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Evaluation of Signal Transduction Pathways in Model Organisms as Critical Mediators of Developmental Toxicity

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Background: Development, when considered at the level of the cell, can be reduced to a series of events leading to the proliferation, migration, differentiation, and in some cases programmed death of the cell. The precise sequence of these events and the nature of the cell differentiation is dependent to some extent on the lineage of the cell, to a greater extent on positional information, and is choreographed by a series of signals passed between cells, and the subsequent responses to those signals. The series of biochemical events leading from stimulus to response is called signal transduction, and the biochemical elements involved are called signal transduction pathways. There are fewer than 20 known signal transduction pathways. These pathways, more than any other aspect of biochemical function, represent the highest level of evolutionary conservation of developmental processes across the animal kingdom. Altered function of a pathway has adverse consequences for development.

Because of the critical role of signal transduction pathways, and because they represent such a convergence in the response of cells to external stimuli, we tested whether alternative assays in lower species that specifically evaluate the outcome of pathways may be highly predictive of developmental toxicity. Because of the highly conserved nature of these pathways across the animal kingdom, and because of the availability of genetically sensitized strains, we chose to use the extremely well characterized developmental biology models zebrafish, *Drosophila*, and the nematode *C. elegans*, as surrogate species for screening for developmental toxicants.

Objectives: The overall objective of the proposed research is to determine whether model organisms with genetically sensitized signal transduction pathways could be used as predictors of mammalian developmental toxicity. Specifically, we evaluated the effect of model developmental toxicants on the morphological development of a number of strains of *Drosophila*, *C. elegans*, and zebrafish that were sensitized for specific signal transduction pathways. Results were compared with wild-type organisms

Results: Strains were chosen that had a sensitizing mutation in a particular signal transduction pathway; the mutation was not severe enough to significantly alter development on its own, but rendered the pathway more sensitive to exogenous perturbation. We evaluated 8 sensitized strains of *C. elegans*, nine of *Drosophila* and three of zebrafish, covering the pathways relevant to development. We tested a panel of developmental toxicants intended to cover a wide range of modes of action. The results indicate that there was interaction between toxicants and signal transduction

pathways, and that there was specificity for these interactions, within species at least. However, the concordance across species was not strong. Analysis of the results suggests that this was not the result of experimental shortcoming, and is most likely attributable to evolutionary disparity in factors that are distant to the signaling pathways themselves. We also evaluated xenobiotic metabolic capability of the tested species. There does appear to be some ability to metabolize xenobiotics, however, not unexpectedly there is a great deal of evolutionary divergence in substrate specificity.

Conclusions: In summary, it appears that the high level of evolutionary conservation and small number of signal transduction pathways did not provide a pragmatic solution for using lower species to predict human developmental toxicity. While there is clear indication of interaction between toxicants and signal transduction pathways, and reasonable support for the specificity of these interactions, there was not a high level of concordance of these responses across species. Therefore, predictions of mammalian toxicity based on the outcome of these experiments would be uncertain.