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.

Figure 1. Key Elements of Accepted Test Guidelines for Prenatal Development Studies

Species	• Rat or Mouse and • Rabbit or Other Species, e.g., Guinea Pig
Route of Administration	• Oral Gavage • Other: Dermal, in Food, in Water, Inhalation
Number of Doses	• Includes Concurrent Control • Minimum of Three Dose Levels
Number of Animals	• ≥20 Females with Implantation Sites at Necropsy

Minimum Length of Exposure	• Daily:
	  • From Implantation to the Day Before Cesarean Section
	  • To Close of Hard Palate (ICH S5(R2))

Maternal Observations	• Daily Clinical Observations • Food Consumption • Gross Necropsy and Examination of Uterine Contents
Fetal Observations	• Sex and Body Weight • Skeletal and Soft Tissue Malformations and Variations

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 (EPA 1998) ICH S5(R2)(ICH 2005), and Test Guideline 414 issued by the Organisation for Economic Co-operation and Development (OECD 2001).
- The search of the peer-reviewed 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 was 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)

Figure 2. Databases Searched

Peer-reviewed Literature Databases	• PubMed • Embase • SciFinder • Scopus • Web of Science
Additional Databases	• California Office of Environmental Health Hazard Assessment Prop 65 List • European Chemicals Agency (ECHA) • Hazardous Substances Data Bank (HSDB) • Micromedex • NTP Electronic Library (NELI) • NTP Office of Health Assessment and Translation (OHAT) • Agency for Toxic Substances and Disease Registry (ATSDR) • Chemical Data Access Tool (EPA) • High Production Volume Information System (EPA) • Integrated Risk Information System (EPA) • Pesticide Registrations (EPA) • Toxicity Reference Database (EPA) • Drug Approval Documents (FDA) • Chemical Effects in Biological Systems (NIEHS)

Figure 3. Example Study Endpoint Data Types 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

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

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

	Rat	Mouse	Rabbit	Total
Inhalation – Whole Body	6	2	1	9
Injection - Subcutaneous	1	0	0	1
Injection - Intravenous	0	1	1	2
Oral – Drinking Water	1	0	1	2
Oral - Feed	8	8	0	16
Oral - Gavage	30	22	19	71
Topical – Direct Application	2	0	2	4

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, this data will provide an important resource for evaluating the performance of alternative methods that measure key events in pathways associated with developmental toxicity.

References

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