaches)
  - ◆ Discuss responses to Charge Questions 2 and 3.
- Discuss possible approaches and identify best approach(es) for group's chemical.
  - ◆ Identify additional data needs and common issues.
- Record supporting information in presentation and notes
  - ◆ See Breakout Group Instructions for list of questions to answer

#### Timing

- 20 minute presentations from each group with 10 minutes for Q&A
- Begin presentations at 2:20
- Groups take 45 minute lunch break

#### Present Approaches

- How would you estimate the quantitative relationship between infant dose from milk and continuous daily maternal dose for both humans and any relevant laboratory species (i.e., species in which health effects have been observed as a result of postnatal exposure to this chemical)?
  - ◆ Rationale
  - ◆ Data gaps
  - ◆ Uncertainties
  - ◆ Extrapolation

---

## C.2. Common Issues and Data Gaps: Group Brainstorming and Approaches Breakout Groups

### Structure of Group Brainstorming

- Prepare notes this evening
- Round robin in which each person presents an issue or data gap
- Maximum of 2 minutes to present, with at least 2 turns per person

### What to present?

- Consider how we would group chemicals based on:
  - ◆ Chemical characteristics
  - ◆ Available data
- What approach could be taken for a given set of chemical characteristics or available data?
  - ◆ Explain rationale.
  - ◆ What input data would be needed?
  - ◆ What uncertainties or data gaps remain?

## Matrix of Approaches

	<b>Potential Approaches</b>			
<b>Chemical or Database Characteristics</b>				
		Input Data: Considerations: Approach: Key Uncertainties:		

## Approaches Breakout Groups

Divide approaches among new breakout groups

Group will draft report text and create presentation to give Friday morning