Non-Mammalian In Vivo models: C. elegans As a Model System to Inform Hazard Identification

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Conflict of Interest Statement

Disclaimer:
The data, views, and opinions presented here are those of the presenter and do not represent FDA policies or positions.

The presenter has no competing interests.
Affiliation
C. elegans biology

Comparison of *C. elegans* model to other models

Established methods for assessing toxicity to *C. elegans*

Utility of *C. elegans* model for hazard identification of mixtures

A novel method for assessing developmental toxicity in *C. elegans*
What is *Caenorhabditis elegans*?

Adult Length ~ 1mm
What is Caenorhabditis elegans?
**Caenorhabditis elegans**

*C. elegans* adult and 1st stage larvae (L1)

Oxidative Stress Response *gst-4p::GFP* transgene
C. elegans Transgenic Strains Can Rapidly Detect Changes in Toxicity Pathways

Transgene Expression with Arsenic Compounds

Oxidative Stress Response with PbOAc + NaAs
Conserved Pathways

Key Elements of the Apoptosis Pathway Are Conserved Between C. elegans and Mammals

(Rastogi et al., 2010)
Conserved Pathways

ATP Synthesis Pathways Are Conserved Between *C. elegans* and Mammals

- Mitochondrial respiratory chain complex, high homology for:
  - Nuclear encoded genes
  - mtDNA encoded genes
  - Bioenergetics
  - Metabolism
  - Structure

(Tsang, Lemire, 2002)
Conserved Pathways

IGF-1 Signaling Is Conserved Between C. elegans and Mammals

(Klotz et al., 2015)

(Christensen et al., 2006)
Conserved Pathways

Conserved Signal Transduction Pathways

• Wnt pathway via β-catenin
• Receptor serine/threonine kinase pathway
• Receptor tyrosine kinase pathway
• Notch-delta pathway
• Apoptosis pathway
• Receptor protein tyrosine phosphatase pathway
• G-protein-coupled receptor pathway
• Ligand-gated cation channel pathway
• Gap junction pathway

(Leung et al., 2008)
C. elegans vs. Rats

Space: 1 rat vs. ~100,000 C. elegans nematodes

Experiment Duration: months vs. days

Both: intact digestive, reproductive, and neuronal systems

Validation studies needed to define domains of applicability

(Hunt, 2017)
## C. elegans vs. Zebrafish

<table>
<thead>
<tr>
<th>Utility for Toxicity Testing</th>
<th>C. elegans</th>
<th>Zebrafish embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>organ systems: reproductive, muscular, nervous</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>organ systems: visual, circulatory, skeletal</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>established assays can be used with non-water soluble, volatile and/or extreme pH compounds</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>neurobehavioral assays</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>mutant and transgenic strains can identify pathways of toxicity</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>life cycle</td>
<td>3 days</td>
<td>3 months</td>
</tr>
<tr>
<td>model</td>
<td>Oral</td>
<td>Absorptive</td>
</tr>
</tbody>
</table>
Public Law 114–182
114th Congress

An Act

To modernize the Toxic Substances Control Act, and for other purposes.

(a) SHORT TITLE.—This Act may be cited as the “Frank R. Lautenberg Chemical Safety for the 21st Century Act”.

SEC. 4. TESTING OF CHEMICAL SUBSTANCES AND MIXTURES.

Section 4 of the Toxic Substances Control Act (15 U.S.C. 2603) is amended—

(9) by adding at the end the following:

“(h) REDUCTION OF TESTING ON VERTEBRATES.—

“(1) IN GENERAL.—The Administrator shall reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures under this title by—

“(B) encouraging and facilitating—

“(i) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under this title;
The nematode *Caenorhabditis elegans* as a model of organophosphate-induced mammalian neurotoxicity

Abstract

Fifteen organic phosphate pesticides were tested by computer tracking for their acute behavioral toxicity with the nematode *Caenorhabditis elegans*. Thirteen of these 15 chemicals are used as insecticides and are anticholinesterase agents. The other two chemicals are used as herbicides. EC50 values for each chemical were compared to the corresponding LD50 acute lethality value in rats and mice. Order of toxicity was found to be significantly correlated in comparisons of *C. elegans* to both rats and mice. Mechanistic investigations were conducted by assaying 8 of the 15 chemicals for anticholinesterase activity in *C. elegans*. Significant cholinesterase inhibition was confirmed for five chemicals that had displayed high behavioral toxicity, while three chemicals of low behavioral toxicity showed no significant decrease in cholinesterase activity. Toxicity for two chemicals that do not inhibit cholinesterase in mammals was linked to pH effects. Detailed comparison of individual chemicals and metabolic issues are discussed. These results have positive implications for the use of *C. elegans* as a mammalian neurological model and support the use of *C. elegans* in early rounds of chemical toxicity screening.

(Cole et al., 2004)
C. elegans and Mammalian Developmental Toxins

The nematode *Caenorhabditis elegans* as a tool to predict chemical activity on mammalian development and identify mechanisms influencing toxicological outcome.

- 72 Chemicals, 57 with Mammalian Reproductive Effects
- Egg Viability Assay
- Cytochrome P450 expression
- RNAi P450 Expression Knock Down

<table>
<thead>
<tr>
<th>Developmental activity in C. elegans</th>
<th>Developmental activity in mammals</th>
<th>Positive predictivity</th>
<th>Negative predictivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active</td>
<td>active</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>inactivle</td>
<td>inactive</td>
<td>40</td>
<td>13</td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

(Harlow et al., 2016)
C. elegans and Mammalian Developmental Toxins

ToxCast™ Phase I and II Libraries (~900 chemicals)

• Assessed C. elegans larval growth data for predicting developmental outcomes in rabbits and rats
  - 58% concordance between rabbit and rat
  - 52% concordance between C. elegans and rabbit or rat

(Boyd et al., 2015)
Growth Assessed by Flow/Laser Technology

COPAS™: Complex Object Parametric Analyzer and Sorter

(Hunt et al., 2013)
C. elegans LC50 Correlates

Table 1: Toxicity ranking as measured by rat oral LD50s (ScienceLab, 2010).

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Rat Oral LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercuric chloride</td>
<td>1</td>
</tr>
<tr>
<td>Sodium arsenite</td>
<td>41</td>
</tr>
<tr>
<td>Cadmium chloride</td>
<td>88</td>
</tr>
<tr>
<td>Copper chloride</td>
<td>584</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>2600</td>
</tr>
</tbody>
</table>

-Hunt et al., 2012-
Correlation of chemical acute toxicity between the nematode and the rodent

The utility of any non-rodent model system for chemical toxicity screening depends on the level of correlation between its responses and toxic reactions in rodents. Toxicity assays in the nematode Caenorhabditis elegans (C. elegans) can be fast and inexpensive; however, few studies have been performed comparing toxic responses in the nematode with data on acute rodent toxicity. We assayed the acute toxicity of 21 types of chemicals in different toxicity categories using C. elegans. The nematodes were exposed to different concentrations of chemicals in 96-well plate for 24 h. The lethality rate was observed at 2, 4, 12 and 24 h, and median lethal concentration (LC\textsubscript{50}) was calculated by the Probit method. The lethality rate was counted at 1, 8, 16 and 20 h additionally at the concentrations of 10,000, 21,500 and 46,400 mg ml\textsuperscript{-1} to acquire median lethal times (LT\textsubscript{50}). The results indicated that the chemical pH could affect the C. elegans LC\textsubscript{50} value. The pH tolerance range for C. elegans was more than 2.75. Excluding 4 types of acidic chemicals, there were positive correlations between LC\textsubscript{50} of C. elegans and LD\textsubscript{50} of mouse/rat (r > 0.72, p < 0.01) after both 12 h and 24 h exposure. As to the LC\textsubscript{50} data following a 24 h exposure in C. elegans, the correlation of C. elegans LC\textsubscript{50} vs. rat LD\textsubscript{50} (r = 0.885) was greater than the correlation of mouse vs. rat LD\textsubscript{50} (r = 0.879), while the correlation of C. elegans LC\textsubscript{50} vs. mouse LD\textsubscript{50} (r = 0.741) was lower relative to that of mouse vs. rat LD\textsubscript{50}. The data were further compared with an LC\textsubscript{50}....
Cadmium Induces Intestinal Abnormalities in Mammals

(Hunt et al., 2012)
C. elegans Developmental Stages

C. elegans Lifecycle

(Altun, Hall, 2008)
C. elegans Developmental Stages
Motility Assessment with Infrared Beam Detection

WMicroTracker™ measures small animal activity in beam interruptions

(Simonetta, Golombek, 2007)
Worm Development and Activity Test (wDAT)

- **6h, Stage: L1**
  - Length: 0.30mm

- **27h, Stage: L2**
  - Length: 0.48mm

- **48h, Stage: L4**
  - Length: 0.74mm

- **72h, Stage: Adult**
  - Length: 1.0mm
Low Level Mercury Exposure Associated with Hyperactivity in Children and Developing Rodents

(Cheuk, Wong, 2006; Gimenez-Llort et al., 2001; Rocha et al., 2001; Sagiv et al., 2012)
Mercury Exposure Associated with Reduced Weight Gain in Developing Rodents

(Fredriksson et al., 1993)
Mercury Exposure Induces Hypokinesia in Mammals

![Graph showing developmental delay and loss of synchronous development with exposure to higher levels of mercury. The x-axis represents time in hours, with values ranging from 1 to 70. The y-axis represents activity in beam interruptions, ranging from 0 to 160. Lines represent different groups: Water, HgOAc 6, HgOAc 8, and HgOAc 10.](image)
Mercury Exposure Induces Hypokinesia in Mammals

Normalized Average Group Activity after treatment with HgCl₂

(Bushana, Hunt, 2014)
C. elegans Larval Development with wDAT vs. Larval Growth by Flow/Laser Technology

* p-value < 0.05  
** p-value < 0.005
Arsenic Exposure Associated with Hyperactivity in Children and Developing Rodents

(Roy et al., 2011; Rodriguez et al., 2010; Chandravanshi et al., 2014)
Arsenic Exposure in Developing Rodents: hyperactivity at low dose, hypoactivity at high dose

(Rodriguez et al., 2010; Bardullas et al., 2009; Itoh et al., 1990)

* p-value < 0.05
* p-value < 0.005
Developmental Delay with Mixtures

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
Developmental Delay with Mixtures

Mercury and Arsenic Together are Additive
Summary

- *C. elegans* assays are rapid and inexpensive, facilitating mixture assessment
- Concordance has been demonstrated between:
  - *C. elegans* LC50 ranking and rat LD50 ranking
  - *C. elegans* motility and mammalian neurotoxicity
  - *C. elegans* larval growth and mammalian developmental toxicity
  - *C. elegans* gene expression and mammalian mechanisms of toxicity
- Validation studies are urgently needed


References


The Office of Applied Research and Safety Assessment (OARSA) at the FDA’s Center for Food Safety and Applied Nutrition (CFSAN) protects public health by providing science-based data to support the FDA’s regulatory food protection and cosmetic safety programs. Current CFSAN priorities include the development of improved methods for the detection of developmental toxicity and assessment of mixture toxicity. Our concordance study findings indicate a potential for the use of data from *C. elegans* assays to quickly provide human-relevant information for agency resource allocation and regulatory decision making. In this way, we seek to increase the number of chemicals and chemical mixtures assessed for safety, while at the same time reducing costs and laboratory mammal use.
Acknowledgements

- Andrew Liem (transgenic gene expression)
- Priyanka Bushana (motility analyses)
- Cory Vaught (morphology analyses)
- Nicholas Olejnik (COPAS assessments)
- Tom Black (lab management)
- Robert Sprando (DOT Director)
- Suzanne Fitzpatrick (CFSAN Senior Advisor for Toxicology)