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RESEARCH TRIANGLE PARK, NORTH CAROLINA

Pre-natal Exposures of Children to Polybrominated Diphenyl Ethers: The Collection of Animal and Human Data Along With the Development and Validation of a PBPK Model

James Raymer, Amy Licata, Ed Garner

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Basis for Study

In-utero exposures and exposures during childhood create potential for adverse health effects.

- **Abnormal or disturbed development of neurological, endocrine systems**
- **Some adverse effects later in life could be linked to early exposures**
- **Adult and childhood cancers, reproductive and development anomalies, behavioral deficits**

In-utero studies to most environmental chemicals lacking.

PBDEs widely occurring; Tetra- and penta- isomers most commonly found in biological media.

Study Objectives

- **Develop a physiologically based pharmacokinetic (PBPK) animal model for 2,2',4,4'-tetrabromo- and 2,2',4,4',5-pentabromodiphenyl ether that can be used to estimate fetal exposure.**

  - **The parameters needed to develop the model will be measured.**

Study Objectives (cont.)

- **Analytical methods for PBDEs in human blood and meconium will be developed and applied to samples to estimate the utility of the model for estimating fetal exposures.**
  - **Determine if cord blood or meconium are appropriate media for measurement of cumulative exposures of newborn babies to PBDEs.**

Specific Hypotheses

1. **A rodent PBPK model for PBDEs can be scaled to be applicable to humans.**
2. **The PBDE concentrations in cord blood and meconium from newborns are proportional.**
3. **Mother's blood concentrations of PBDEs are predictive of the cord blood and/or meconium concentrations in newborn babies.**
4. **Meconium is a useful medium for assessing cumulative dose to the developing fetus.**

Potential Benefits

- **This work will provide a PBPK model that can be used to estimate exposures of unborn children to the target PBDEs.**
- **The most appropriate matrix for the assessment of *in-utero* exposure will be obtained.**
- **The developed PBPK model can be used in future work to study different aspects of exposure, including the impact of chronic and intermittent exposures on time-sensitive, developmental events.**
- **The exposures of a group of mothers and their newborn children to the target analytes will be determined.**

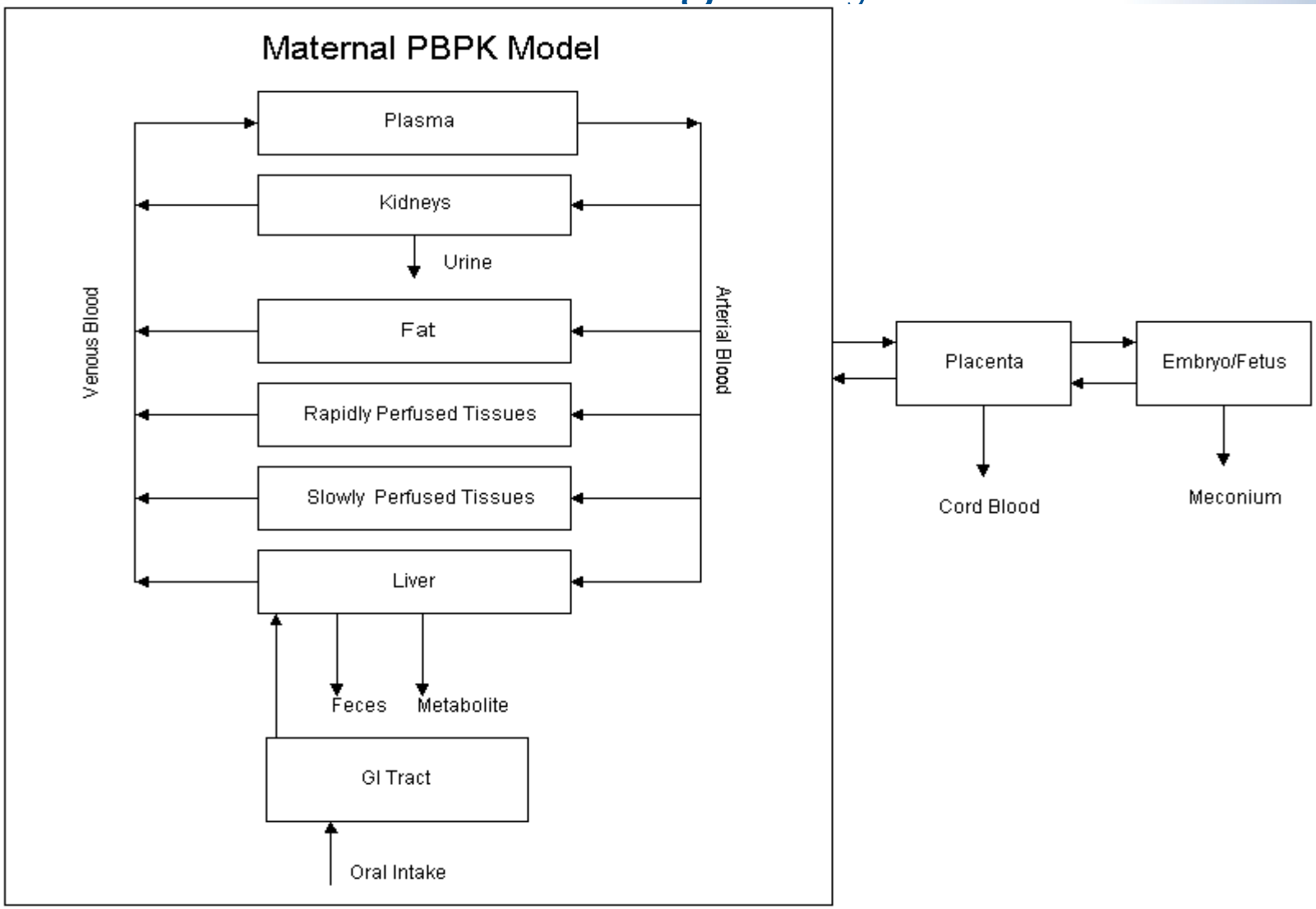
Approach

- **A PBPK model will be developed and validated for the target PBDEs that includes the gestational component.**
- **A chronic exposure scenario akin to that anticipated in humans will be used.**
- **Necessary partitioning and metabolic parameters will be measured using *in-vivo* and *in-vitro* experiments.**
- **Analytical methods for the PBDEs in all matrices under study will be validated. (Extraction, fractionation followed by GC/ECD, GC/NCI MS).**
- **The model will be scaled to humans and the applicability will be tested using biological samples collected from mothers and newborn infants.**

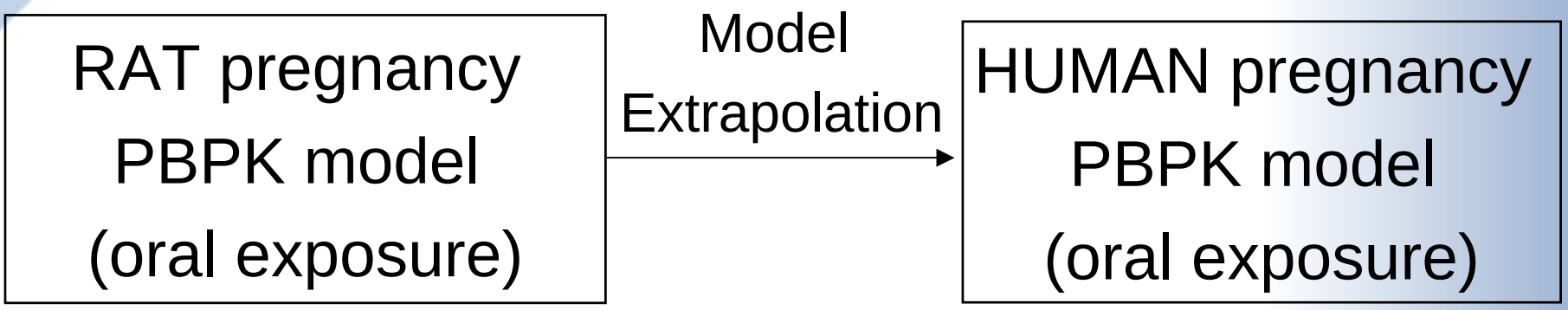
PBDE PBPK Pregnancy Model

- **Develop PBPK rat pregnancy model for PBDEs that can be used to estimate human fetal exposures to PBDEs**
- **At present, no published PBPK pregnancy model for PBDEs**
- **RAT model – oral exposure:**
  - **Animal studies (RTI): PCs; Metabolic rates; Single oral and repeated oral experiments; Single iv experiments; PBDE concentrations will be determined in plasma, urine, feces, and tissues**
- **HUMAN model – oral exposure:**
  - **Human data (RTI via local medical center): mother's blood (shortly before birth), cord blood, meconium, mother's and baby's weight, mother's age, linkage between mother/baby**

Schematic for Preliminary PBDE PBPK Pregnancy Model



PBDE PBPK Pregnancy Model



Model representation

Model parameterization

Model simulation

Model validation

Animal Studies

Experiment	*Goal*	*Sample Size*	*Dose (mg/kg)*
Partition Coefficient	PDBE-Specific Solubility in Tissues	5	N/A
Metabolic Rates in Hepatic Microsomes	PDBE-Specific Metabolic Rate Constants	3	N/A
Single Dose PO	Assess PK Parameters, Develop Model	3/dose/group	0, 1, or 50
Single Dose IV	Assess PK Parameters	3/dose/group	0, 1, or 50
10 day Repeat dose PO	Assess PK Parameters, Develop Model	3/dose/group	0, 1, or 50
Single Dose PO	Validate Model	3/dose/group	20
10 day Repeat dose PO	Validate Model	3/dose/group	20

Comparison of Rat and Human Hepatic P450 Content

	% of Total P450	
P450	***Human***	***Rat***
1A2	13 ± 7	<5
2A6	4 ± 4	6
2B6	0.15 ± 0.26	
2C	20 ± 8	60
2D6	1.7 ± 1.2	2
2E1	6.6 ± 3.1	10
3A4	29 ± 10	20

Differences between rat and human in substrate specificity and isoforms suggest that activities in both species should be studied.

Shimada et al. (1994) *Carcinogenesis*. 15, 2523-2529.

Geungerich, F.P. “Human Cytochrome P450 Enzymes” in *Cytochrome P450: Structure, Mechanism, and Biochemistry*, Ortiz de Montellano ed., Plenum, NY 1995.

Progress to date

Preliminary PBPK Model Development (Model Representation)

Synthesis of 2,2',4,4'- tetrabromodiphenyl ether (≈ 99.5% pure)

Synthesis of 2,2',4,4',5-pentabromodiphenyl ether (≈ 65% pure)

Method development started (solvent extraction/ASE; fractionation)

In-life animal studies begin July 2004

Current Tissue Method (Brain)

- Grind tissue with anhydrous sodium sulfate
- Add surrogate (PCB 198)
- Extract 3 times with Hexane (5 mL) – vortex, shake, vortex, centrifuge, decant
- Adjust volume to 15 mL; take 3 mL for lipid determination
- Concentrate remaining 12 mL to 1 mL using KD/micro Snyder
- Fractionate with Florisil
- Reduce Volume (25 mL to 1 mL)
- Add internal standard (PCB 119)
- Analyze by GC/ECD (GC/NCI-MS)

Spiked Brain Recovery

Triplicate aliquots of brain (29 mg) were spiked with 500 ng of tetra- and penta- BDE (17 ppm or μg/g); Extract, fractionate, and analyze using method. Analyze all fractions (1-3).

Recoveries of 80-82% of each target analyte; Majority in fraction 1.

Responses from blank approximately 2% of targets.

The background is probably acceptable for dosing studies; Might need to be a bit lower for human samples from environmental exposures (to detect low exposure situations rather than non-detect or no difference from background).

Future Work

Evaluate method for rat tissues; Modifications are likely, especially in the homogenization step.

Evaluate potential for necropsy contaminations (dust, etc.)

Determine partition coefficients

Perform animals studies; tetra-bromo (begin July); penta-bromo after acquisition of material

Determine metabolic parameters

Continue model development and apply as described

Scale to humans and apply to human samples

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