**Case Study Summary**

**Title: Building Confidence in the Use of NAMs data for Risk Analysis: *C. elegans* as a Case Study**

**Version: 3**

**Presented by: Piper Reid Hunt**

**Contributors: Piper Reid Hunt, Jessica Camacho, Robert L. Sprando, Suzanne Fitzpatrick**

**Panel Advisor: A. Wallace Hayes**

## 1. Provide a few sentences summarizing the method illustrated by the case study.

Oral exposure to arsenic from contaminated drinking water and food is associated with a variety of poor health outcomes, including developmental delay and neurotoxic effects. Molecular form alters the toxicity of arsenic, complicating both monitoring and regulation efforts [1]. The predominant forms of arsenic in rice, a common constituent of commercial baby foods, are arsenite and dimethylarsinic acid (DMA) [2, 3]. While organic forms of arsenic are generally considered to be less toxic than inorganic forms, experimental data on the effects of DMA exposure are lacking [3-5]. DMA is found in the progeny of mouse dams fed inorganic arsenic [6], indicating that DMA may cross the placenta and raising concerns that more information is needed to fill data gaps concerning the developmental toxicity of this organic form of arsenic.

Small animal models such as *Caenorhabditis elegans* can provide mammalian-relevant toxicity data with *in vitro* expense and time parameters [7-9]. Mammals and *C. elegans* share many genetic pathways involved in embryonic patterning, organismal development, and neuronal function [7, 10, 11]. While *C. elegans* data cannot replace a rodent study, substantial evidence supports correlations in chemical effects between this non-pathogenic, microscopic roundworm and mammals for developmental and neuronal toxicity [12, 13].

Exposure to inorganic arsenic retards early development in humans and *C. elegans* [3, 14]. Relative to sodium arsenite (NaAsO2), we found that approximately 20-fold higher concentrations of DMA were required to induce similar levels of developmental delay in *C. elegans* [15]. Activation of oxidative stress resistance gene expression is a conserved effect of exposure to inorganic arsenic across phyla [16]. More than 10-fold higher concentrations of DMA relative to NaAsO2 were required to induce similar levels of oxidative stress resistance gene expression in *C. elegans* for both native and transgenes [15]. Additionally, a biomarker for activation of conserved AIRAP/AIP-1, an arsenite inducible gene involved in unfolded protein response and resistance to arsenic toxicity [17], was strongly activated by NaAsO2, but not DMA at tested concentrations [15]. These data are consistent with reduced toxicological concern for oral exposures to DMA relative to inorganic arsenic.

2. **Describe the problem formulation(s) the case study is designed to address. How is the method described in the case useful for addressing the problem formulation?**

Developmental toxicity studies in laboratory mammals are expensive and time consuming. Human cell based *in vitro* and microphysiological system studies can provide human specific mode of action data, but not information on apical endpoint effects such as juvenile growth and behavior, or effects that are dependent on an intact digestive system [8, 13]. Funding for toxicity assessment is limited and sometimes action is required in a timely manner that does not allow for a rodent study that may take a year or more to complete. An alternative, whole animal toxicology model such as *C. elegans* can provide apical endpoint information relatively quickly and at reduced cost, potentially providing a bridge between *in vitro* and laboratory mammal studies. However, a simple model can only provide limited information, so determination of fit-for-purpose is essential. In this case, we needed to know more about the toxicity of DMA, and we used a model in which effects for related chemicals, sodium arsenate and sodium arsenite, are concordant to mammalian effects for developmental toxicity [14], oxidative stress [16], and AIRAP/AIP-1 activation [18].

3. **Comment on whether the method is general enough to be used directly, or if it can be extrapolated, for application to other chemicals and/or problem formulations. Please explain why or why not.**

*C. elegans* is a well-studied, but simple and therefore limited model. The benefits of the model (conserved effects for inorganic arsenic on development and perturbation of genetic pathways plus rapid testing) indicated that *C. elegans* was a good fit for generating useful information in this case. It may be that for new approach methodologies (NAMs), flexible criteria need to be developed so that useful hazard information can contribute to regulatory decision making. The selection of what data from each model system would most likely be useful/predictive should be made on a case-by-case basis. In this instance, our findings of developmental delay and changes in gene expression induced by NaAsO2 in *C. elegans* correspond with effects in humans and human cell cultures [3, 16, 18]. The reduced toxicity of DMA relative to NaAsO2 for these endpoints in *C. elegans* is consistent with 1. epidemiological reports correlating urinary inorganic arsenic, but not DMA, with reduced growth and developmental toxicity in babies and children [19, 20], and 2. the fact that consumption of seafood, which contains significant levels of organic arsenicals but not inorganic arsenic, has not been linked to toxicity in humans [3, 21].

4. **Discuss the overall strengths and weaknesses of the method.**

Strengths: Many genetic pathways involved in organismal development, neuronal function, aging, and stress resistance are conserved from worms to humans [9-11, 22-24]. There is also a high degree of conservation for toxic modes of action [7, 9]. Data support the use of *C. elegans* within mammalian-predictive toxicity assessments for developmental toxicity [10, 12], neurotoxicity [11, 13, 25], and acute toxicity [26, 27]. Most *C. elegans* assays can be completed in a week by a single technician using standard laboratory equipment.

Limitations: *C. elegans* is primarily an oral toxicity model that is exposed to test articles in a liquid medium. Therefore, as with *in vitro* and aquatic testing, the chemical testing space is limited by solubility and pH. *C. elegans* lack many mammalian organs such as eyes and livers as well as skeletal, circulatory and adaptive immune systems. *C. elegans* lack a heart as well as voltage gated sodium channels [11], indicating that the species is not a likely model for cardiotoxicity, for example. While many genetic pathways involved in carcinogenesis are well conserved, adult *C. elegans* somatic cells are postmitotic, making them a poor choice for tumorigenicity assessment in the absence of mode of action information. Additionally, at approximately 1mm in length, *C. elegans* small size limits toxicokinetic and tissue distribution analyses.

Summary: The use of rapid, lower-cost results from *C. elegans* toxicity testing must be balanced with a thorough understanding of toxicodynamics in the model as well as endpoints for which the model is, and is not, likely to provide mammalian-predictive results.

**5. Outline the minimum data requirements and describe the types of data sets that are needed.**

Reducing the use of mammals in toxicity testing for chemical risk evaluation is a priority for the U.S. Food and Drug Administration [28]. At the same time, there is an urgent need to increase the generation of useful information to fill data gaps, especially on the effects of mixtures. No one model or assay is going to replace rodent testing. Instead, test batteries and integrated approaches that tailor best practices to each new approach methodology (NAM) will maximize applicability. If *C. elegans* data correlates with mammalian data on compounds in particular chemical classes, can data from those same endpoints be used for risk assessments on other, similar compounds? If findings in *C. elegans* correlate with mammalian data for components of mixtures, can those same endpoints be used towards decisions on the risks of exposure to mixtures? Case studies such as this one can help define how and when NAMs can be used to support regulatory decisions [29].

**Does your case study:**

**A. Describe the dose-response relationship in the dose range relevant to human exposure?**

We found that the *C. elegans* developmental delay LOELs for methylmercury chloride and mercury(ii) chloride were 0.5 and 2.0 µg/mL, respectively [15], and these relative values are close to the relative TWIs of 1.3 and 4.0 µg Hg/kg for these two compounds [30]. The developmental delay LOELs for NaAsO2 and DMA were 10 and 200 µg/mL, respectively [15]. While TWIs have not been established for these two compounds [31], the 20-fold difference in developmental delay LOEL values is consistent with reported rodent LD50s of 41 mg/kg for NaAsO2 and 700 to 1,200 mg/kg for DMA [32].

Several studies have shown strong correlations between *C. elegans* and rodent toxicant ranking for LD50 and neurotoxicity [25-27, 33-35]. As more data are acquired, it may be possible to estimate toxic mg/kg body weight mammalian effects from *C. elegans* mg/L responses through

a comparison to other ranked chemicals. Currently, however, there isn't enough data to support this type of estimation method.

**B. Address human variability and sensitive populations?**

*C. elegans* have been used extensively to study genetics, organismal development, and neurobiology for over 60 years. These studies have revealed strong conservation between worms and mammals for molecular and cellular pathways relevant to toxic modes of action [7, 9]. A comparison of the human and *C. elegans* genomes revealed that the majority of human disease genes and disease pathways are present in *C. elegans* [36, 37], suggesting a high likelihood that genes of interest for specific susceptible human populations are conserved. Supported by the NIH, the *Caenorhabditis* Genetics Center (CGC) maintains a collection of over 20,000 genetically distinct strains of *C. elegans* which can be purchased for a nominal fee [38]. Additionally, given the short life cycle and lifespan of *C. elegans*, it is relatively easy to assess toxic effects at multiple life stages and in multiple generations. Therefore, *C. elegans* has the potential to be used as a relatively inexpensive and rapid test model for a variety of sensitive human populations, both genetic and life-stage based.

**C. Address background exposures or responses?**

The ease, low cost, and potential for higher-throughput toxicity testing in *C. elegans* allow for a variety of dosing paradigms to be tested simultaneously. Approximately 200,000 *C. elegans* can be housed in the space of a single rat cage. Using microfluidics and laser technology, acute, chronic and/or multigenerational exposure scenarios can be rapidly assessed for effects on multiple endpoints and life-stages at an array of concentrations and/or mixtures. This also allows for cost-effective range finding and follow up studies in *C. elegans*, as well as assessment of effect amelioration using drugs or nutraceuticals.

**D. Address incorporation of existing biological understanding of the likely mode of action?**

Depending on the bioinformatics approach used to assess homology, 60-80% of human genes have *C. elegans* homologs [7]. Similarly, 78% of human inborn errors of metabolism genes have *C. elegans* homologs [37]. *C. elegans* is often used to evaluate modes of toxic action via labeled transgenes, RNAi, RT-PCR, and gene expression microarrays. Toxicodymamic assessments indicate that the mechanisms of action for a wide array of chemicals are conserved from worms to mammals [9]. The *C. elegans* literature on conserved pathways of toxicity such as oxidative stress response, apoptosis, and insulin/IGF-1 signaling is extensive, allowing the suitability of the model for different purposes to be assessed on an ad hoc basis. In this case, inorganic arsenic effects of developmental toxicity, oxidative stress, and protein folding are conserved in humans and *C. elegans*. Approximately 20-fold higher concentrations of DMA relative to NaAsO2 induced similar levels of developmental delay and oxidative stress response, and DMA did not activate a conserved biomarker of unfolded protein response at tested concentrations [15]. Additionally, gene expression microarray analysis at LOEL concentrations for developmental delay did not identify significant activation by 10 µg/mL

NaAsO2 or 200 µg/mL DMA of other pathways of toxicity such as DNA repair, MAPK/AMPK, or xenobiotic response ([15] and unpublished results), indicating a low likelihood of unassessed effects from DMA, at least in this model.

**E. Address other extrapolations, if relevant – insufficient data, including duration extrapolations, interspecies extrapolation?**

In a study of the developmental effects of the ToxCast$^{TM}$ chemical libraries of approximately 900 chemicals, the balanced accuracy concordance between rat and rabbit was 58% [12]. Using a medium-throughput juvenile growth assay that can be completed in less than a week by a single technician, the concordance between *C. elegans* and rat or rabbit for the same chemicals was 52% [12]. We would like the panel to comment on what additional information and/or studies are needed to move towards *C. elegans*-to-human extrapolation for developmental toxicity.

*C. elegans* has been used to assess concordant neurotoxicity effects using biochemical, morphological, behavioral, and gene expression analyses [11, 13, 25, 39, 40]. Unlike the juvenile growth assay evaluated above, however, published studies on *C. elegans* neurotoxicity effects have so far been limited to assessment of just one or a few test articles. Given the need for better tests for developmental neurotoxicity (DNT), what additional studies would best support the use of *C. elegans* data within a DNT testing battery or strategy?

**F. Address uncertainty?**

Do we need defined uncertainty factors to use information to contribute to hazard assessment? Are calculated sensitivity and specificity required, and if yes, for how many chemicals? What is the bar for the acceptability of a data source? If we don't have data from multiple labs to establish reproducibility on a given assay, can data from a different tool suffice? For example, if data from individual reliable labs show that *C. elegans*, zebrafish, and Drosophila all have similar adverse outcomes after exposure to compound X, would that be sufficient to move forward with guidelines? If not, what would be sufficient?

**G. Allow the calculation of risk (probability of response for the endpoint of interest) in the exposed human population?**

Not applicable at this time.

**H. Work practically? If the method still requires development, how close is it to practical implementation?**

In this case study, experimental data from *C. elegans* developmental timing and pathways of toxicity gene expression were used to support epidemiological evidence indicating low toxicological concern over DMA in food. For the questions asked in this case, the model is well developed. Given that a rodent study would have taken at least two years to put together and complete, it was also practical.

Studies correlate *C. elegans* to mammalian data for developmental toxicity, acute toxicity, and neurotoxicity responses, as well as aging effects. In the absence of funding for ring trials, how can this model be used along with other tools to help fill data gaps related to those endpoints? Do we need to keep the model as a screening tool for hazard identification? What additional information is needed to move beyond screening to include *C. elegans* data in risk assessments?

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