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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

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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

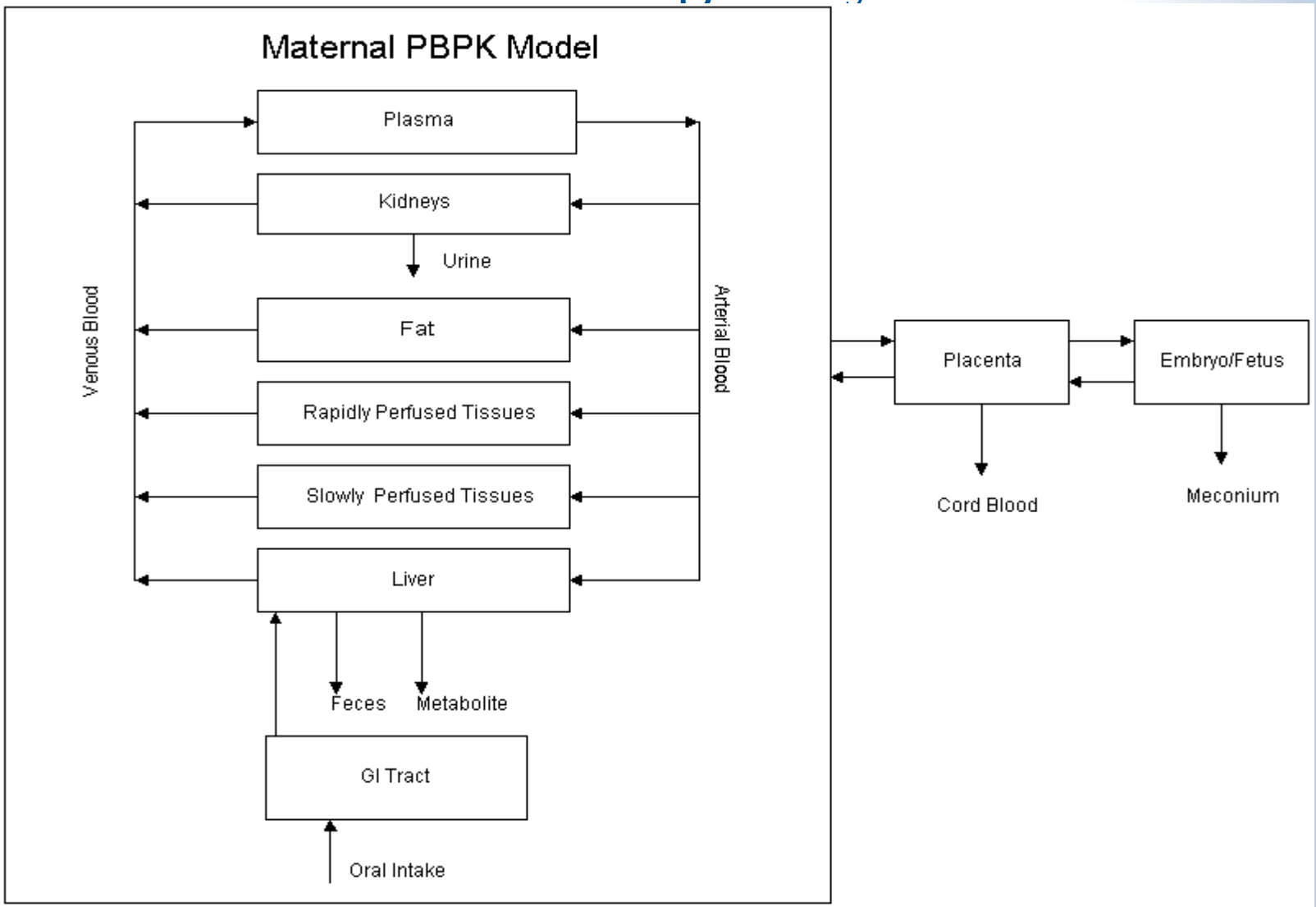
- **Develop PBPK rat pregnancy model for PBDEs that can be used to estimate human fetal exposures to PBDEs**

- **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

RAT pregnancy PBPK model (oral exposure)

Model Extrapolation →

HUMAN pregnancy PBPK model (oral exposure)

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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