Development of a Curated Database of In Vivo Developmental Toxicity Data

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Introduction

Developmental Toxicology

- Developmental toxicology is the study of adverse effects on the developing organism resulting from chemical exposures to (EPA 1991):
  - Either parent before conception
  - The mother during prenatal development
  - The offspring to the time of sexual maturation

- Developmental toxicity testing is used by chemical producers and drug developers to inform product development decisions and by regulators for decisions about registration and product labeling.

- There are four major manifestations of developmental toxicity:
  - Mortality
  - Altered growth
  - Structural abnormalities
  - Functional deficits

- Conventional prenatal developmental toxicity studies evaluate increased mortality, altered growth, and structural abnormalities that occur during the gestational period.

Alternative Assays

- Currently accepted in vivo testing protocols (EPA 1998; ICH 2005; OECD 2001) used to generate prenatal developmental toxicity data are time- and resource-intensive and require the use of animals (Figure 1).
Using these protocols, it could take extensive time and resources to run studies on even a small percentage of the thousands of chemicals for which there is no significant developmental toxicological data (Judson et al. 2009; Richard et al. 2016).

The U.S. Environmental Protection Agency (EPA) ToxCast™ and the U.S. interagency Tox21 programs use quantitative high-throughput screening (qHTS) assays to assess how human cells and tissues are affected by exposure to chemicals. Both programs are applying these approaches to address the need for developmental toxicity data on uncharacterized chemicals.

In vitro and qHTS methods used to prioritize and screen chemicals must be scientifically sound and able to effectively detect endpoints relevant to developmental toxicity; therefore,
high-quality in vivo reference data are critical to establishing the biological relevance, usefulness, and limitations of these approaches.

- In 2015, the National Toxicology Program (NTP) initiated a project to identify a list of developmental toxicants supported by publicly available data. As part of that effort, the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) conducted a systematic search for high-quality mammalian developmental toxicity studies.

- This effort focused on identifying agents associated with a range of developmental toxicity effects, ranging from subtle effects on fetal weight, to increased incidence of variations, to frank terata and post-implantation loss.

- This poster summarizes the current status of this ongoing effort and describes the criteria used to identify studies, the data extracted from them, the chemicals assessed, and summary information about the data set.

**Literature Searches and Data Extractions**

- To limit the size of the chemical space being evaluated, NTP established an information panel of experts from industry, academia, and government, who identified several hundred chemicals to evaluate.

- Once this initial list of chemicals was established, we began searches of the publicly available scientific literature. To determine the scope of the available literature, our initial searches focused on the peer-reviewed scientific literature (Figure 2) for 17 chemicals.

- Searches were designed to identify “regulatory guideline-like” studies, which were defined as using protocols adhering as closely as possible to accepted test guidelines: EPA OPPTS 870.3700 (1998) ICH S5(R2)(2005), and Test Guideline 414 issued by the Organisation for Economic Co-operation and Development (OECD 2001).

- The search of the peer-review literature returned thousands of citations describing studies on very few chemicals. Most of the protocols used in these studies were not guideline-like.

- In an attempt to obtain useful guideline study data for a broader range of chemicals, we returned to the original list identified by the information panel and expanded the search to include non-peer-reviewed literature and data from studies that have not yet been peer-reviewed/published. The complete set of databases searched is presented in Figure 2.
The second set of searches identified fewer studies overall but returned data on a broader range of chemicals. For example, our search of the NTP Electronic Library (NELI) returned guideline-like studies for about 70 chemicals.

- Reports for 42 of these studies are currently available to the public through the National Technical Reports Library (https://ntrl.ntis.gov/NTRL/).
- Reports of the remaining studies are not yet publicly available, but data were extracted from these studies to help expand the database.

Protocol data collected for each study included:

- Chemical name
- Chemical Abstracts Service Registry Number
- Study year (where possible, the end date of the in-life study)
- Species and strain of test organism
- Route of administration
- Diet
- Chemical lot and purity
- Dosing duration (including gestational ages at dosing initiation and conclusion)

- Some of the study endpoint data types collected are presented in Figure 3.

**Figure 3** Example Study Endpoint Data Types Collected from Studies Obtained from NELI Studies

Maternal Toxicity
- Body Weight and Body Weight Change
- Organ Weights (e.g., Uterus, Liver)
- Clinical Observations (e.g., Piloerection, Convulsions)
- Gross Necropsy Findings

Altered Fetal Growth
- Number of Implantation Sites per Dam
- Number of Litters, and Live Offspring per Litter
- Number and Percent of Late Fetal Deaths
- Sex Ratio
- Fetal Body Weights

Fetal Structural Abnormality
- Number and Percent of Fetuses with External, Visceral, and Skeletal Malformations and/or Variations
- Number and Percent of Litters with External, Visceral, and Skeletal Malformations and/or Variations

- Of the 70 chemicals initially identified in NELI, 15 were considered insufficient for data extraction because they only had range finding study data, were underpowered, and lacked detailed evaluations of fetal structural abnormalities.

- The remaining 55 chemicals (Table 1) had developmental toxicity data from guideline-like studies contained in 105 files.
Table 1  Chemicals with Complete Developmental Toxicity Data in NELI

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CASRN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3,4-Butanetetracarboxylic acid</td>
<td>1703-58-8</td>
</tr>
<tr>
<td>1,3-Butadiene</td>
<td>106-99-0</td>
</tr>
<tr>
<td>1,4-Butanediol</td>
<td>110-63-4</td>
</tr>
<tr>
<td>2-Ethylhexanol</td>
<td>104-76-7</td>
</tr>
<tr>
<td>8-Methoxypsoralen</td>
<td>298-81-7</td>
</tr>
<tr>
<td>Acetone</td>
<td>67-64-1</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>75-05-8</td>
</tr>
<tr>
<td>alpha-Methylidopa</td>
<td>555-30-6</td>
</tr>
<tr>
<td>Bendectin</td>
<td>8064-77-5</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>119-61-9</td>
</tr>
<tr>
<td>Berberine chloride dihydrate</td>
<td>5956-60-5</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>80-05-7</td>
</tr>
<tr>
<td>Butylbenzyl phthalate</td>
<td>85-68-7</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>75-15-0</td>
</tr>
<tr>
<td>Chloroprene</td>
<td>126-99-8</td>
</tr>
<tr>
<td>Codeine</td>
<td>76-57-3</td>
</tr>
<tr>
<td>d-Camphor</td>
<td>464-49-3</td>
</tr>
<tr>
<td>Diethyl phthalate</td>
<td>84-66-2</td>
</tr>
<tr>
<td>Diethylene glycol</td>
<td>111-46-6</td>
</tr>
<tr>
<td>Diethylene glycol diethyl ether</td>
<td>112-36-7</td>
</tr>
<tr>
<td>Diethylene glycol dimethyl ether</td>
<td>111-96-6</td>
</tr>
<tr>
<td>Dimethyl phthalate</td>
<td>131-11-3</td>
</tr>
<tr>
<td>Diphenhydramine hydrochloride</td>
<td>147-24-0</td>
</tr>
<tr>
<td>Emodin</td>
<td>518-82-1</td>
</tr>
<tr>
<td>Ethylene chlorohydrin</td>
<td>107-07-3</td>
</tr>
<tr>
<td>Ethylene glycol diethyl ether</td>
<td>629-14-1</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>107-15-3</td>
</tr>
<tr>
<td>Gallium arsenide</td>
<td>1303-00-0</td>
</tr>
<tr>
<td>Gentian violet</td>
<td>548-62-9</td>
</tr>
<tr>
<td>Glyoxal trimeric dehydrate</td>
<td>4405-13-4</td>
</tr>
</tbody>
</table>
### Chemical Name | CASRN
---|---
Hydrochlorothiazide | 58-93-5
Isoeugenol | 97-54-1
Isoprene | 78-79-5
Isoproterenol | 51-30-9
L-5-hydroxytryptophan | 4350-09-8
Melatonin | 73-31-4
Methacrylamide | 79-39-0
Methacrylonitrile | 126-98-7
Methyl ethyl ketone | 78-93-3
Methylene blue trihydrate | 7220-79-3
Methyleugenol | 93-15-2
N,N'-methylenebisacrylamide | 110-26-9
Nitrofurazone | 59-87-0
Oxytetracycline hydrochloride | 2058-46-0
Phenol | 108-95-2
Polyoxyethylene sorbitan monolaurate | 9005-64-5
Polyoxyethylene sorbitan monoooleate | 9005-65-6
Scopolamine hydrobromide | 114-49-8
Sodium chlorate | 7775-09-9
Sodium fluoride | 7681-49-4
Sodium thioglycolate | 367-51-1
Sulfamethazine | 57-68-1
Tetrahydrofuran | 109-99-9
Thiophenol | 108-98-5
Triethylene glycol dimethyl ether | 112-49-2

CASRN = Chemical Abstracts Service Registry Number

### Current Status

- Data extraction from the 105 NELI study reports containing developmental toxicity data from guideline-like studies on 55 chemicals has been completed. Table 2 summarizes species and route of administration information from this data set.
Table 2  Number of Studies for Each Species and Dosing Route

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Mouse</th>
<th>Rabbit</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation – Whole Body</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Injection - Subcutaneous</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Injection - Intravenous</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Oral – Drinking Water</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Oral - Feed</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Oral - Gavage</td>
<td>30</td>
<td>22</td>
<td>19</td>
<td>71</td>
</tr>
<tr>
<td>Topical – Direct Application</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Future Directions

- Data cleaning, standardization, and annotation is ongoing to prepare the data for submission to the NTP Chemical Effects in Biological Systems (CEBS) and Integrated Chemical Environment (ICE) databases.
  - More information about CEBS is available online https://www.niehs.nih.gov/research/resources/databases/cebs/
  - More information about the ICE database will be presented elsewhere at SOT:
    - Bell et al., Abstract 2935, Poster Board P429, presented on Wednesday, March 15, 1:15-4:30 p.m., CC Exhibit Hall
    - Exhibitor-hosted session, “ICCVAM Tools for Validation and Regulatory Application of Alternative Methods,” Wednesday, March 15, 1:30-2:30 p.m., Room 337
- Once available in both databases, these data will provide an important resource for evaluating the performance of alternative methods that measure key events in pathways associated with developmental toxicity.

References


ICH. 2005. International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH harmonised tripartite guideline S5(R2)
Detection of toxicity to reproduction for medicinal products & toxicity to male fertility. Geneva, Switzerland.


Acknowledgements

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