# Signal Transduction Pathways: Potential Foundation for Developmental Toxicity Alternatives

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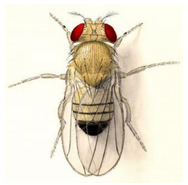
Abstract: The purpose of this project was to test the hypothesis that signal transduction pathways are integral to the toxicity of developmental toxicants. These pathways are highly conserved through animal evolution, and only a few are active during development. If these pathways are a target for toxicity, it should be possible to screen for human developmental toxicants using the embryos of lower species. We used embryos of three species, the nematode C. elegans, the insect Drosophila, and the zebrafish. The developmental genetics of these species is thoroughly characterized. 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 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 upstream of 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.

| Developmental Stage | Signal Transduction Pathways |
|---|---|
| **Early development** | Wingless-wnt<br>TGF-beta (Receptor serine kinase and receptor threonine kinase)<br>hedgehog<br>Receptor tyrosine kinase<br>Notch-delta<br>Cytokine receptor (cytoplasmic tyrosine kinases; STAT) |
| **Organogenesis/ differentiation** | Interleukin-1/Toll/NFkappaB<br>Nuclear hormone receptors<br>Apoptosis<br>Receptor phosphotyrosine phosphatase |
| **Post-differentiation** | Receptor guanylate cyclase<br>NO receptor<br>G-protein-coupled receptor integrins<br>Cadherins<br>Gap junctions |

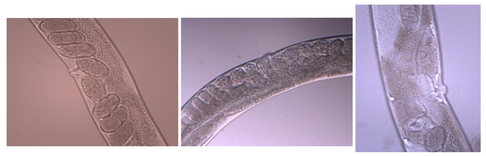
| Signaling pathway | Mutant | | |
|---|---|---|---|
| | C. elegans | Drosophila | zebrafish |
| *Early development* | | | |
| TGF-beta | Sma-6 | Dpp397, tkv427 | Bmp4 |
| Wnt-wingless | WT74 | Wg(spd1005) | |
| hedgehog | Not present | Hh646 | smo |
| JAK-STAT | RB796 | Os79 | |
| Receptor tyrosine kinase | flk-1 | Ras1475 | Erbb3 |
| Notch-delta | RB1337 | Notch118 | |
| *Organogenesis/ differentiation* | | | |
| apoptosis | LET-23, MT3002 | DIAP5783 | |
| IL-1/Toll | IG-10 | Toll3238 | |

C. elegans



Drosophila

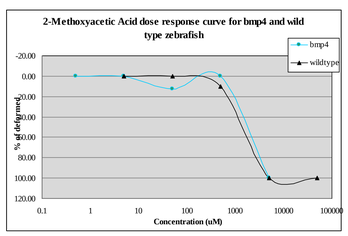
zebrafish

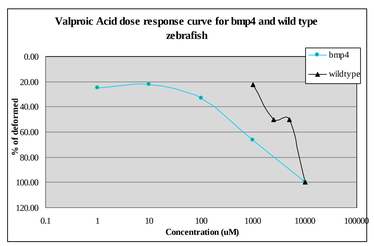


Interaction between a toxicant (diphenylhydantoin) and the apoptosis signal transduction pathway in C. elegans, resulting in vulval narrowing and decreased reproductive capability.

Example of interaction between retinoic acid (50 nM) and a mutation in the hedgehog signaling pathway. All abnormal zebrafish in this image are homozygous for the mutation. The morphologically normal fish are either heterozygous for the mutation or wild-type.


Wing vein phenocopies that occur spontaneously in strains of fruit flies that have a sensitizing mutation in the TGF-beta pathway, but are increased in frequency by toxicant exposure. The wing vein gap (upper right) was observed in the dpp strain; the loop (lower left) in the tkv strain.




Typical dose-response data indicating interaction between sensitizing mutation and chemical (right) and no interaction (left)

| Chemical | mode of action |
|---|---|
| 6-aminonicotinamide | Inhibitor of intermediary metabolism via interference with NADPH supply |
| 5-fluoruracil | interferes with DNA and RNA synthesis |
| cadmium | Interferes with sulfhydryl groups in metalloenzymes, transcription factors and cytoskeleton |
| dexamethasone | Activates glucocorticoid receptor |
| 5,5-diphenylhydantoin | Enhances GABA activity in neurons, may induce macromolecule damage in embryos via an epoxide intermediate |
| 2-ethylhexanoic acid | Unknown, possible HDAC inhibitor |
| 2-methoxyacetic acid | Active metabolite of 2-methoxyethanol, possibly acting on cell proliferation |
| methotrexate | Folate antagonist, inhibition of one-carbon metabolism |
| Valproic acid | HDAC inhibitor |
| Retinoic acid | Retinoic acid receptor agonist, affects numerous developmental processes |
| 2-methoxyethanol* | Unknown, possible effects on proliferation |
| Cyclophosphamide* | Alkylating agent (DNA and proteins) |

* - these compounds require metabolic activation to be toxic

**Significant interaction of toxicants with developmentally relevant signal transduction pathways.**

| Signaling pathway | Chemical | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6-AN | 5-FU | Cd | Dex. | DPH | 2-EHA | 2-MAA | MTX | VPA | RA |
| *Early development* | | | | | | | | | | |
| TGF-beta | D | D,Z | | C,Z | D | Z | | Z | D,Z | D |
| Wnt-wingless | D,C | | | D,C | D,C | | | | | |
| Hedgehog* | Z | Z | | | | D | | | | Z |
| JAK-STAT | D | | | D,C | | D | | | | |
| Receptor tyrosine kinase | D,C,Z | | | | D,Z | | | Z | | |
| Notch-delta | | | | D | D | | | | | |
| *Organogenesis / differentiation* | | | | | | | | | | |
| apoptosis | | | | D,C | D,C | | | | | |
| IL-1/Toll | | D,C | | | | | | D | | |

* - pathway not present in C. elegans
C = C. elegans, D = Drosophila, Z = zebrafish

**Interactions between toxicant and pathway are replicated in strains with mutations in different elements of the same signaling pathway**

| Strain/Pathway | Chemical | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6-AN | 5-FU | Cd | Dex. | DPH | 2-EHA | 2-MAA | MTX | VPA | RA |
| *C. elegans/ apoptosis* | | | | | | | | | | |
| Let-23 | | | | X | X | | | | | |
| MT3002 | | | | X | X | | | | | |
| *Drosophila/ TGF-beta* | | | | | | | | | | |
| dpp | X | X | | | X | | | | X | X |
| tkv | | * | | | X | | | | X | X |

X indicates significant interaction between the toxicant and the sensitized strain.
* - there was an interaction between toxicant and strain for this chemical, but it did not reach the 5-fold criterion that we had set a priori for “significant interaction”

## Conclusions

- Developmental toxicants interact with signal transduction pathways
  - Almost all chemicals tested interacted with one or more pathways
- Within species, this interaction appears to have specificity
  - Sensitizing mutations in different elements of the affected pathway still predisposed the embryos to developmental toxicity by toxicants active on that pathway
- Concordance across species was not strong, however
  - This indicates that the initial interaction of the chemical with the biological system is upstream of the signal transdution pathways, where evolutionary conservation is less rigorous
- In sum, these results indicate that the approach of using genetically sensitized non-mammalian species will not be useful in predicting human developmental toxicity
- However, given the clear role of signal transduction pathways in mediating developmental toxicity across a wide range of chemical classes and modes of action, these models may be very useful for investigating mechanisms of developmental toxicity.