

## Systems Biology Approaches for Dose-Response Assessments – An Example with an Androgen Synthesis Inhibitor

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Systems biology is the quantitative evaluation, through laboratory experiments and computer modeling, of the manner in which the components of biological systems are organized together to give rise to biological function. Toxicity is a perturbation in those biological processes leading to adverse responses. Here, we begin to apply a systems biology approach with the goal of creating a mechanistic dose-response model of perturbations of Leydig cell steroidogenesis by one class of androgen synthesis inhibitors – phthalic acid monoesters (PAMEs). The approach has two broad components: (1) biological and computational studies to create a model of the molecular biochemical pathways that controls steroidogenesis in the mouse Leydig cell, and (2) laboratory research to identify the site(s) of action through which PAMEs perturb this circuitry. Our research plan, now amended, examines the hypothesis that PAMEs block steroidogenesis by specifically targeting an enzyme, cytosolic phospholipase A2 (cPLA2), involved in arachidonic acid release from nuclear membranes. This project has three specific aims (SAs). SA 1 examines the dose-response for inhibition of phospholipase A2 (PLA2) activity and steroid synthesis in a mouse Leydig cell tumor line (MA-10) by PAMEs and chloroquine, a known PLA2 inhibitor. SA 2 uses inhibitory RNA and gene overexpression technology to specifically alter cell dosage of PLA2s, in order to more directly assess the roles of PLA2s in steroidogenesis and in actions of PAMEs. SA 3 develops computational modeling efforts that couple altered cPLA2 activity and arachidonic acid release and steroid production. SA 3 includes further development of a physiologically based pharmacokinetic model for phthalates and analysis of various *in vitro* study results for phthalate effects on male rat pup development. The expected outcome of the project is a mechanistic dose-response model for toxicant inhibition of steroidogenesis that will aid in risk assessments through low-dose, interspecies, dose-route, and, eventually, life-stage extrapolation. The research in this proposal lays the foundation for a systems biology approach to dose-response assessments for a wide variety of compounds with diverse endpoints. The complete systems biology model for steroidogenesis is not a goal in this project. However, promising *in vitro* models have been developed that should assist structure activity modeling of phthalate ester inhibition of steroidogenesis.

**Implications:** Systems biology combines laboratory experimentation and computer modeling to understand perturbation of biological processes by toxic chemicals. Here, a systems biology approach was evaluated for creating dose-response models for perturbation of steroidogenesis by phthalate esters. The approach had two components: (1) biological and computational studies to create a model of the molecular circuits for steroidogenesis, and (2) laboratory research to identify site(s) of action for the phthalates. This project produced a dose-response, dosimetry model useful in guiding low-dose, interspecies, dose-route, and life-stage extrapolations. Modes-of-action studies identified promising pathways for structure activity modeling for diverse phthalate in mouse MA-10 cells.

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### Presentations:

Andersen, M. E. (2005). Computational systems biology and dose response assessment. Web seminar, Dose Response Specialty Group, Society of Risk Analysis, September 6, 2005.

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Clewell, R. A., Kremer, J. J., Williams, C. C., Campbell, J. L., Andersen, M. E., and Borghoff, S. J. (2008). Tissue exposures to free and glucuronidated monobutylphthalate in the pregnant and fetal rat following exposure to di-n-butylphthalate: Evaluation with a PBPK model. *Toxicological Sciences* 10: 241–259.

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Andersen, M. E. (2005). Enhancing interspecies extrapolation with quantitative pharmacokinetic and pharmacodynamic models. Risk Policy Report-[www.insideepa.com](http://www.insideepa.com)-September 6, 2006, p. 12–13.

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