

Incorporating Gene-Environment Information into Kinetic Models: Lessons Learned and Future Challenges

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Outline

- I. Statement of the Need
- II. Discussion of Case Study with CP
 - A. Building Toxicokinetic (TK) models for evaluating genetic polymorphisms
 - B. Building simple TK models for extrapolating across rodent studies
- III. Final Comments

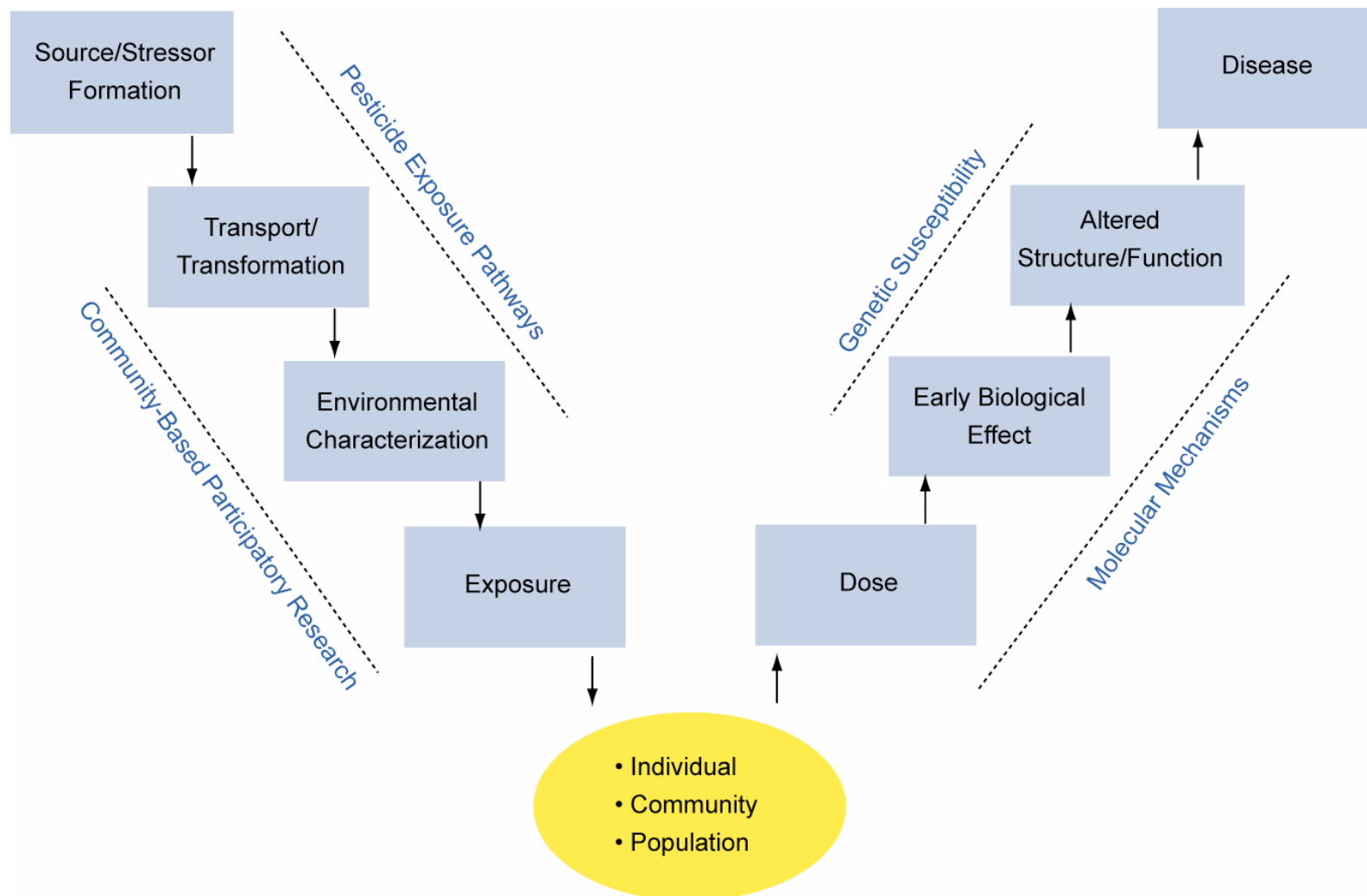
Introduction

Examples of Toxicokinetic models including information about genetic polymorphisms

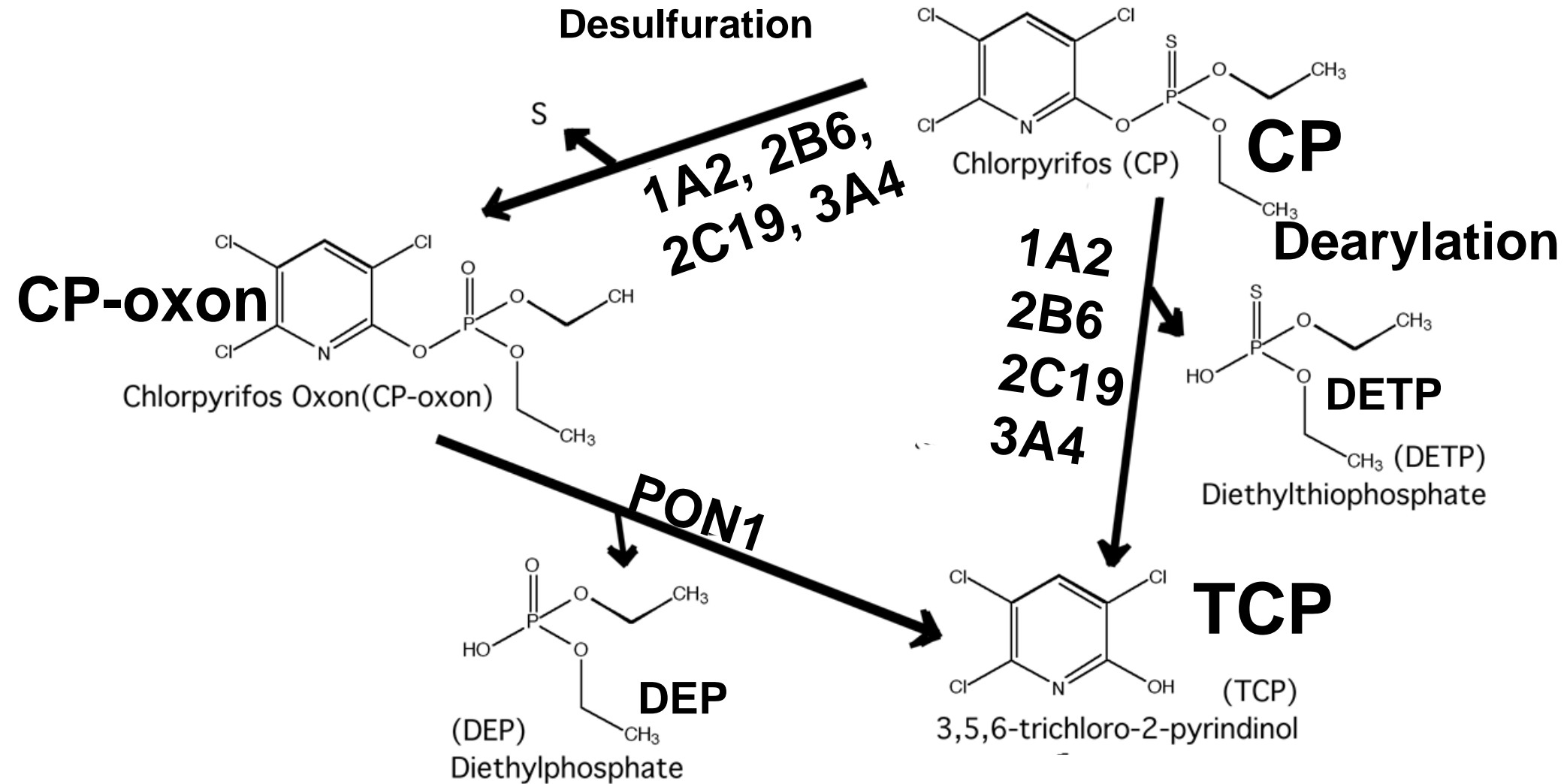
<u>Polymorphism</u>	<u>Chemical</u>	<u>Reference</u>
GST T1	Dichloromethane	El-Masri, et al 1999 Jonsson, et al 2001
PON1	Chlorpyrifos	Timchalk, et al 2002
ALDH2	Acetaldehyde	Teeguarden et al 2008
GST1M1	Acrylamide	Walker et al 2007
CYP2D6	Aripiprazole	Koue et al 2007
MDR1, CYP3A5	Indinavir	Solas et al 2007

NIEHS/EPA Center for Child Environmental Health Risks Research

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Metabolism of Chlorpyrifos



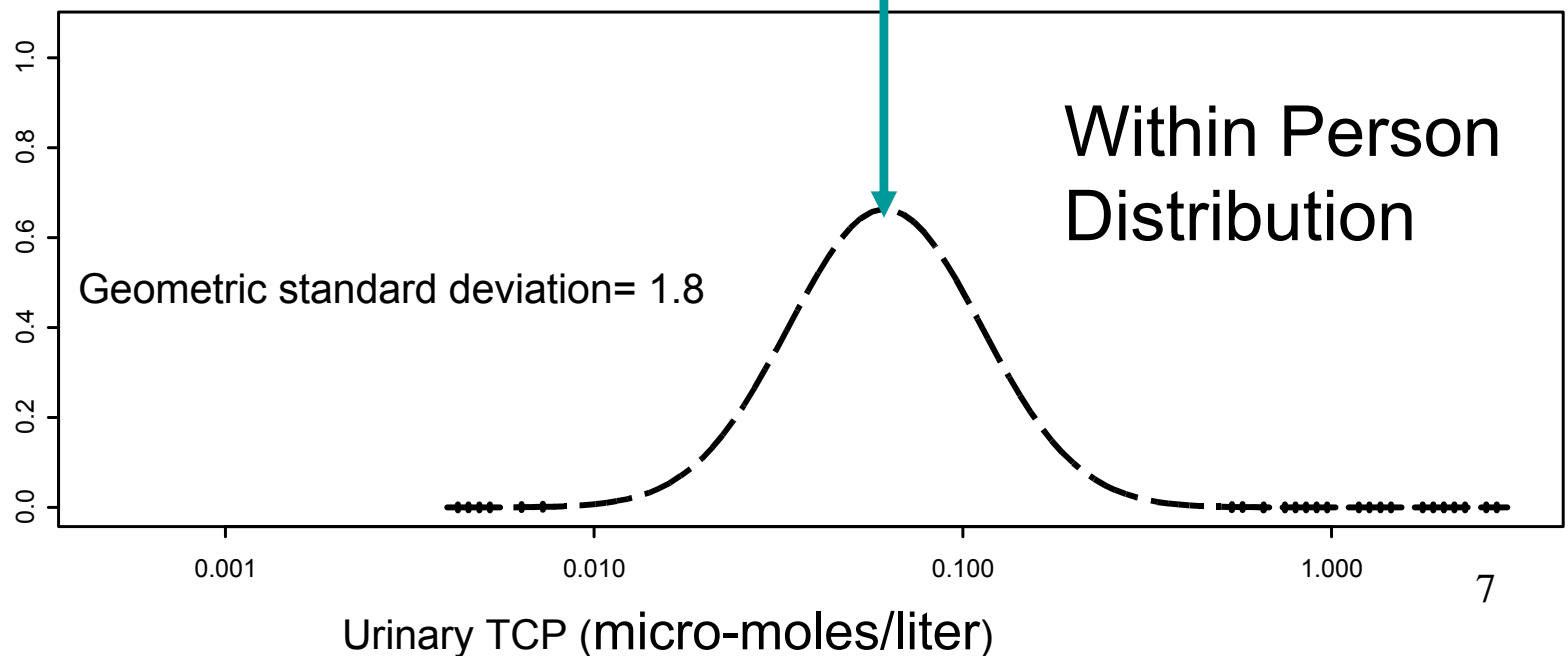
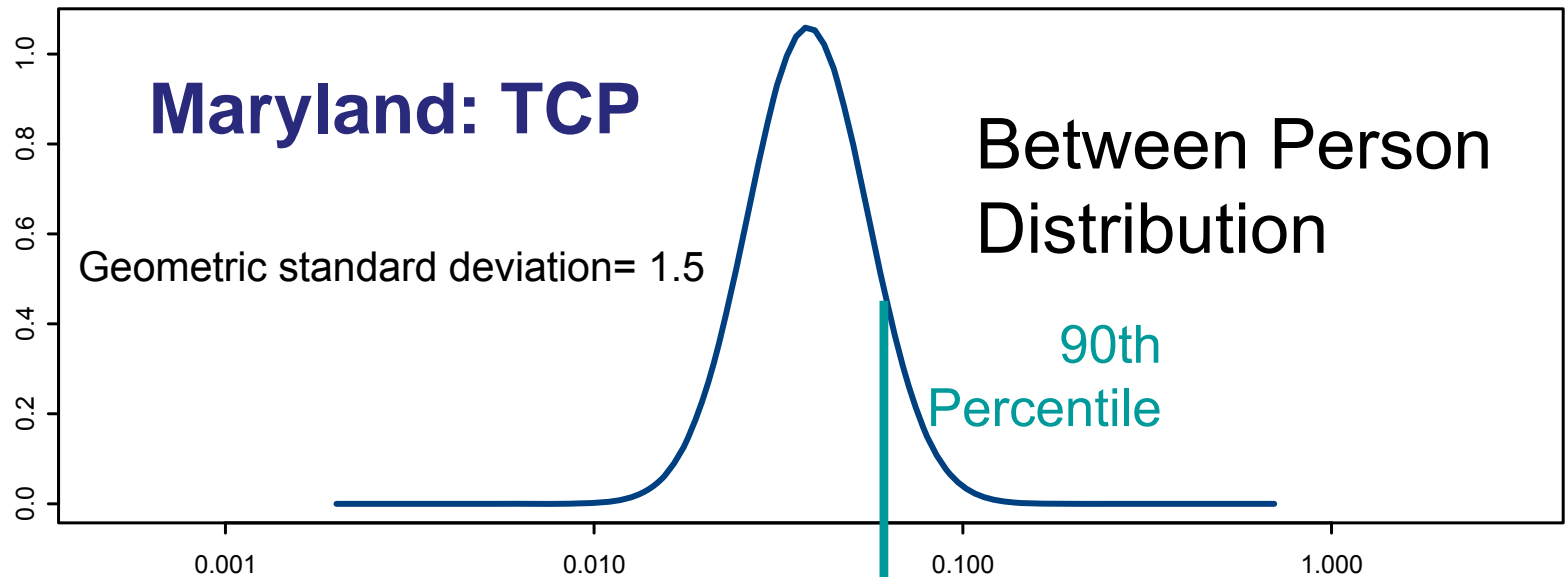
Metabolites of Organophosphate Pesticides

- Biomarkers of exposure
- Nonspecific Diakyl Phosphate (DAP) metabolites
 - Six DAP Metabolites
 - Each metabolite can be produced by multiple OPs
 - Divided into two groups
 - Dimethyl metabolites
 - DMP, DMTP, DMDTP
 - Diethyl metabolites
 - DEP, DETP, DEDTP
- Specific metabolites
 - Chlorpyrifos metabolites
 - TCP, DEP, DETP
 - Chlorpyrifos-methyl metabolites
 - TCP, DMP, DMTP

Within and Between Person Distributions for TCP

Macintosh, et al., JEAEE, 1999

NHEXAS database

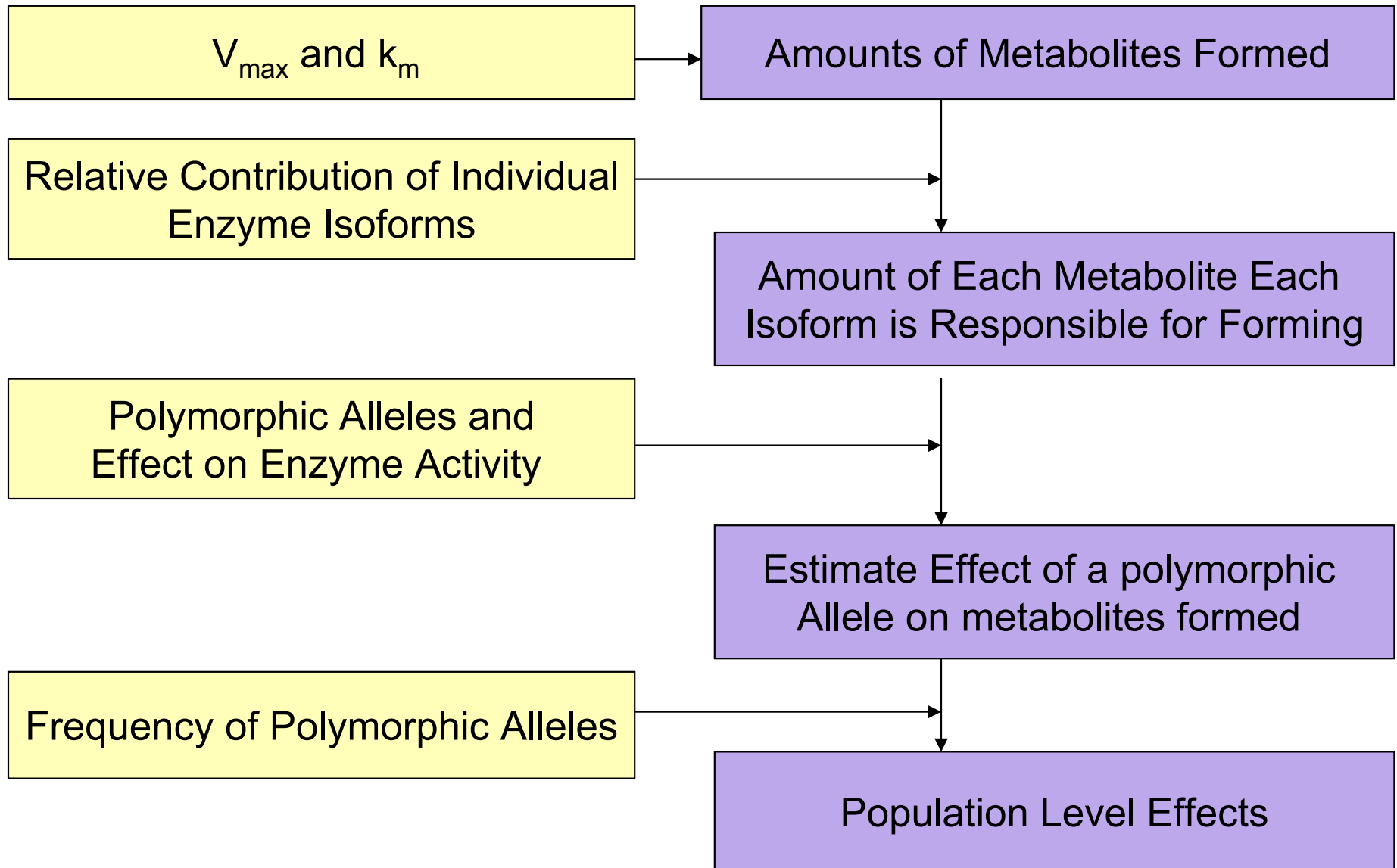


Simulating the effects of polymorphisms and abundance of CYP-450 enzymes in metabolism of chlorpyrifos.

*William C. Griffith , Jaya Ramaprasad, Ann Bradley, Elaine M
Faustman*

Objective is to survey limited CYP-450 literature on reactions with CP and determine how polymorphisms and amounts of CYP-450s may affect the amount of the toxic metabolite, CP-oxon produced

Data Needed for Modeling



Three types of studies were assessed to obtain parameters for the TK model

1. Immunoinhibition Studies - Evaluated the contribution of CYP450's by using antibodies for each specific CYP450 and measuring inhibition of reaction in human liver microsomes
2. Reconstituted Systems Expressing a Single Human Enzyme Isoform - Measured contribution of adding individual CYP450 isoforms to the overall concentration of As found (rates of reaction)
3. Recombinant System – Uses specific human CYP's expressed in ecoli and baculovirus infected insect cells (Supersomes)

The rate at which a CYP-450 metabolizes CP in a volume m by dearylation or desulfuration is

$$\frac{V_{\max} A \text{ CP}/m}{k_m + \text{CP}/m}$$

where V_{\max} and k_m are the Michaelas-Menten rates and A is the abundance of the CYP-450. At low concentrations of CP this simplifies to

$$\frac{V_{\max} A \text{ CP}/m}{k_m} = A \lambda \text{CP}/m$$

For the mixture of the four CYP450s $i= 1A2, 2B6, 3A4, 2C19$ with both dearylation, λ_{ai} , and desulfuration, λ_{si} , reactions the rate of metabolism for CP is

$$\frac{d \text{ CP}}{dt} = \frac{\text{CP}}{m} \sum A_i (\lambda_{si} + \lambda_{ai})$$

The rate of CP-oxon formation is

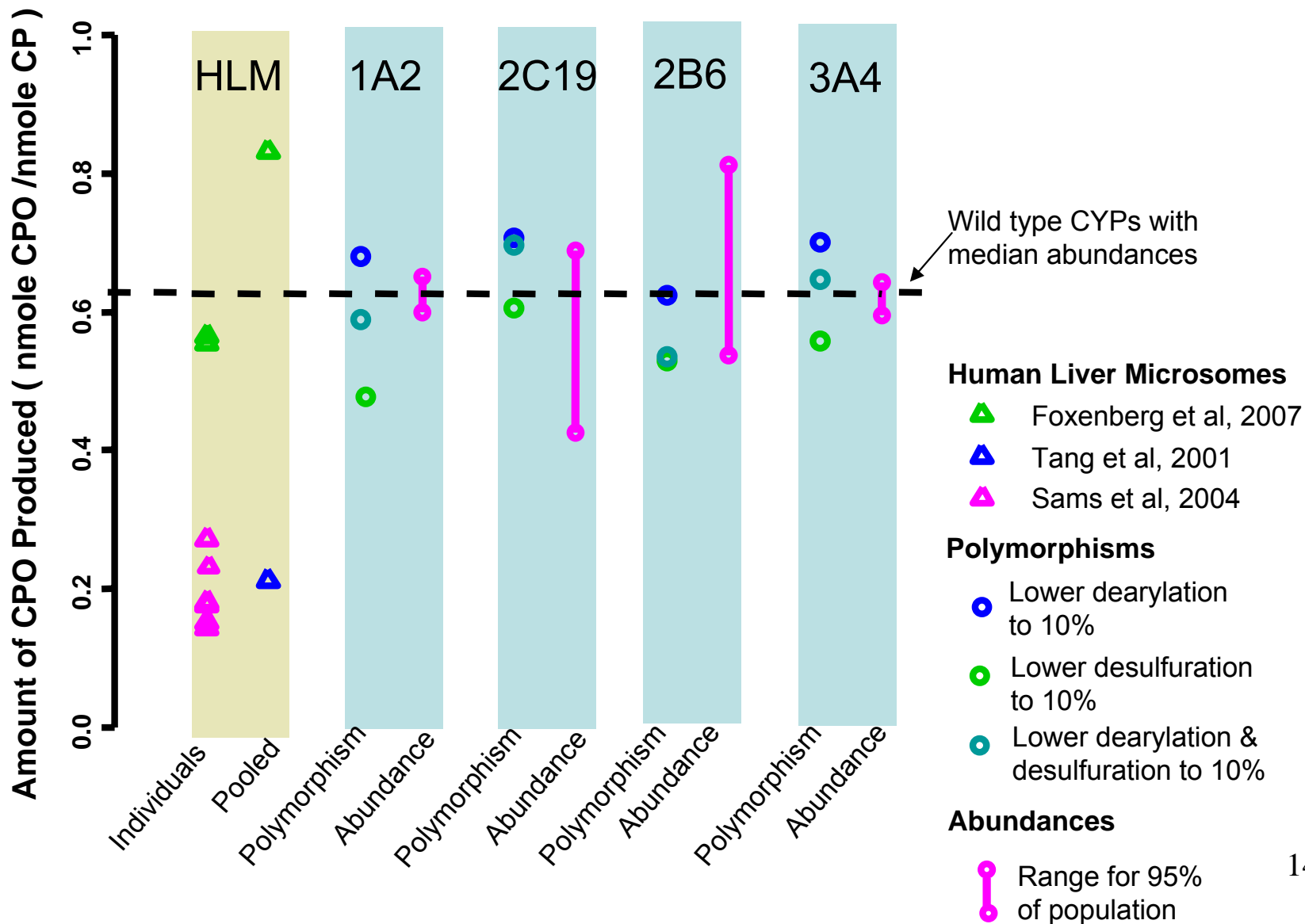
$$\frac{d \text{ CPO}}{dt} = \frac{\text{CP}}{m} \sum A_i \lambda_{si}$$

Parameters used in Toxicokinetic Calculations

Parameter	CYP450			
	2C19	3A4	1A2	2B6
A_i Abundance (pmol P450 /mg protein)	9.6 (2.4)	130 (1.8)	43 (1.8)	6.2 (2.9)
λ_{ai} Dearylation Rate (nmol oxon/ min/ nmol P450)	9.6 (1.9)	0.4 (1.4)	1.6 (1.6)	0.8 (1.5)
λ_{si} Desulfuration Rate (nmol oxon/ min/ nmol P450)	2.8 (2.3)	0.4 (1.2)	5.3 (2.8)	15.6(1.2)

Observed and predicted variability in CPoxon levels

Sensitivity Analysis for potential effects of polymorphism and abundance of CYP450



Conclusions

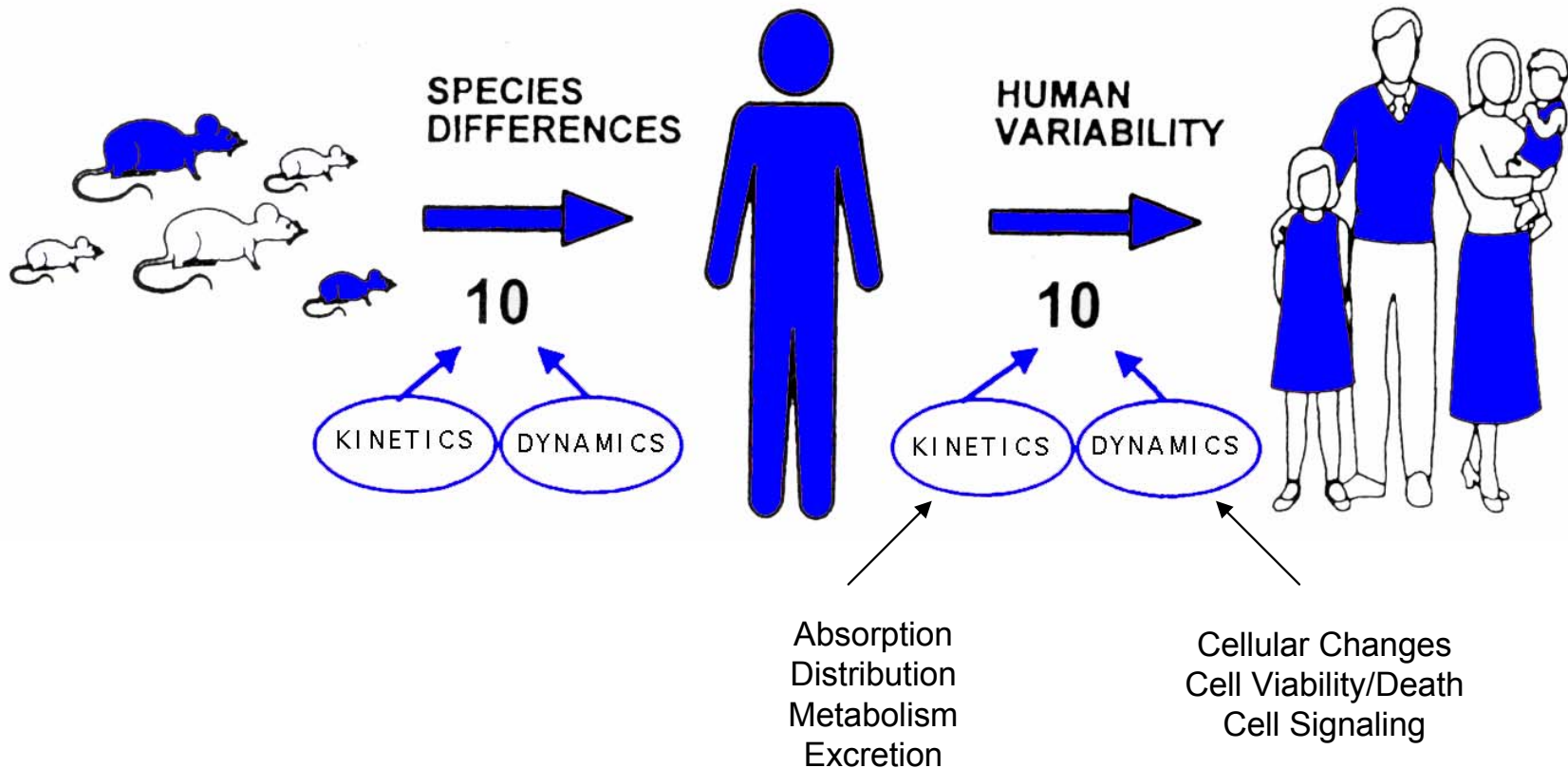
- Variability in the abundance of the individual CYP-450s is likely to have as large an effect on variability in the amount of CP-oxon produced as are genetic polymorphisms of CYP-450s
 - This occurs because all four enzymes exert an effect on the rate of CP-oxon production and when a polymorphism occurs in one CYP-450 the other enzymes are still effective in producing CP-oxon
 - These results suggest that observations made for PON1 may also hold for complex multi-enzyme metabolism of organophosphate pesticides (geno-phenotyping)

Conclusions (Cont'd)

- Literature on the abundances of CYP-450s is limited
 - Most studies have small sample sizes and do not report correlations among CYP-450s which could have a large effect for estimation of production of metabolites in multi-enzyme reactions
- More studies are needed for reaction rates of CYP-450 polymorphisms using organophosphate pesticides as substrates
- There are few studies on induction of CYP-450s by organophosphate pesticides
- These observations have implications for biomarker studies and storage of materials for biorepositories

Determining Acceptable Risk

The Use of Uncertainty Factors in Risk Assessment



Toxicokinetic and Toxicodynamic Factors Affecting Chlorpyrifos Developmental Toxicity

Kelly Schumacher, Bill Griffith, Jaya Ramaprasad, Elaine M Faustman

Example Challenges for Interpreting Rodent Studies for Children's Health Risk Assessment

- Availability of Rodent Studies with multiple experimental designs
- Experiments can be performed across wide range of doses and times of exposure
- Variability is seen between study outcomes examined and observed

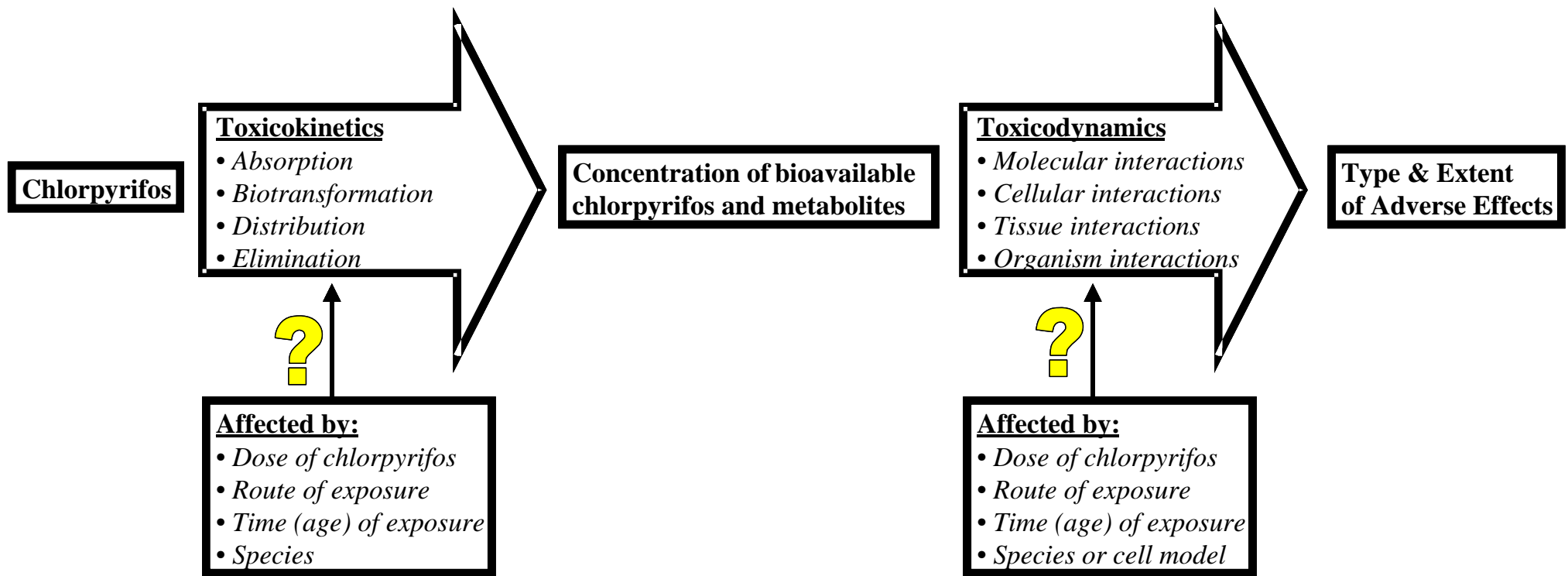
Can Models Help?

- ***Can toxicokinetic models help us evaluate the kinetic & dynamic factors affecting the neurodevelopmental toxicity of chlorpyrifos?***

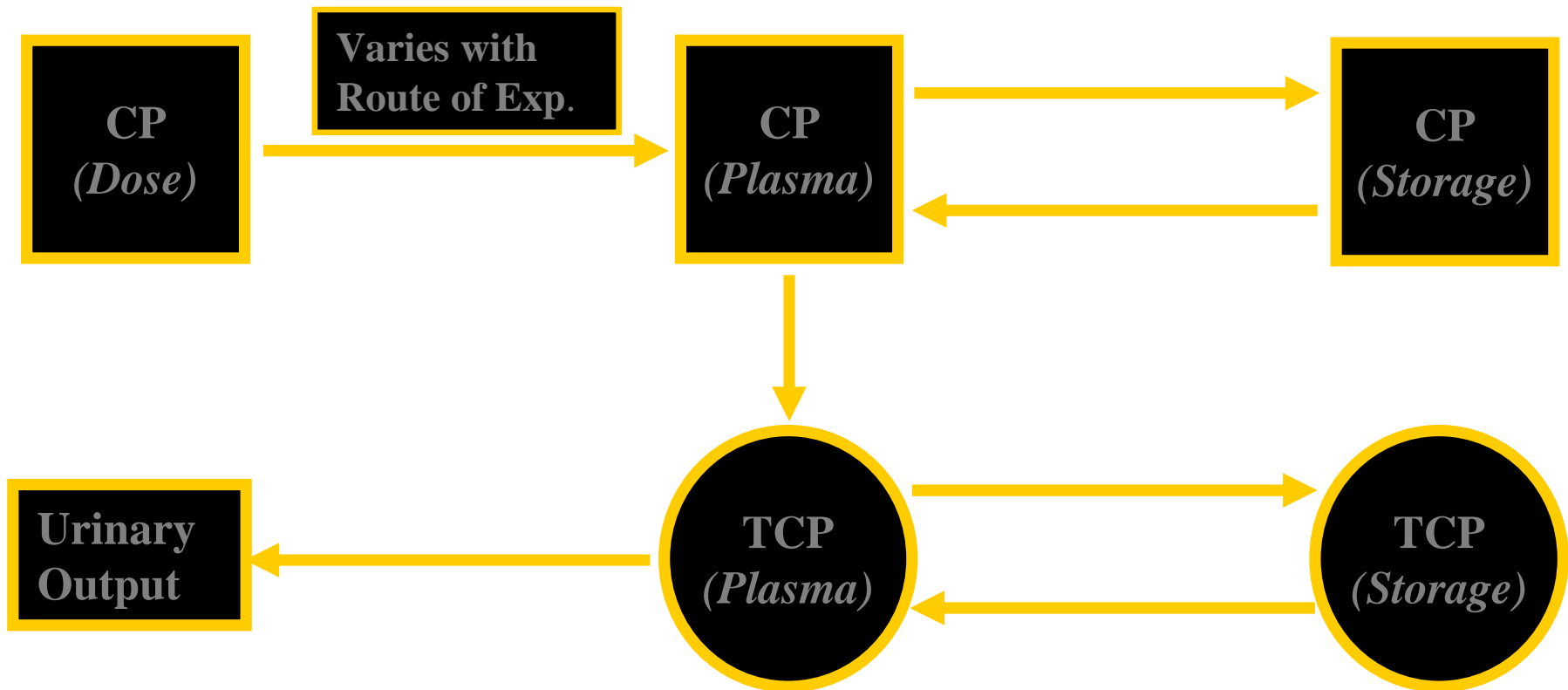
Methods

- ***Identification & assessment of animal studies that evaluate the neurodevelopmental toxicity of chlorpyrifos***
- ***Development of a toxicokinetic model***
- ***Data analysis based on toxicokinetic model***

Kinetic & Dynamic Considerations for Chlorpyrifos Neurodevelopmental Toxicity



Two Compartment Toxicokinetic Model



Toxicokinetic Modeling and the Evaluation of Factors Affecting Developmental Toxicity

- We modified the 2 compartment model by including different absorption half lives to distinguish between various rates of exposure.
- To implement the model the investigator sets:
 - The dose(s) of CP administered to the rodent;
 - The frequency of exposure (e.g. one every 24 hours);
 - The number of doses
 - The ending time (i.e. time of measurement) and;
 - The rate of exposure
- SAAM II program was used to calculate the plasma AUC for CP for each study design
- Maternal and neonatal plasma AUC were calculated at the time of each endpoint measurement.

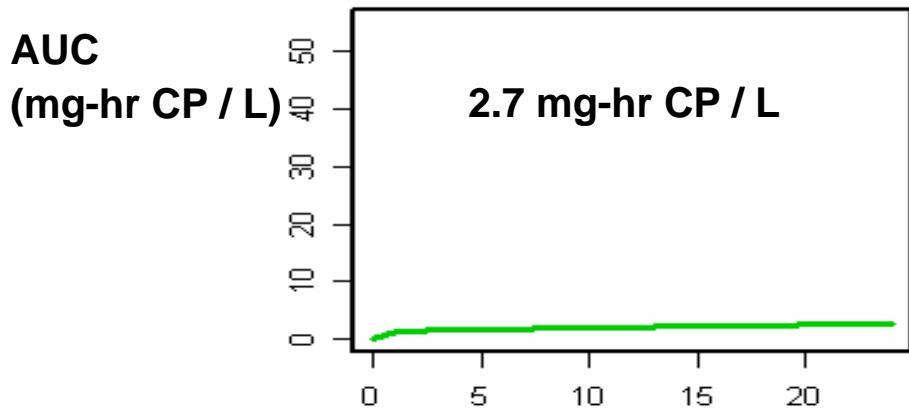
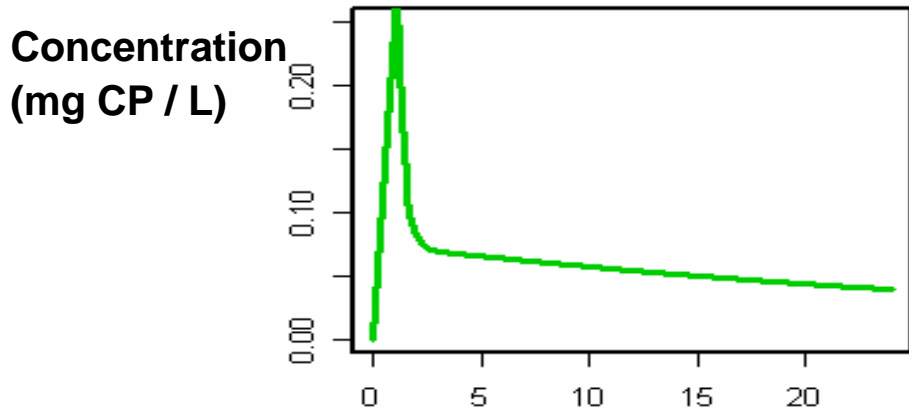
Modeling (Continued)

- For in utero experiments we used maternal AUC rates as a surrogate in these simple kinetic assessments to approximate fetal conditions.
- We calculated the AUC's using the toxicokinetic models for:
 - Multiple exposure routes across studies
 - Multiple doses within/across studies
 - Discontinuous timing of dosing
 - (e.g., different than every 24 hours)

Toxicokinetic Modeling

- **Example 1**

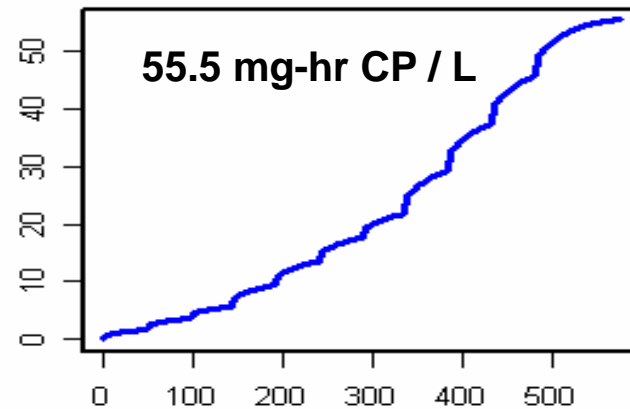
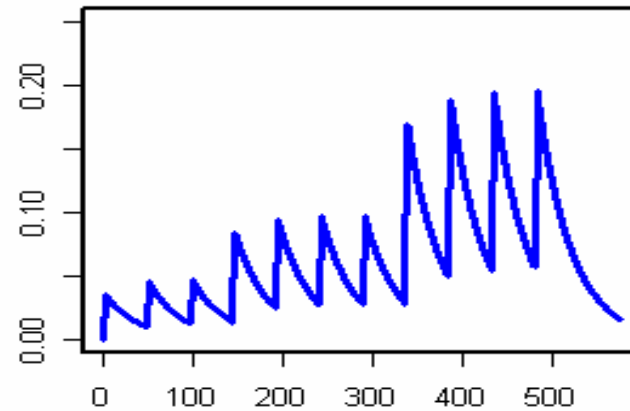
- Single dose
 - 5 mg/kg chlorpyrifos
- Subcutaneous injection
- Adverse effects measured 24 hours later



Time (hours)

- **Example 2**

- Dosing scenario
 - 3 mg/kg on PND1, 3, & 5
 - 6 mg/kg on PND7, 9, 11, & 13
 - 12 mg/kg on PND15, 17, 19, & 21
- Oral gavage
- Adverse effects measured on PND25



Graphical Representation of the Data

- **Axes**
 - y-axis: % change
 - x-axis: time of exposure
- **Points**
 - Shapes: route of exposure
 - **Colors: dose or AUC**
- **Arrows**
 - Duration of exposure
 - Timing of exposure

Administered Dose (mg/kg)	Color	Plasma AUC (mmol/L hr)
> 50	Red	> 75
40 to 49	Orange-red	50 to 75
30 to 39	Orange	25 to 50
20 to 29	Light orange	15 to 25
10 to 19	Yellow-orange	10 to 15
6 to 9	Yellow	8 to 10
4 to 5	Light yellow	6 to 8
3	Light green	4 to 6
1 to 2	Green	2 to 4
< 1	Bright green	0 to 2

▲	dermal
◆	i.p. injection
■	oral gavage
◆	s.c. injection

→ Exposed every 24 hrs

Identification & Assessment of Chlorpyrifos Developmental Toxicity

Animal Studies

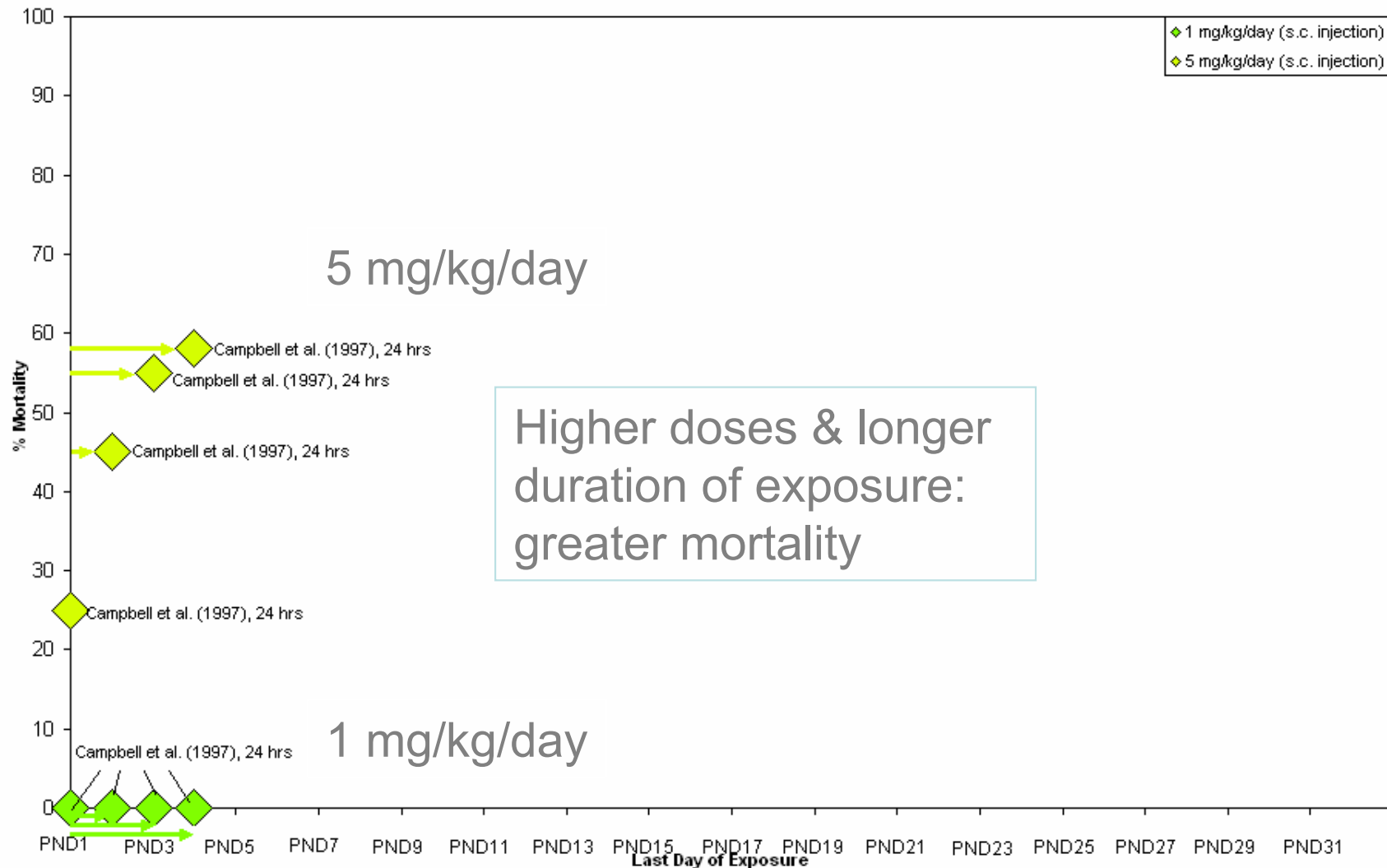
- Studies: 34
 - Endpoints
 - Mortality: 5
 - Body weight: 6
 - Brain morphology: 4
 - Macromolecules: 4
 - AChE: 4
 - BuChE: 4
 - CaE: 6
 - Total ChE: 21
 - Neurotransmitters: 10
 - Behavior: 9
- Rat strain
 - Sprague-Dawley: 26
 - Long-Evans: 8
- Route of exposure
 - s.c. injection: 18*
 - oral**: 15*
 - dermal: 1
 - i.p. injection: 1
- Time of exposure
 - Prenatal: 12*
 - Postnatal: 24*
 - Pre- and postnatal: 2*

* Study used more than one route or time of exposure

** Includes 14 oral gavage and 1 oral (vanilla wafer)

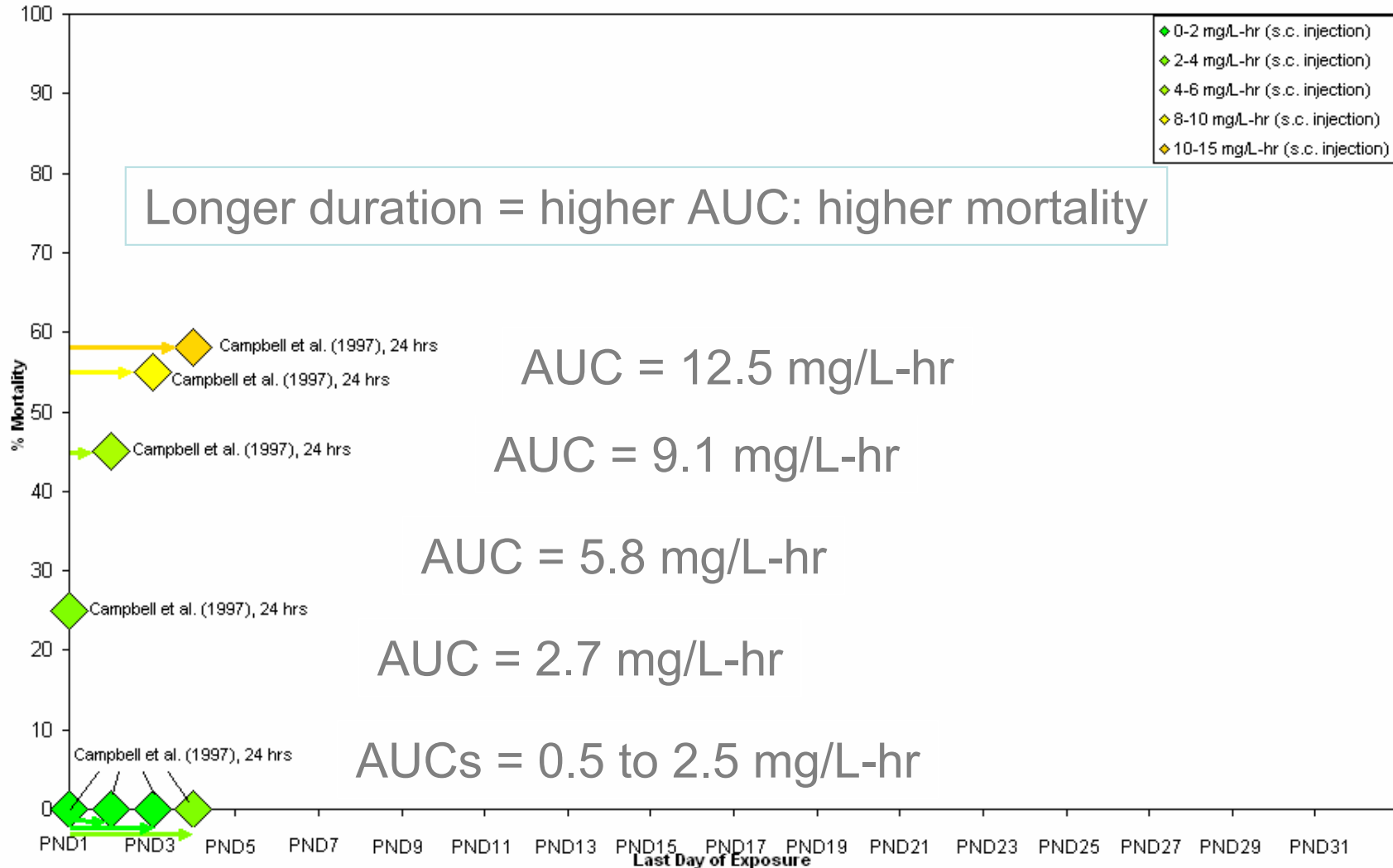
Neonatal Mortality – Admin. Dose & Duration

Neonatal Mortality Following Postnatal Exposure to Chlorpyrifos vs. Last Day of Exposure
grouped by administered dose chlorpyrifos



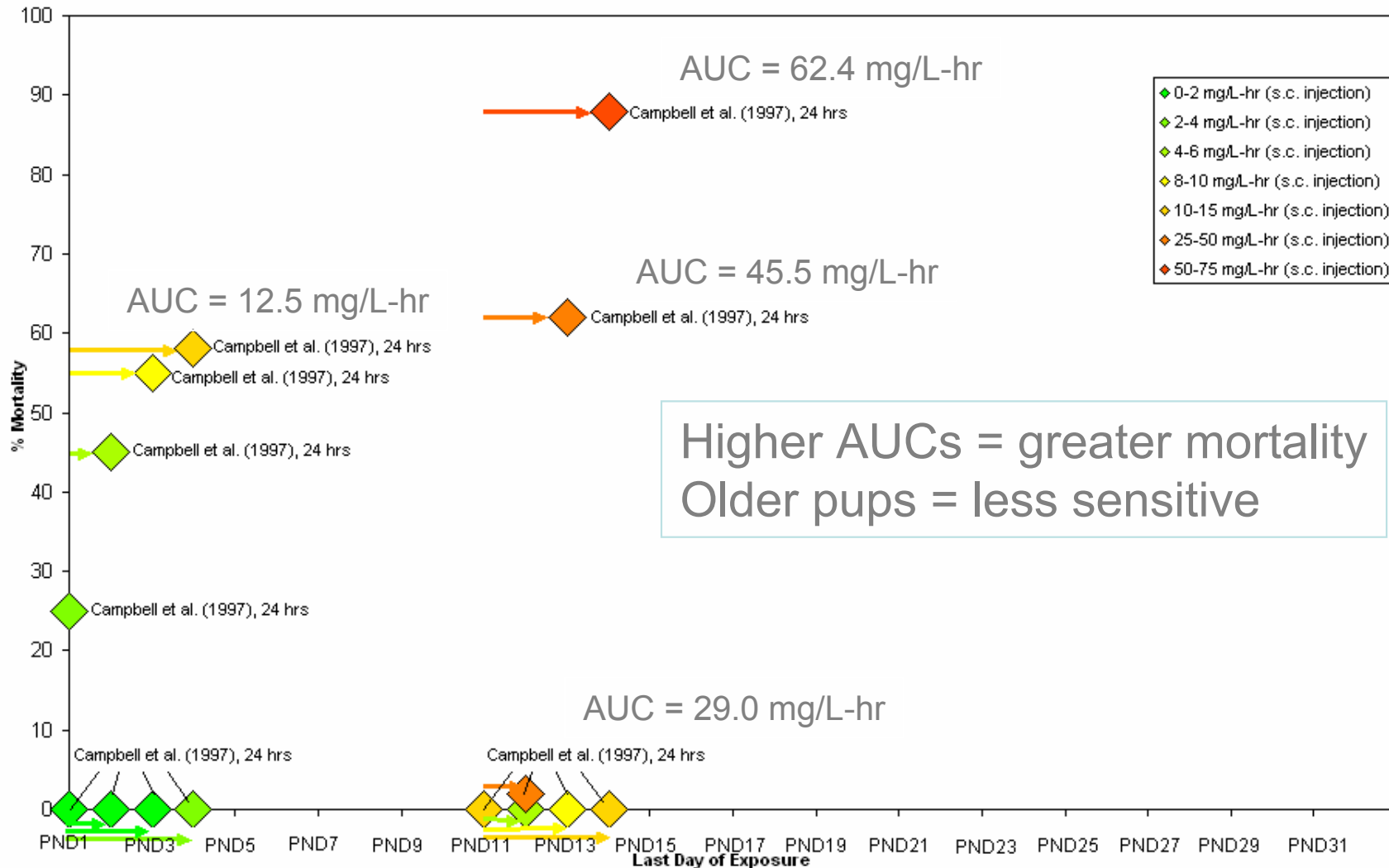
Neonatal Mortality – AUC & Duration

Neonatal Mortality Following Postnatal Exposure to Chlorpyrifos vs. Last Day of Exposure
grouped by neonatal plasma AUC chlorpyrifos



Neonatal Mortality – AUC, Duration, & Time

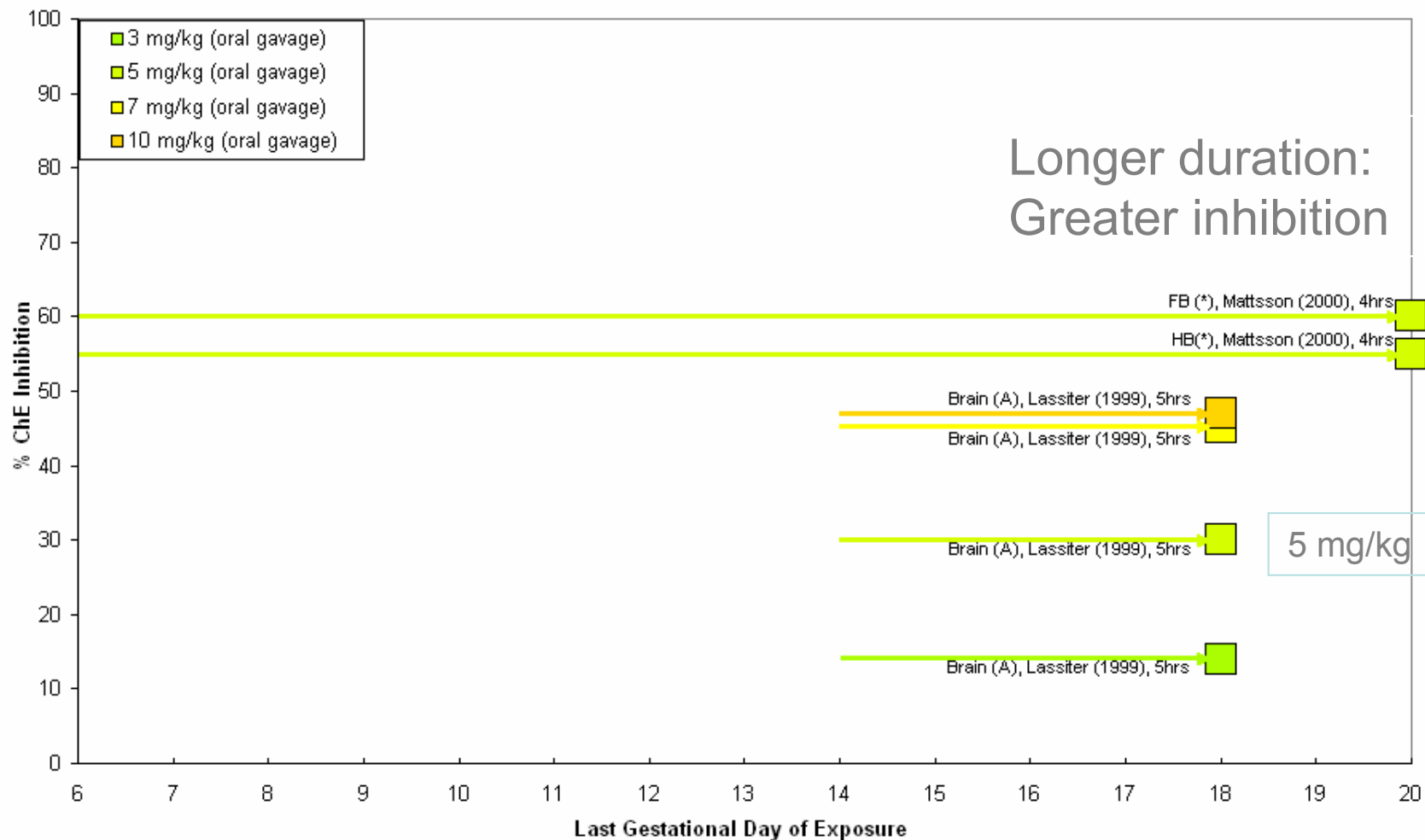
Neonatal Mortality Following Postnatal Exposure to Chlorpyrifos vs. Last Day of Exposure
grouped by neonatal plasma AUC chlorpyrifos



Fetal Brain ChE Activity – Admin. Dose & Duration

4-5 hours after last dose

Fetal Brain ChE Inhibition Following Prenatal Exposure to Chlorpyrifos vs. Last Day of Exposure
grouped by administered dose chlorpyrifos; measured 4-5 hours after last dose



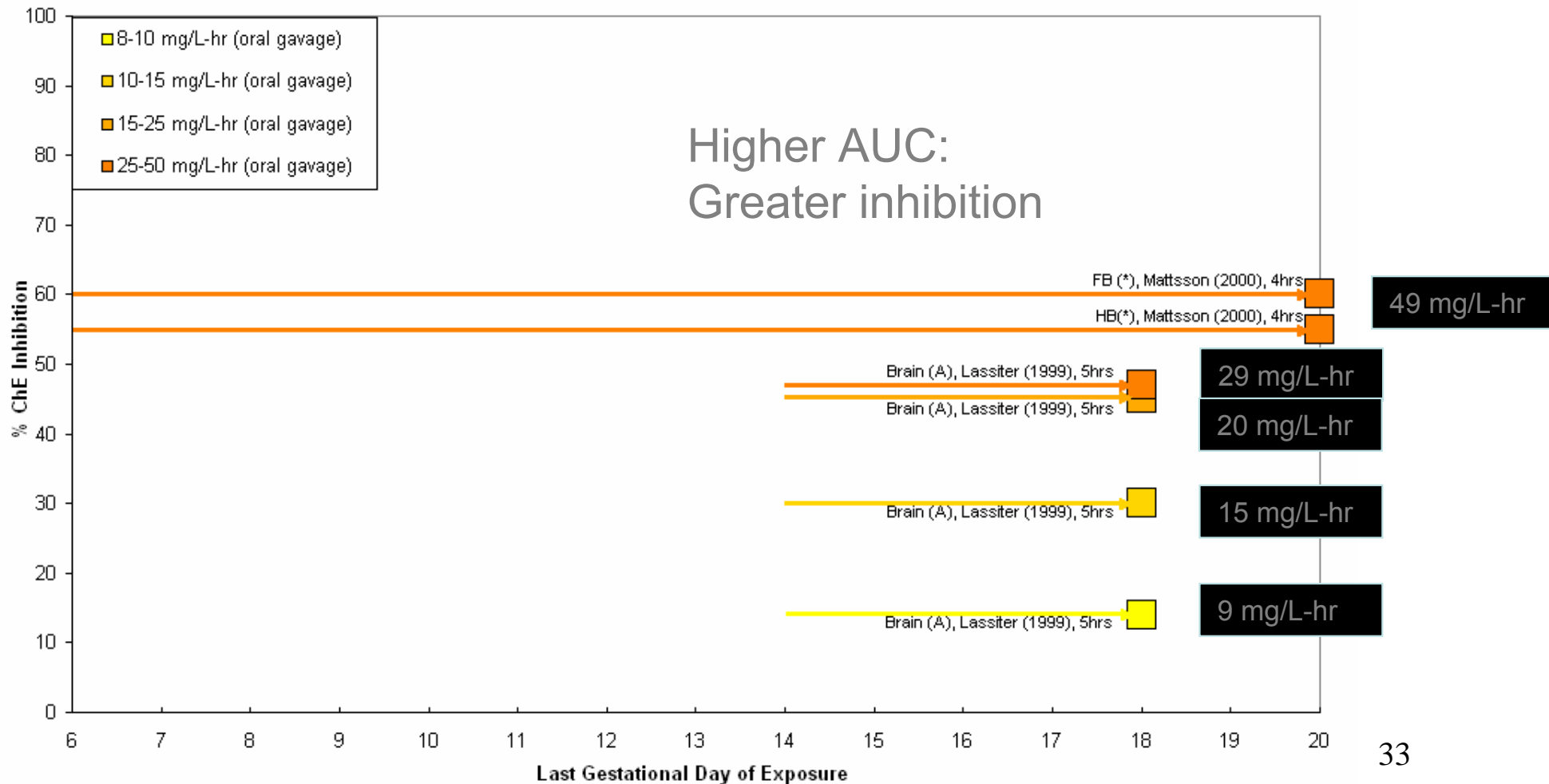
5 mg/kg

5 mg/kg

Fetal Brain ChE Activity – AUC

4-5 hours after last dose

Fetal Brain ChE Inhibition Following Prenatal Exposure to Chlorpyrifos vs. Last Day of Exposure
grouped by maternal plasma AUC chlorpyrifos; measured 4-5 hours after last dose



Observations

- This simple kinetic model allowed us to better compare across rodent experiments especially among different designs
- Application of this approach for other compounds
 - A fairly simple pharmacokinetic model can help us compare these studies
 - We have a way to create graphs to visually represent data
- In combination with our polymorphism analyses we have demonstrated 2 examples of how toxicokinetic models can improve our ability to inform quantitative risk assessment

Acknowledgements

- US Environmental Protection Agency Biomarkers grant (RD-83273301) and contract (2W-2296-NATA)
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- The Institute for the Evaluation of Health Risks