Building a Prenatal Developmental Toxicity Ontology

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Contents

SUMMARY

This report discusses the need for a systematic representation of knowledge about developmental toxicity (i.e. an ontology) that would enable computer-based prediction of which chemicals are likely to induce human developmental toxicity. The focus of the report is on ways of building a foundation for such an ontology, based on knowledge of developmental biology and mode of action/adverse outcome pathways (AOPs) in developmental toxicity. The ontology should include as much biology and signalling mechanism pathway content that current science allows. It will be necessary to build a Resource Description Framework (RDF) database to house not only the ontology but also the chemical data needed to assess perturbations to developmental processes. In order to start, terminology and relationships from qualitative, putative AOPs will need to be assembled. Ultimately, with the application of quantitative chemical bioassay data, we will be able to populate quantitative, confirmed AOPs. The implementation of this ontology-described database will also require consideration of the non-linearity of dynamic biological systems, the influence of critical periods in development, and, ideally, the influence of maternal toxicity. This report discusses some of the challenges in building a developmental toxicity ontology and RDF database. It also discusses some of the currently available, web-based resources for building AOPs. Case studies on one of the most well understood morphogens and developmental toxicants, retinoic acid, are presented as examples of how such an ontology may be built up. The potential for the use of the rapidly expanding data in the ToxCast program is also explored.

1. PREFACE

1.1 Definitions

Adverse Outcome (AO)[1]: A specialised type of key event (KE), measured at a level of organisation that corresponds with an established protection goal and/or is functionally equivalent to an apical endpoint measured as part of an accepted guideline test. Generally at the organ level or higher. Anchors the "downstream" end of an adverse outcome pathway (AOP).

Adverse Outcome Pathway (AOP): A conceptual framework that organises existing knowledge concerning biologically plausible, and empirically supported, links between molecular-level perturbation of a biological system and an adverse outcome at a level of biological organisation of regulatory relevance.[1] AOPs are informed by, but independent of, the chemicals that may affect a pathway. An AOP is usually described as a linear sequence of key events, starting from a **molecular initiating event**, followed by various key intermediate events, as compensatory mechanisms and feedback loops are overcome, linked by defined **key-event relationships**, and ending with an adverse outcome. Thus, an AOP encompasses increasing levels of complexity from the molecular initiating event, via the biochemical, cellular, tissue and organ levels to the **adverse outcome** at the entire organism or population level (see Figure 1).

Apical Endpoint: Traditional, directly measured, adverse whole-organism outcomes of exposure in *in vivo* tests. In this context, generally death, reproductive failure, or developmental dysfunction.

Developmental Toxicity Ontology (DTO): This is an application ontology built for the specific purposes of organising existing information about modes of action of developmental toxicants and their relationships with adverse outcomes.

Integrated Approaches to Testing and Assessment (IATA): A structured approach that strategically integrates and weights all relevant data to inform regulatory decisions regarding potential hazard and/or risk and/or the need for further targeted testing and therefore optimising and potentially reducing the number of tests that need to be conducted.[2]

Key Event (KE)[1]: A measurable change in biological state that is essential, but not necessarily sufficient, for the progression from a defined biological perturbation toward a specific adverse outcome. KEs are represented as nodes in an AOP diagram or AOP network and provide verifiability to an AOP description.

[1] Taken from Villeneuve *et al*. (2014a,b). Adverse Outcome Pathway (AOP) Development I: Strategies and Principles, Toxicological Sciences 142:312-320 and/or the OECD (2013b), Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs (ENV/JM/MONO(2013)6.

[2] Working definition taken from OECD (2015). Report of the workshop on a framework for the development and use of integrated approaches to testing and assessment Series on Testing and Assessment No. 215. ENV/JM/MONO(2015)22, 22 July 2015.

Key Event Relationship (KER)[1]: A scientifically-based relationship between a pair of KEs, identifying one as upstream and the other as downstream. It facilitates inference or extrapolation of the state of the downstream KE from the known, measured or predicted, state of the upstream KE.

Molecular Initiating Event (MIE)[1]: A specialised type of KE, defined as the point where a chemical directly interacts with a biomolecule within an organism to create a perturbation that starts the AOP – as such, by definition, it occurs at the molecular level. Anchors the "upstream" end of an AOP.

Mode Of Action (MOA): A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A mode of action describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed effect—in a logical framework[3]. A mode of action starts with the molecular initiating event. Unlike AOP, it does not (usually) include consideration of exposure or effects at higher levels than the individual (see Figure 1).

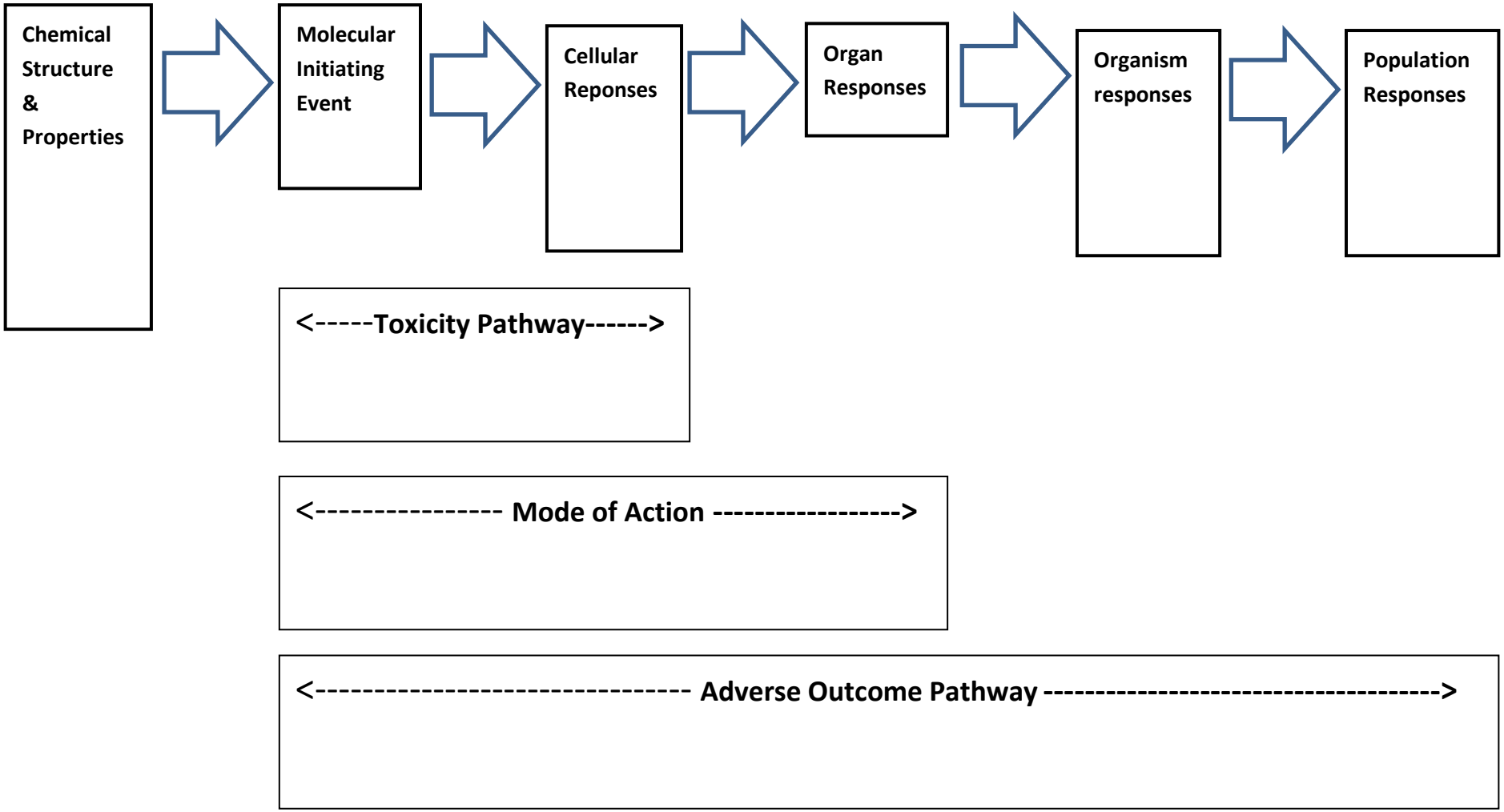
Ontology: An ontology is an organised representation of a domain of knowledge consisting of concepts and information, generally referred to as classes, and relationships between classes. Ontologies are useful in organising information into a structure that makes the information more understandable and facilitates hypothesis generation.

Resource Description Framework (RDF): An infrastructure for storing information, usually in triplestore or RDF triple format. The relationships in an RDF are organised and described by an ontology. The ontologies **themselves can be stored in an RDF.**

Toxicity pathway: Perturbation of a normal biochemical pathway from the molecular initiating event to the cellular effect (see Figure 1).

[3] Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W (*2006).* IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol* **36***:*781–792.

Figure 1: Schematic representation of the concept of the Adverse Outcome Pathway showing relationship to Mode of Action and Toxicity Pathway. (adapted from OECD (2013a) Guidance Document on Developing and Assessing Adverse Outcome Pathways, ENV/JM/MONO(2013)6)



2. INTRODUCTION

2.1 The value of ontologies

As toxicology begins to move towards high-throughput and high-content screening, scientists are becoming deluged with data on the effects of chemicals at a molecular/mode-of-action level. This information has the potential to be used for prediction of adverse effects at an organismal level, but these predictions would be facilitated by systematic organisation of data by presumed mode of action (MOA). Such a systematic organisation (an ontology) would (1) provide linkage of molecular data to traditional toxicology study outputs and to human disease states, (2) provide clarity as to whether existing high-throughput or high-content approaches are sufficiently inclusive of the universe of MOAs for toxicity, and (3) serve as an organising structure for constructing adverse outcome pathways (AOPs). Ontologies provide one means to deal with such data in a structured manner, while also providing a mechanism for integrating with larger IT-infrastructures to facilitate decision making.

Ontologies are often used when data from disparate sources need to be integrated. This allows investigators to make complicated queries of the data encoded by the ontology. Ontologies can be foundational, forming the basis upon which other ontologies will be built, or application-specific, where there is a specific scope or purpose for the ontology. Although ontologies use a controlled vocabulary (i.e. a distinct set of words is used to describe each concept), it is the relationship between concepts that sets it apart from a controlled vocabulary and make it useful for computing and learning.

Scientists will appreciate the ability of ontologies to quickly build hypotheses that can be followed up. The ontology may even be able to provide insight that can be used in a decision-making system for evidence integration. For example, the ontology may hypothesise that a particular chemical causes issues with eye development; this leads to experimental follow-up and those results can also be managed in the ontology. All of this, and other information, could be placed in a decision-support tool, which integrates additional information, such as historical information that this class of chemicals causes developmental toxicity at high doses that are not seen in other vertebrates. All of the computational aspects can be fully automated, allowing decisions to be made more quickly, while managing a larger portfolio of chemicals. A similar set-up combining the ontology with a decision-support tool can be used for other applications, such as streamlining the evidence integration process in various risk assessments.

Ontologies also allow for the automated prediction of AOPs. Once the ontology contains several AOPs, there may be interconnections, where common key events (KEs) exist. This allows for the automated construction of AOP networks. As the number of AOPs grows, the complexity of the network will also grow, generating novel AOPs that have not been previously explored. These computationally predicted AOPs (cpAOPs) are new AOP hypotheses that can be followed up experimentally. The experimental results can be used to fine-tune/prune relationships within the network, potentially graduating an AOP from being a cpAOP to a putative or accepted AOP.

This report focuses on the need to explore the field of developmental toxicology in this way, by creating a formal system (i.e. ontology) that organises the knowledge of chemical structure, developmental biology and

developmental toxicology so that it becomes possible to predict and explain which chemicals are likely to induce human developmental toxicity. The authors believe this is needed for the following reasons:

- It would overcome some of the limitations of current safety testing by exploiting the state of the science and the increasing amounts of data that can inform us about MOAs that lead to adverse outcomes.
- It would improve public health protection through increased relevance and accuracy of testing.
- It would facilitate the design of pharmaceuticals and other chemicals so that they are unlikely to have the potential for developmental toxicity in humans.
- It would save resources (time, animals).

2.2 The need for a developmental toxicity ontology

Developmental biology is characterised by a complex interplay between a multitude of processes at the molecular, cellular, tissue and organism level, which change continuously with time in development and location in the conceptus. These processes need to be mapped, at least to the extent that is necessary for understanding developmental toxicity. In the OECD AOP terminology, this implies that a network of mutually interacting AOPs needs to be defined that would lead to the identification of a limited number of KEs in the network. These KEs, in turn, can be used as biomarkers of developmental toxicity and can be represented in a limited number of test systems in an integrated testing strategy, which aims to cover developmental toxicity in its entirety. The genesis of this approach begins with, and is critically dependent on, an integral description of the developmental toxicity ontology. The ontology will provide an overview of the essential physiological/toxicological routes (and their interrelationships) leading to developmental toxicity, providing a blueprint for a comprehensive integrated approach for testing and assessment (IATA) for developmental toxicity. A successful IATA of the complete ontology would, by definition, detect all developmental toxicants, providing confidence for scientists and regulators that application of the IATA will be sufficient for hazard and risk assessment. The ontology will also be useful as an organising principle for expanding understanding in the field, such as defining novel KEs for *in vitro* testing and a refinement/reduction or replacement for *in vivo* testing. The ontology should be formatted such that it is available for computational approaches for risk assessment. Whereas the integral approach provided by the developmental toxicity ontology would provide significant merit over individual IATA and AOP approaches, it will be, by definition, limited by the state of knowledge of the mechanisms of embryofoetal development, and will require continuous update as scientific knowledge progresses.

2.3 Purpose of this report

The purpose of this report is to develop organisational principles and frameworks that could be used to build a developmental toxicity ontology that would help in the creation of AOPs and an IATA to predict developmental toxicity. Whereas the ultimate goal would be to produce an ontology that encompasses quantitative AOPs, in this report we propose that the starting point has to be a state-of-the-science MOA ontology. From the computer science perspective, the structure of an AOP ontology will be similar to an MOA ontology. However, moving from an approach using qualitative molecular initiating events (MIEs) and

qualitative KEs towards a quantitative AOP approach requires consideration of the non-linearity of dynamic biological systems and critical periods in development (e.g. the same MIE at different time points in development might produce different outcomes). By starting with an MOA, containing as much biology and signalling mechanism as current knowledge allows, we will move closer to building a quantitative AOP.

In this report, we aim to explore how this may be done by demonstrating how relevant qualitative and quantitative information from structured data (formal data sets) and unstructured data (from literature) can be organised into a logical ontology framework. Relevant Information will include existing knowledge and interrelationships between developmental biology, developmental defects caused by known chemicals, molecular pathways, molecular targets, and models that describe interrelationships. While the benefits of understanding and linking complex biological information in a structured format to understand and predict developmental toxicological outcomes are clear, the challenge in developing an ontology is to make it user-friendly and understandable to health scientists.

The report also aims to show how case studies of well-understood developmental toxicants can be used to elucidate the elements to be incorporated into formalised developmental toxicology.

Currently, there is no single source of information providing a comprehensive ontology of developmental toxicity linked to the MIEs and AOPs responsible for these effects. A developmental toxicity ontology (DTO) would be invaluable for scientists as it would contribute knowledge and understanding for:

- Development of *in vitro* approaches (including high-throughput screening) and *in silico* models for developmental toxicity.
- Development of AOPs, to elucidate what is known, what are the data gaps, and what are the potential inter-relationships between different biological pathways.
- Generation of hypotheses around critical events underlying adverse developmental outcomes, including the complex relationships between environment, genetics and host factors (e.g. nutritional status).
- Development of biomarkers of developmental toxicity.
- Hazard and risk assessment of chemicals for developmental toxicity.
- Furthering of research on the biology of reproduction.

Use of rational design in product development (e.g. computerised structural design to create molecules).

3. AOP/ MOA ONTOLOGY CONCEPT DEFINED

Ontologies are used in biology as a way to classify terms and their relationships to broader concepts and their interrelationships. Once these concepts and their relationships have been formally defined, new relationships between concepts may emerge, and classifying one concept as a type or subclass of another becomes possible. Formally, concepts are generally called “classes”; relationships are called “relationships”. Generally, ontologies operate as a system of triples. Triples consist of a subject-predicate-object. The subject and objects are classes, while the predicate is the relationship that connects them.

For example, consider a pizza ontology. Within this ontology, there is a class called ‘pizza’, defined as a thing with a crust and toppings (note that sauce is optional, as there are some pizzas which lack sauce, such as white pizzas). ‘Toppings’ has three subclasses: (1) meat, (2) vegetable, and (3) cheese. There is also a subclass of pizza called a ‘vegetarian_pizza’, which is defined as a pizza with vegetable toppings, no meat toppings, and it may or may not have cheese toppings. Thus, we could develop a specific instance of vegetarian_pizza from Joe’s Pizza Shack called, “Veggie Supreme.” In subject, predicate, object form, we would have “veggie_supreme is_a vegetarian_pizza”. Here the subject is “veggie_supreme”, “is_a” is the predicate, and the object is “vegetarian_pizza.” An example of a developmental biology illustration of a triple would be an increase in retinoic acid level (subject) enhances (predicate) cell differentiation (object), or in AOP general terms, KEx (subject) leads to (predicate) change in KEx+1 (object).

An ontology will allow scientists to begin to ask questions. For instance, we could identify the assays associated with the minimal suite of KEs within an AOP that are sufficient to infer an adverse outcome with high confidence. We could also consider a set of parameters, such as the gestational age at exposure and a series of high-throughput screening data, and query the ontology to identify potential adverse outcomes for chemical screening decisions.

Having the data encoded in an ontology also makes it easy to store and manage. Data can be obtained from various sources, including that already encoded in other ontologies, and easily encoded into the developmental toxicity ontology. In some instances this may require parsing the data and re-encoding it. In other instances it may be as easy as a simple import. Once the data are encoded, it can be easily queried and analysed using a number of freely available or commercial, off-the-shelf tools. A number of standards exist for querying data within ontologies built upon existing standards, such as the Web Ontology Language (OWL) for encoding the ontology and its associated data, and SPARQL (SPARQL Protocol and RDF Query Language) for querying the data within the ontology.

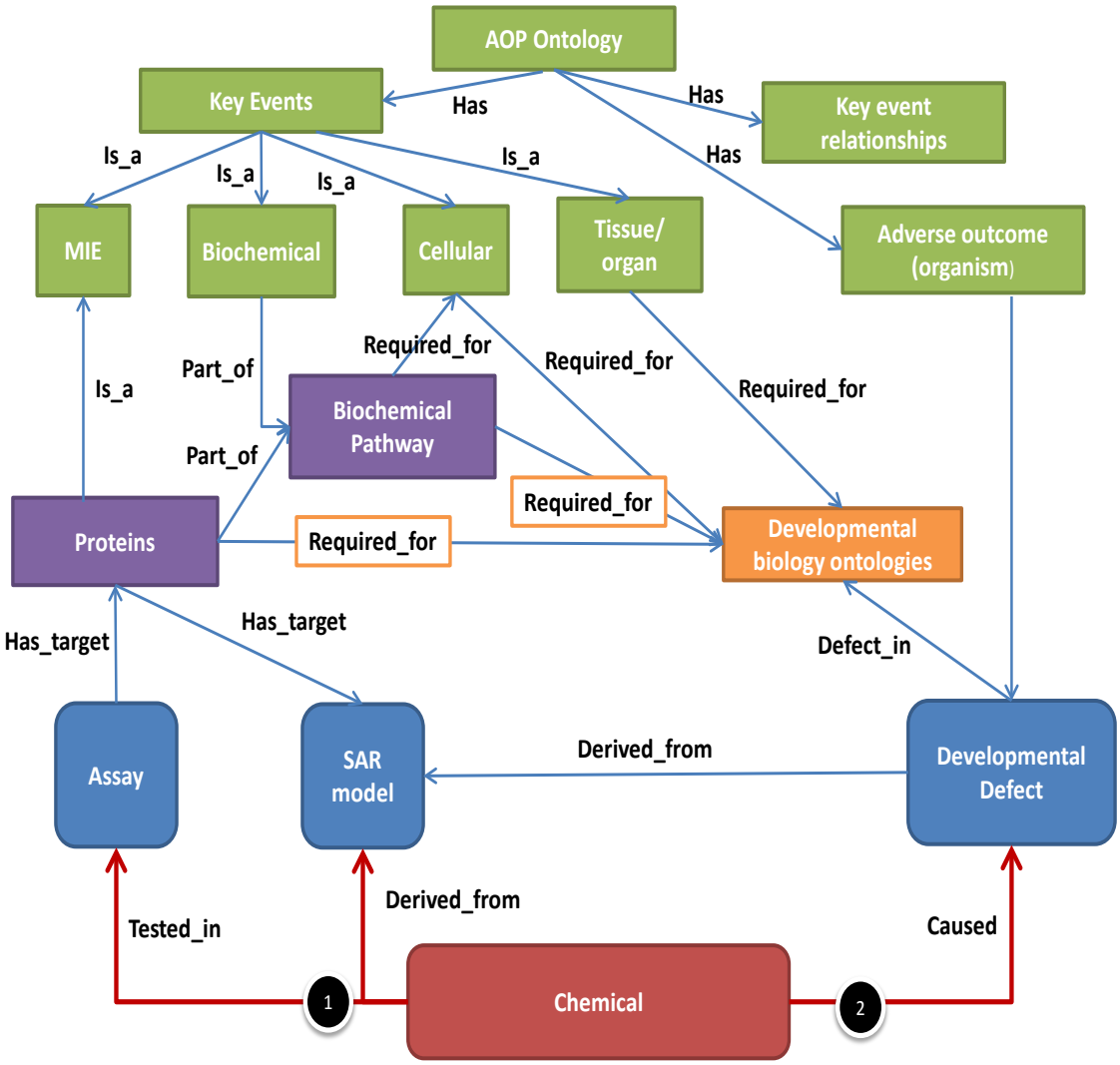
The ontology can be stored in an RDF (Resource Description Framework) database. The same RDF database can be populated with data from biological assays and chemical assays such as ToxCast or Connectivity Map. If the data are entered following prescribed ontologies, the relationship between chemical activity and perturbation of development can be predicted or captured. To continue with the pizza metaphor: if a chemical has the effect of disrupting meat production, a pizza normally covered with meat might become a vegetarian pizza. Ideally, the reduction in meat and its relationship to the phenotype of the pizza could be expressed in quantitative terms. When the ontology and the chemical perturbation data are stored appropriately, SPARQL queries should be able to reveal phenotypic outcomes like this one. To move the discussion into more relevant space, let us suppose the developmental ontology links palate growth to

retinoic acid (RA) signalling. The RDF triple store will contain the connections between palate growth and RA and between the RA receptor and levels of retinoic acid. The RDF may also have assay information showing that an environmental chemical binds and blocks the RA receptor with affinity. A SPARQL query should be able to reveal that this chemical activity disrupts palate growth.

The RDF format facilitates the merging and integrating of data and concepts. The RDF database, for instance, could integrate chemical structural information from a chemistry source. By employing a chemical structure ontology, a query could be constructed that reveals that many chemicals with this feature are linked to the same developmental perturbation.

It is important to note that the developmental ontology and data are separate, even though they are both stored in the RDF. The developmental observations are organised by the developmental ontology and the assay data, for instance, will be organised by an assay ontology. Reasoning with the ontologies on the data is a function of SPARQL query language. The potential contribution of AOPs to the building of developmental ontologies and the identification of appropriate high-throughput assays and *in silico* models for prediction of developmental toxicants is shown in Figure 2.

Figure 2: Interrelationships between the building of AOPs, developmental ontologies and potential screening assays



4. RESOURCES FOR BUILDING A DEVELOPMENTAL TOXICITY ONTOLOGY

A number of groups have developed ontologies for human development as well as genetic and other developmental abnormalities. Portals for biological ontologies and for toxicological or adverse effects, as well as those based on the developmental effects of specific chemicals are available. In parallel with the development of ontologies, some of them specific to developmental toxicity, there have been considerable advances in determining mechanisms/MOAs for adverse effects on developmental outcomes, including in some cases associated toxicological information on the causative chemical, and the compilation of this information into publicly accessible repositories (See Table 1).

Table 1: Summary of some publicly available resources on ontologies of human and animal development and information on developmental effects and developmental toxicants

Ontologies for Human Development include:	
Uberon (Mungall *et al*., 2012)	Primarily based on anatomical relationships
EHDAA2 (Bard, 2012)	
Gene Ontology (GO; http://geneontology.org/)	
AmiGO 2 from University of Berkeley (http://amigo2.berkeleybop.org/amigo),	
National Library of Medicine MeSH headings (2015)	
Human Phenotype Ontology (http://www.human-phenotype-ontology.org/)	
Ontology for Biomedical Investigations (http://purl.bioontology.org/ontology/OBI).	Links information on several aspects, including function and pathology
Existing ontologies on genetic and other developmental abnormalities include:	
OMIM (Online Mendelian Inheritance in Man; http://omim.org/)	
GWAS Central (http://www.gwascentral.org/phenotypes/tree)	
Portals for biological ontologies, including aspects of development	
The Open Biological and Biomedical Ontologies (http://www.obofoundry.org/)	
BioPortal (http://bioportal.bioontology.org/)	
Ontologies for toxicological or adverse effects, that include developmental effects	
MEDDRA (Medical Dictionary for Regulatory Activities) (http://bioportal.bioontology.org/)	
International Classification of Diseases (http://www.cdc.gov/nchs/icd.htm)	Currently in transition to the 10th revision
Ontologies based on the developmental effects of specific chemicals	
US EPA ToxRefDB (http://epa.gov/ncct/toxrefdb/files/ToxRefDB_DevTox_10Feb2009.xls) (http://actor.epa.gov/toxrefdb/faces/Home.jsp)	Also hosts associated toxicological information on the causative chemicals
DevTox initiative in Germany (http://www.devtox.org/index.htm)	Also hosts associated toxicological information on the causative chemicals
OECD QSAR Toolbox (Reproductive/developmental toxicity ontology, http://www.qsartoolbox.org/ontologies)	
Efforts are underway to construct a comprehensive toxicological ontology (OpenToxipedia OntologyBrowser, http://www.opentoxipedia.org/index.php/Special:OntologyBrowser) but as yet the section on developmental toxicity has to be started.	
Additional databases of toxicological information on developmental effects (amongst others)	
RepDose (http://fraunhofer-repdose.de/repdose/)	
ACToR..(http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=D37C5BDFE4 B361E108FD2BD56FE48770),	
ECHA (http://echa.europa.eu/information-on-chemicals) OECD eChemPortal (http://www.echemportal.org)	
Background information on design and conduct of developmental toxicity studies in the rat and rabbit	
Leroy and Allais , 2013	
Allais and Reynaud, 2013	
Barrow, 2013	
Data generated using non-animal methods is being compiled into publicly accessible databases	
US EPA ToxCast (http://epa.gov/ncct/toxcast/data.html)	
European Bioinformatics Institute Chemical Entities of Biological Interest (http://www.ebi.ac.uk/chebi/)	
The OECD QSAR Toolbox (http://www.qsartoolbox.org/)	
Open TG-GATEs (http://toxico.nibio.go.jp/english/index.html)	
Comparative Toxicogenomics Database (http://ctdbase.org/)	
Chemical Effects in Biological Systems (http://www.niehs.nih.gov/research/resources/databases/cebs/index.cfm)	
Data from the DiXa project (http://www.dixa-fp7.eu/)	
DrugMatrix (https://ntp.niehs.nih.gov/drugmatrix/index.html)	
PubChem (https://pubchem.ncbi.nlm.nih.gov/)	

Following work by the WHO International Programme on Chemical Safety on MOA (WHO, 2007), the OECD commenced an activity to map AOPs for the adverse effects of chemicals in humans and other species, particularly those of ecotoxicological relevance. A key action was to establish a public repository (AOP wiki) of established and proposed AOPs (see Table 2). The intention is to cover all toxicological effects, including developmental toxicity. At present, the AOP wiki contains only a relatively limited number of AOPs, and very few of these are on mammalian development. The expectation is that the wealth of information being generated on the biological and toxicological effects of chemicals using non-animal methods will provide the substrate and impetus to develop a far greater number of AOPs, particularly when linked with the adverse outcome data available in some of the databases listed in Table 1.

A number of efforts are now underway to integrate this information into adverse outcome or toxicity pathways for developmental effects. For example, Knudsen *et al*. (2009), Kleinstreuer *et al*. (2011) and Sipes *et al*. (2011) have utilised data from high-throughput screening to develop predictive algorithms for a number of adverse effects on prenatal development. Others such as Robinson *et al*. (2010, 2013) and van Dartel *et al*. (2011) have investigated the use of toxicogenomics data for this purpose. Bal-Price *et al*. (2015) have reported on putative AOPs for developmental neurotoxicity. Some aspects of these approaches were discussed at a workshop of the Neurobehavioral Teratology Society[4] in 2009 (Bushnell *et al*., 2010). In very few instances were AOPs, as defined by the OECD, elaborated for any of the effects in question. One example where this was the specific focus of the study can be found in the work of Zhang *et al*. (2014) from the Hamner Institute.

A number of websites provide information on effects of chemicals on signalling pathways and other biological processes that might be relevant to AOPs (Table 2).

Table 2: AOP resources

AOP databases	
OECD resource for AOPs of chemicals in humans and other species	(http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm).
OECD AOP wiki (public AOP knowledge base)	https://aopkb.org/aopwiki/index.php/Main_Page).
Effects of chemicals on biological processes relevant to AOPs	
NIH LINCS project	http://www.lincsproject.org/)
Connectivity Map from the Broad Institute	https://www.broadinstitute.org/cmap/),

Despite the considerable work being undertaken in all of these areas, there is no single source of information providing a comprehensive ontology of developmental toxicity linked to the MIEs and AOPs responsible for these effects.

[4] Now renamed Developmental Neurotoxicology Society.

5. APPROACHES TO BUILDING AN AOP/ MOA ONTOLOGY

There are several possible approaches to create an ontology of developmental toxicity. Perhaps the most straightforward approach would be to mine the literature for reports that link chemicals with MIEs, and then on through the biological responses that result from these initial interactions. For this approach, the only information needed is chemical structure, putative MIE, and adverse outcome.

Another approach would be to take advantage of multi-scale modelling approaches, especially AOPs that define the KEs from MIE to ultimate outcome, as a starting point for such an ontology. Unfortunately, there are still too few AOPs that have been documented to date, as they also must rely on mechanistic data from the literature and require considerable effort to construct and validate. It would be impractical to wait for a critical mass of relevant AOPs before embarking on a developmental toxicity ontology, particularly given that the latter can inform and expedite AOP development.

For most chemicals or small molecules, the chemical structure is known information. Given that a critical component of the chemical-target interaction that constitutes an MIE is the chemical, a practical starting approach for ontology construction is to group developmental toxicants by chemical structural features that contribute to their MOA (e.g. known or inferred interaction with specific receptors, reactive characteristics that lead to DNA damage, etc.). The decision tree for developmental and reproductive toxicity end points, recently published by Wu *et al*. (2013), provides a structure for starting on an ontology. It is supported by the first approach (mechanistic studies from the literature) to add strength to conclusions about MOA.

In summary, we are considering two possible approaches to building an AOP ontology: (1) start from the chemicals and potential MIEs and work forwards through our knowledge of developmental biology to an adverse outcome, or (2) start from the adverse outcomes and work backwards to AOPs through our knowledge of developmental biology.

6. HOW CAN IT BE DONE?

6.1 Terminology

The use of a harmonised and internationally accepted nomenclature for developmental toxicity is a requirement for all database operations. Such a nomenclature would offer a comprehensive description of adverse developmental outcomes from traditional animal testing.

A harmonised terminology effort was undertaken through a series of "Workshops on the Terminology in Developmental Toxicology" to eliminate ambiguities and inconsistencies within the terminology and to establish working definitions for malformations and variations (Wise *et al*., 1997; Chahoud *et al*., 1999; Solecki *et al*. 2001, 2003; Makris *et al*., 2009; Solecki *et al*. 2013, 2015). Adaptations for a better use in computerised systems were made by dividing a teratological diagnosis into a *localisation* term and an *observation* term, by eliminating topographical descriptions from the apical endpoints and adding a hierarchical structure for the anatomical localisations, based on observational *modes* (External, Skeletal, SoftTissue). The USEPA's Toxicity Reference Database (ToxRefDB) slightly enhanced the annotation system, joining 895 terms from the harmonised nomenclature (version 1) with standardised terms from the OECD-OPPTS vocabulary to generate a thesaurus of 982 non-redundant terms (Knudsen *et al*., 2009). In the enhanced system, 'description' annotates the particular apical endpoint condition or phenotype (*observation*) and 'target' annotates coarse regional anatomy (*localisation*). The website for this DevTox nomenclature, together with other potential sources of terminology for developmental toxicology, is listed in Table 3.

Table 3: Literature and Data Sources

Scientific literature sources	
Medline	
Pubmed	
Databases	
Gene Ontology (GO) project	http://www.geneontology.org/
Edinburgh Mouse Atlas Gene Expression (EMAGE) database	http://www.emouseatlas.org/emage/
Mammalian Phenotype Ontology (MPO) Browser	http://www.informatics.jax.org/
Zebrafish Model Organism Database	http://zfin.org/cgi-bin
Online Mendelian Inheritance in Man (OMIM) database	http://www.ncbi.nlm.nih.gov/omim/
Potential sources of terminology and data to address developmental toxicology	
DevTox – a public website for internationally harmonised terms	http://www.DevTox.org
Licensed database	http://www.LeadScope.com
USEPA Toxicology Reference Database housing reference *in vivo* animal toxicology data for the ToxCast research program	http://www.epa.gov/ncct/toxrefdb/

There are several limitations with the DevTox vocabulary that need to be considered for an AOP-based approach. One is that it is observational rather than embryological. For example, hypospadias is mapped to

'trunk'. This coarse parent, although technically correct - the perineum, is not informative of the underlying biology leading to the defect. Hypospadias should rather be annotated as genitourinary (system), urethra (tissue), and penis (location). The latter triad maps an informative relationship between embryology and defect. A second caveat is that common conditions are missing. For example, the term coloboma appears as 'ocular coloboma' and 'palpebral coloboma'. The former misses a more specific diagnosis localised to the iris, retina, or choroid. Since DevTox adaptations made for computability divide a diagnosis into localisation and observation terms, while eliminating topographical descriptions, 'retinal coloboma' does not appear in the lexicon despite being the most common coloboma. Also, the DevTox terminology does not consider larger syndromes. For example, the CHARGE syndrome (Colobomas, Heart defects, Atresia of the choanae in the nasal structures, Retarded growth and mental development or CNS abnormalities, Genital hypoplasia in males, and Ear anomalies and/or deafness). The hierarchical relationship of these malformations in the DevTox lexicon identifies a need for a stronger developmental ontology.

6.2 Specification

Here we can move descriptions into related defects based on the embryology of the target system. In this way, the unique view of DevTox as an observation-based ontology system is extended with new concepts and relations derived from an embryology-based ontology. What is most useful is to map apical endpoints to developmental ontology that gives order and timing to pathogenesis. Descriptions are first integrated into elementary concepts (one and only one 'parent' and distinctive 'children': "necessary" must occur in order to define the relationship, and "sufficient" may be enough to define the relationship). For example, a 'disproportionate reduction in size of the optic globe' is an elementary concept; it is a necessary criterion for microphthalmia, but not sufficient because complete apparent anophthalmia may also apply. More information is needed to decide between these phenotypes (e.g. some evidence of optic tissue).

The medical ontology for human birth defects does not have the necessary specificity in this regard. Consider the classification system for human malformations adopted for the National Birth Defects Prevention Network, based on The Metropolitan Atlanta Congenital Defects Program (MACDP) (Correa-Villaseñor *et al.*, 2003). This is a population-based, birth defects surveillance program motivated by the thalidomide tragedy. It tracks approximately 50,000 births per year for 35 years to monitor trends over time and find co-occurrence patterns that elucidate etiology. Information collected on each infant for over 100 individual defects is classified using a six-digit code modified from the British Paediatric Association (CDC-BPA codes) based on the WHO *International Classification of Diseases, 9th revision, Clinical Modification* (ICD-9-CM codes). Codification queries the databases by anatomic specificity and medical classification of defects and is driven by the need to assess prevalence rates (e.g. cases per 10,000 births) and syndromes (e.g. CHARGE). Although CDC-BPA and ICD-9-CM meet the needs of large, population-based birth defects surveillance programs, the classification is not linked to a developmental ontology. As such, these classifications do not systematically address embryology.

Text-based systems such as the Medical Dictionary for Regulatory Activities (MedDRA) have been used to group human birth defects by anatomical location or clinical condition in smaller databases. A related classification system proposed for signalling teratogenic clusters has basically condensed and rearranged the familiar CDC-BPA codes into a three-tiered hierarchy: <organ system>, <preferred defect term>, and

<reported defect term> (Scheuerle and Tilson, 2002). The first level uses traditional medical reporting, modified where necessary to impose current embryological considerations to 20 basic categories of organ systems and specified syndromes. The second level attempts to define defects by the most standard and recognisable terminology as defined in the diagnosis, which is the third level. By aggregating individual defects into pathogenic groups, the system improves diagnostic specificity for multiple terms used to describe the same defect. A three-tiered system allows the database to be queried at different levels of specificity to make inferences across smaller population sizes. Although this can increase the visibility of an early signal of developmental toxicity, it is still basically an anatomy-based observational system.

An example of what can be done is the work of Georgas *et al*. (2015) who have developed a definitive spatiotemporal description, at the level of organ, tissue and cell type, for the developing lower urinary and reproductive tracts in the mouse. The information has been incorporated into a text-based anatomical ontology spanning developmental time, space and gender.

Formalising associative relationships between anatomical structure and spatial location, functional system and chronological stage in the embryo requires hierarchical information. Bard (2005) discussed the difficulties imposed in trying to formalise abnormal anatomy across organisms.

6.3 Ontology development

Formalising the associative relationships between an anatomical structure and its spatial location, functional system and chronological stage requires hierarchical information. Ontologies link facts as a triad of related terms that can be integrated with other data using common controlled vocabularies (Smith *et al*., 2007). This can be done using web-accessible resources such as CARO (Common Anatomy Reference Ontology), CL (Cell Type), ZFA (Zebrafish Anatomy and Development), and EMAP (Mouse Gross Anatomy and Development), which can be found at The OBO and OWL hot-links found at http://obofoundry.org/.

Building a formal system that unambiguously makes explicit the knowledge to be included in the ontology of developmental processes and toxicities is not a trivial task (Bard, 2005). To bring together the vertical observational series (e.g. phenotype ontology) with a longitudinal embryological series (e.g. the forward progression of outcomes as development advances) is a composite task. For example, existing ontologies can be merged and thus arrange information by embryology (EMAP) and developmental toxicology (DevTox). Thus ToxRefDB taxonomises 982 terms (level-5) into 51 embryological targets (level-4), 24 embryological systems (level-3), 141 tissue localisations (level-2), and 3 observational modes (level-1 modes). The model combined DevTox ontology (levels-1, -2 and -5) with developmental ontology from EMAP (level-3, embryological system; level-4, embryological target). The Open Biomedical Ontology (OBO) website[5] in Web Ontology Language (OWL)[6] format has also been used to write developmental ontologies for Theiler Stages

[5] OBO website: http://obofoundry.org/

[6] OWL is a language of the semantic web to express natural language (used on the world-wide web) in machine-readable form. It uses a triad structure to define classes and interrelationships to annotate taxonomic hierarchy <**classes**><**properties**><**individuals**>:

(see Bard, 2007) and Carnegie Stages (see Hunter *et al*., 2003), describing mouse and human development, respectively.

Having a sound DevTox ontology will codify the organisation of facts and concepts into useful descriptions based on embryology and some degree of common pathogenesis and interoperability with other resources. For example, an emerging mouse/mammalian phenotype ontology resource using OBO is being developed for the Mammalian Phenotype Ontology (MPO) browser as part of the Mouse Genome Informatics (MGI) project at The Jackson Laboratory (http://www.informatics.jax.org/). The MPO browser deconstructs mammalian phenotypes into their constituent terms using a schema proposed as phenotype/attribute (PATO[7], EUMORPHIA[8])/value, as shown in the example below.

MGI EXAMPLE: microphthalmia (199 genotypes, 199 annotations)

<MP term> microphthalmia

<synonym> small eyes

<MP id> MP:0001297

<Definition> reduced average size of the eyes

The same condition is represented in OBO as:

[Term]

id: MP:0001297 ! microphthalmia

intersection_of: PATO:0000587 ! decreased size

intersection_of: inheres_in MA:0000261 ! eye

A main advantage of using PATO is the ability to express phenotypic ontologies based on concept relationships, rather than instances. A PATO-compliant zebrafish database is being developed by The Jackson Laboratory to manage morpholino-induced phenotypes (morphants)[9] (Knowlton *et al*., 2008).

<**classes**> unit of taxonomy; sets, collections, or types of objects

<**properties**> features that objects have and share (attributes)

<**individuals**> basic, ground level objects (entities); instances in a class

[7] PATO: Phenotypic Attribute Trait Ontology.

[8] EUMORPHIA: A project that connects mouse mutants to human disease.

[9] A type of molecule used in molecular biology that alters the development of genes by preventing access by other molecules (knockdown of targeted gene expression).

6.4 Informal ontologies

Informal ontologies that include less explicit information can make a useful contribution when the end-user is somewhat knowledgeable about the field (Bard, 2005). For example, mapping gene expression identifiers (GeneIDs) by stage, tissue and region in development and extracting this information for a sensitive period of development to a particular chemical or class of chemicals can provide information about pathway-level responses to exposure. An informal ontology defining target tissue can then include detailed tissue geometry and morphogenetic boundary conditions drawn from conventional histology (Bard, 2005). Interoperability can be built with ontology tools such as Protégé.

[Term]

id: MP:0001297 !	microphthalmia
intersection_of:	EMAP:304 !TS12, eye
vulnerability_start:	EMAP:304 !TS12
vulnerability_end:	EMAP:3003 !TS18
associated_with:	Pax6, Fgf8

The distribution of a particular phenotype or combination of features can be summarised by 'frequency' and 'redundancy'. We can define *frequency* as any reference to the term in a document, which may be positive (exposure-related), negative (mentioned but not observed), or noise (not exposure-related). We can define *redundancy* as the number of occurrences for each record. Using redundancy as a quantitative metric, we can apply multivariate clustering to give information on the association of a particular organ system with a chemical, group of chemicals, or animal model. Essentially it is a measure of sensitivity. The pattern of terms appearing together (co-occurrences) can give information on syndromes for a chemical or species as a measure of specificity. Thus, some pairwise statistics would be useful to assess how often two particular terms appear jointly in an experimental condition or dose group.

6.5 Natural Language Processing (NLP)

Literature text-mining is an important aspect of informal ontology development. Whereas many database projects are underway to manually curate data from developmental endpoints, unstructured data presents a different challenge. This information often holds the key to the major themes or ideas associated with the structured data but must be extracted within proper context and managed differently than structured data. NLP can capture a good deal of information about molecular and pathway activity from the scientific literature, starting with curated databases (e.g. GO – gene ontology, EMAGE – mouse embryo gene expression, GXD – mouse gene expression, MPO – mammalian phenotype ontology, ZFIN – zebrafish model organism database, OMIM – online Mendelian inheritance in man). NLP enhances the coarse semantic search for specific concepts and then provides a way to automatically extract the key facts, relationships and quantitative information from literature. The results are then presented to an analyst to perform manual

quality assurance/quality control (QA/QC) and data cleaning. Extensible Markup Language (XML) conveys information about text or other data using embedded codes not easily read by humans. Since XML syntax rules functionally represent data from any subject domain, unstructured data must be parsed with common software tools that read universal XML syntax rules. Rule-based indexing, extensible thesaurus, document classification and document filters go beyond simple keyword searches to summarise information as *major themes* or *main ideas* for developmental processes and toxicities. It is important to use consistent terms when populating the ontology with such information.

As a specific example of NLP, consider:

> <observation>: “over expression of” | “under expression of” | “co-regulation of”
>
> <gene>: “PKA” | “PKB” | “PCNS” | “RAP” | (any gene related to development)
>
> <stage or location>: “in the liver” | “in gastrulation” | “during gastrulation”
>
> <effect>: “causes” | “resulted in” | “activates” | “controls” | “regulates”

When a regular expression parser is applied to abstracts available in PubMed, entries such as the following excerpts are flagged as potentially important:

> “... Overexpression of PCNS resulted in gastrulation failure but conferred little if any specific adhesion on ectodermal cells. Loss of function accomplished independently with two non-overlapping antisense morpholino oligonucleotides resulted in failure of CNC migration, leading to severe defects in the craniofacial skeleton. ...” (Rangarajan *et al*., 2006).
>
> “...We used Affymetrix microarrays to examine temporal gene expression patterns during chondrogenic differentiation in a mouse micromass culture system. ... One gene that was up-regulated at later stages of chondrocyte differentiation was Rgs2. Overexpression of Rgs2 in the chondrogenic cell line ATDC5 resulted in accelerated hypertrophic differentiation, thus providing functional validation of microarray data. ...” (James *et al*., 2005).

NLP can reliably capture the complex relationships for an <observation><gene><stage or location><effect>. However, QC issues must be concerned with information that is either not relevant to the model under investigation (noisy data), or whether key information is not being identified (incomplete data). NLP can also assist with running deeper queries. For example, a formal ontology of embryo development that is fact-based can be used as an automated core for the application of an informal ontology that is easier to navigate but less automated.

This can be illustrated by considering the case for <hypospadias>. The advantage of a simple hierarchy linking the defect to a functional system, such as <genitourinary system>, is the straightforward path to define a subhierarchy for each part, the <urethra> for example, that can be easily navigated by non-experts. Triples can describe almost any concept and can be described in standard formats that are recognised by machines (Murray-Rust, 2008):

Hypospadias {is a defect of genitourinary development that affects the male urethra}.

Written in this way, the sentence is about hypospadias (subject) and the {predicate} tells something about it. The same sentence can be written in different ways with the same meaning. As a triple, we can consider the subject <hypospadias> and the predicate <is a defect of genitourinary development that affects> linked to an object <male urethra>.

In a broader sense the relevant endpoints that comprise critical effects in developmental toxicology studies traditionally include a search string that might be modified from ToxML[10] language, of the form:

<observation>: “malformation of” | “litter size” | “evaluation of” | “weighing” |

<target>: “eye” | “face” | “liver” | “foetal weight” | (term in keywords_target) |

<description>: “hydronephrosis” | “microphthalmia” | (term in keywords_description) |

<effect>: “reduced” | “results in” | “increased” | “deficiency” | “duplication of” |

6.6 Gene Networks

Because mutations in gene regulatory networks underlie many human congenital anomalies [Bard 2007], it follows that developmental toxicants may also produce their adverse effects by altering these same developmental networks. Mouse is the most used mammalian model for understanding the connectivity between genes and human disease and its role is demonstrated by the inclusion of a goal for constructing genetic and physical maps for the mouse genome within the Human Genome Project. Online encyclopaedias are available to support this knowledge exchange. Consider, for example, the Mouse Genome Informatics (MGI) database[11] that provides integrated access to data on the genetics, genomics, and biology of the laboratory mouse. Users may search or browse the database for a Mammalian Phenotype Ontology (MPO) term to view term details and relationships among terms, including links to genotypes annotated with each term or any sub term. The MPO is a structured vocabulary aimed at standardising annotations and describing unambiguous clinical phenotypes in mice using terms derived from ~100 physiological systems, behaviours, developmental phenotypes and survival/aging conditions (Smith *et al*., 2005). For example, searching the MPO browser using the term <eye> returned 79 MPO terms, including abnormal eye development, abnormal anterior segment morphology, microphthalmia, anophthalmia, and so forth. An important use of text-mining will be to build conceptual network models of interacting genes affiliated with morphogenesis and differentiation of specific structures. Resources such as EMAGE, a curated histological database based on gene expression in mouse embryos, and The Jackson Laboratory’s GDX database, a compendium based on phenotypes, provide resources to identify relevant genes.

To filter linkages that are biologically meaningful we could specify threshold occurrences or use strings that reliably extract developmentally-relevant grammar. For example, CoPub (Frijters *et al*., 2007, 2008) can be

[10] ToxML: Open source data exchange, XML-based standard that represents toxicological data in a structured electronic format.

[11] http://www.informatics.jax.org/

used to calculate keyword over-representations from text-mining of the literature, based on gene-gene co-occurrences. This assumes co-citation of gene + keyword in the same abstract indicates strength of relationships. The CoPub 'relevance score' (R-score) describes the strength of a co-association between two keywords given their individual frequencies of occurrence and the number of co-occurrences between every pair in the set. The formula for the raw score is:

$$S = PAB/PA*PB$$

where PA is the number of hits from item 'A' divided by the total number of PubMed identifiers (PMIDs), PB is the number of hits from item 'B' divided by the total number of PMIDs, and PAB is the co-occurrences of items A and B divided by PMIDs. The R-score basically scales these values and transforms them to log10 scale for ranking:

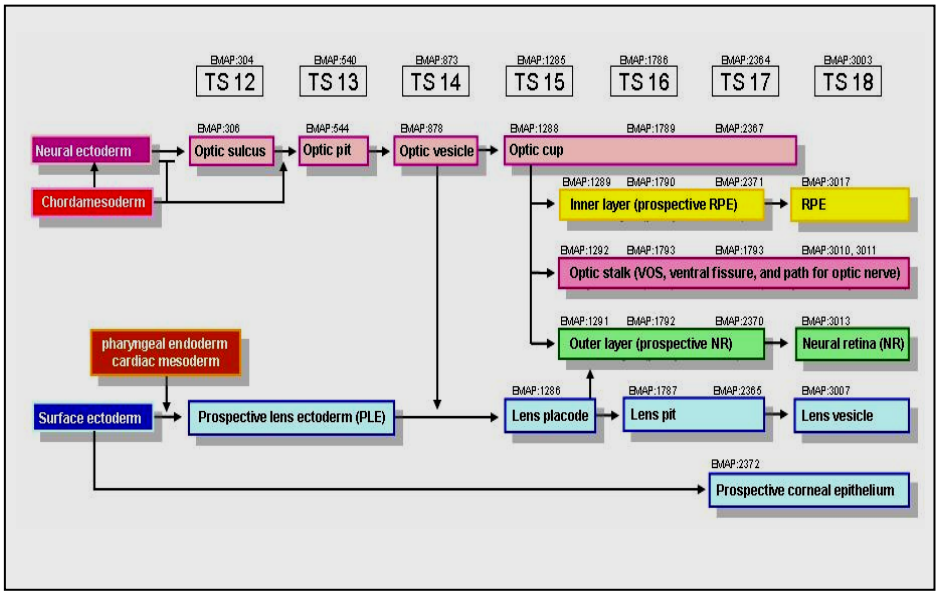
$$R=10\log S$$

Example: Ontology for early eye development in the mouse

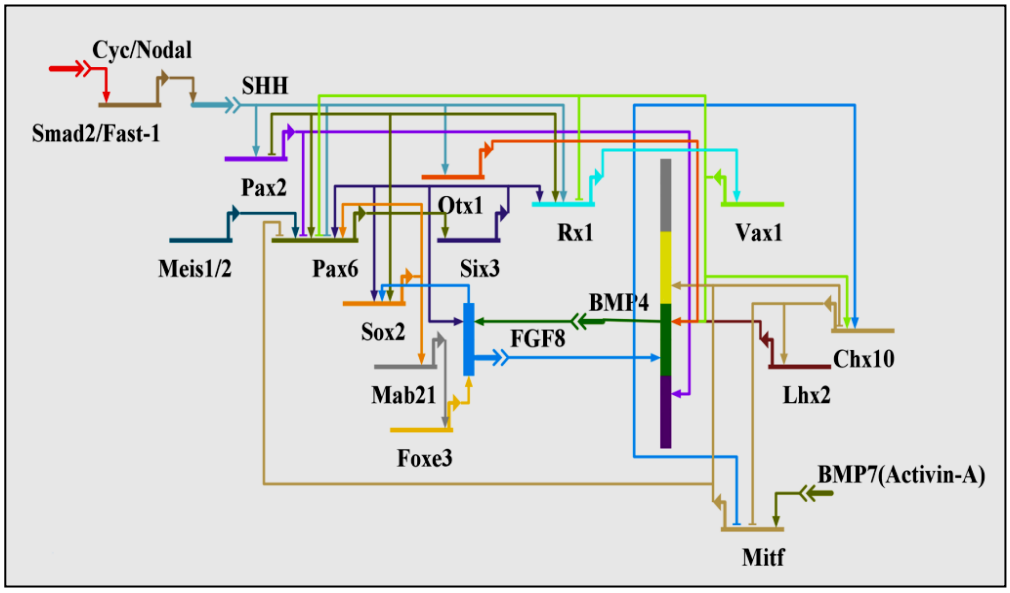
Eye development can be perturbed by genetic mutations and environmental exposures, leading to malformations such as anophthalmia, microphthalmia, coloboma, and cataract. These defects occur in more than a million children worldwide (6.8 per 10,000 live births, ~28,000 annually in the USA). An OVID search of the Medline database revealed specific reference to ocular malformations in 2% of teratology literature in general and 25% of the mouse teratology literature in particular. This implies broad susceptibility of the eye to diverse agents. In the mouse, gestational days 7 to 11 encompass the window of vulnerability to eye reduction malformations such as microphthalmia/anophthalmia, aphakia/aniridia and coloboma. In modelling the formation of a system such as the eye the first step is to lay out its normal pattern graphically. There is a good deal of ontology information already available for this purpose (Baldock *et al*., 1992; Bard 2007). The Edinburgh Mouse Atlas Project (EMAP) (http://genex.hgu.mrc.ac.uk/intro.html) is mapping successive stages of mouse embryonic development to catalogue gene expression domains. Consider, for example, the EMAP ontology for early eye development over Theiler stage (TS)12 to TS18 (see Figure 3).

Figure 3: Ontology for eye development in the mouse

Developmental ontology (*EMAP*)



Wiring diagram (*EMAP*) *BioTapestry*



Annotations are based on the EMAP system (Bard, 2007) over TS 12-18. Prior specification of the optic field is initiated in the anterior neural plate by interactions between ectoderm, mesoderm + endoderm at gastrulation, giving the ectoderm lens-forming 'competence'. Subsequently, eye development is induced from the neural ectoderm and surface ectoderm.

Descriptive embryology has shown the importance of reciprocal tissue inductions over this period. Although annotating terms with standard ontology IDs carries no molecular data, the currently available gene-

expression information associated with a particular developing mouse tissue at a given TS is computationally accessible from the mouse gene expression database, GXD (http://www.informatics.jax.org/mgihome/GXD/aboutGXD.shtml), through ID interoperability (Bard, 2007).

7. CHALLENGES TO BUILDING AND APPLYING AN AOP/ MOA ONTOLOGY

The challenges to building an AOP/MOA developmental toxicity ontology (DTO) include (1) the role of potency (and separating adaptive from adverse response), (2) the importance of maternal toxicity as a driver/confounder of *in vivo* responses, and (3) the importance of developmental stage susceptibility. Identifying a parsimonious testing strategy for identifying a specific AOP is a challenge to applying an AOP/MOA ontology. Additionally, most of the toxicology literature is descriptive and evaluates effects at the organ and organism level and generally does not contain information on mechanism of action, a least not at the granularity that is needed to support a relatively complete ontology.

Translation of an AOP/MOA ontology into a testing strategy containing assays covering the KEs (qualitatively and quantitatively) is necessary for an efficient assessment of the possible developmental toxicity potential of chemicals. Translation of the response magnitude in each KE-representing assay, in terms of adaptation versus adversity, is also required. In other words, thresholds of adversity need to be defined, either for individual assays or for combinations thereof. Moreover, the outcome of a developmental toxicity IATA should be accompanied by an uncertainty analysis, for which tools and approaches need to be defined and put in place.

AOPs describe physiological/toxicological routes as the elements of the ontology. Thus, AOPs can be seen as the bricks needed for building the ontology house, leading to an IATA. Components of IATA outside the domain of the ontology are chemical-related approaches such as structure/activity, physicochemical properties, *in silico* modelling, grouping, read-across, ADME, etc. This approach is fit for purpose if applied case-by-case in an iterative process of initially limited testing and deciding on the next testing step based on interim findings. This process ends if sufficient information has surfaced to underpin hazard and risk assessment for the tested compound.

The acceptability of a DTO-driven IATA for mechanistically based developmental toxicity hazard and risk assessment is heavily dependent on whether the DTO is comprehensive. Comprehensiveness is not necessarily determined by the level of detail of the description of the biology involved, but rather by the extent to which the DTO leads to an IATA that is sufficient to detect developmental toxicants with sufficient sensitivity and specificity, as agreed by risk assessors and risk managers. This would be facilitated by complete and open sharing of all *in vivo* toxicology data to ensure the comprehensiveness of the DTO.

7.1 Maternal factors

A further aspect that needs consideration is the interaction between the mother and conceptus. It is clear that this interaction is not immediately included in the DTO. The strength of the *in vitro*/*in silico* assays is considered by many to be the absence of the confounding influence of maternal organism/placenta. This influence may in some cases be a confounder in animal (*in vivo*) testing, i.e. masking the potential intrinsic developmental toxicity of a compound by species-specific, high maternal toxicity. However, the intact interaction of mother and conceptus also is an essential component of risk assessment, taking into account

bioavailability, metabolism and placental transfer. Moreover, some additional factors, such as the availability of essential nutrients necessary for development may also be influenced, leading to toxicity, which can only be identified *in vivo*.

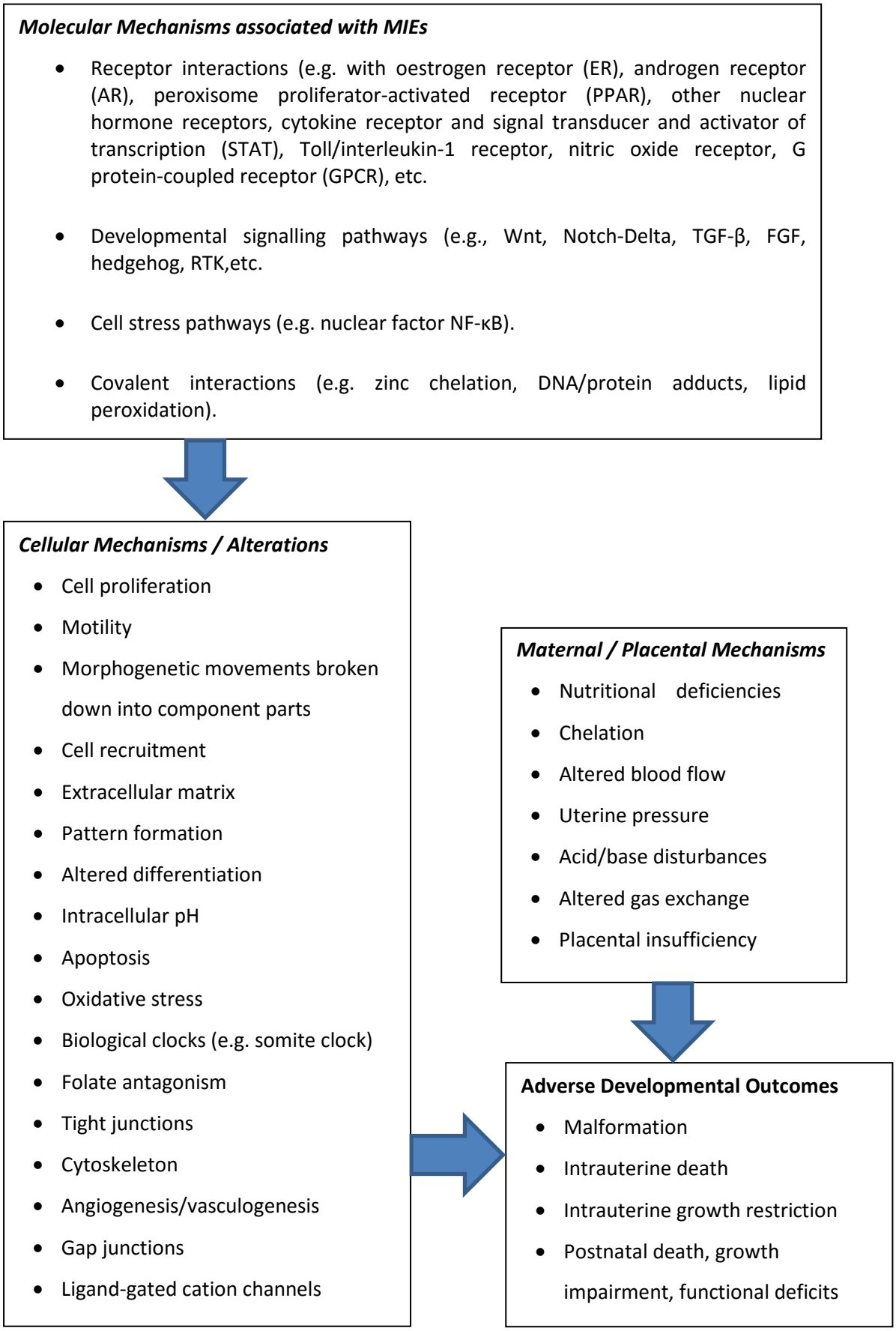
Thus, for risk assessment, the role of the mother, frequently condensed in the term “maternal toxicity”, needs to be considered and is an essential component in an IATA. The presence of the mother and placenta are major strengths of the intact animal tests because the exposure of the human conceptus to potential insult is indeed via the mother, through the placenta; absorption, distribution, metabolism, and elimination of the chemical in the mother and in the placenta determine and control the exposure of the conceptus — when it is exposed, and how long it is exposed. The health of the mother affects the growth and development of the conceptus(es) *in utero*, the success of delivery, and the continued postnatal growth and development of the offspring neonatally, during the lactational period and beyond. The term “maternal toxicity” covers a variety of maternal effects which may or may not affect development, depending on the mode/mechanism of action of the chemical, the dose, the severity of the effect(s), and the timing of exposure. Information on the interactions between the mother and the conceptus may also provide answers on how KEs in the cascade of developmental processes are regulated, or whether they are perturbed or delayed by “outside events”, or whether there are interactions between different AOPs.

Advances in the prediction of *in vivo* developmental toxicity have been made by combining an *in vitro* model using embryonic stem cells with a simple *in vitro* model for placental transfer (Li *et al*., 2015). This demonstrates the importance of maternal factors (such as the placental barrier function) but also indicates the possibility to include these in a more complex model. Physiologically-based pharmacokinetic (PBPK) modelling should be an essential part of the final risk assessment. However, by itself, PBPK modelling only describes the concentration of the compound causing developmental toxicity, and is not a DTO per se. Some other maternal factors, such as the transport and availability of (micro)nutrients, stress hormones, and oxygen, can be direct-acting developmental toxicants and would need to be taken into account at some stage.

7.2 Potential MIEs and KEs for building AOPs and IATA for Developmental Toxicity

Simply defining the level of biological organisation at which the initiating event for toxicity occurs can be a challenge. As indicated below, some toxicity may be the result of an exogenous chemical interacting with a specific biomolecule, such as a receptor or enzyme, and it is this KE (i.e. sufficient occupancy of the receptor, or inhibition of the enzyme) that is the necessary step to initiate the subsequent cascade of events at the molecular, cellular and tissue level that produce the adverse outcome. In other cases, the effect may be at the level of the cell, such as a covalently reactive electrophile that has no specific molecular target, but does sufficient damage to many macromolecules within cells that it leads to cell death or dysfunction at a critical developmental stage. As noted above, even factors external to the embryo, such as placental dysfunction or maternal physiological perturbations (maternal toxicity), which may also have no distinct molecular target, can also be the KE that initiates adverse development. Examples of molecular, cellular, and maternal/placental mechanisms that may be involved in MIEs and KEs in AOPs for developmental toxicity are shown in Figure 4.

Figure 4: Examples of molecular, cellular, and maternal/placental mechanisms that may be involved in MIEs and KEs in AOPs for developmental toxicity



8. CASE STUDIES FOR AOP DEVELOPMENT

A Developmental Toxicity Ontology (DTO) was built for the purposes of these case studies. The DTO builds upon the Adverse Outcome Pathway Ontology (AOPO)[12]. AOPO builds upon the Bioassay Ontology (BAO)[13], the Human Phenotype Ontology (HPO)[14], and the Chemical Entities of Biological Interest Ontology (ChEBI)[15], as well as their dependencies.

8.1 Role of Retinoic Acid during Embryogenesis

Retinoic acid (RA) is a morphogen that plays a key role in vertebrate embryogenesis. It is produced from provitamin A in mesodermal tissues that express representatives of the retinaldehyde dehydrogenase family of enzymes. RA is primarily a differentiation inducer. It competes with growth stimulating factors, such as those of the FGF family, and with other developmental regulators, such as those belonging to the Wnt and Hox families, to exert its effects.

The balance among a host of interacting factors, which changes with time during embryogenesis and is dependent on localisation within the embryo, determines the fate of individual cells in individual locations at distinct time points during development. RA plays a key role in the formation of the vertebrate body plan, being involved in anterior-posterior patterning, axial differentiation of the neural tube, caudal-ventral specification within the central nervous system as well as hindbrain development. Moreover, it regulates neural crest cell migration, contributing to the formation of a host of tissues and organs, such as facial structures, heart, the hematopoietic system, limb innervation and peripheral ganglia. RA activity is determined by the local presence, subtypes, and density of retinoid receptors, which have been grouped in RAR and RXR receptor families. Though RA receptors seem ubiquitous throughout the embryo, specific representatives of these receptor families have specific spatial distributions within embryonic tissues (Rowe *et al*., 1992; Viallet and Dhouailly, 1994; Elmazaar *et al*., 1996; Romand *et al*., 1998; Mandal *et al*., 2013). This may explain differences in embryotoxic characteristics among various embryotoxicants that all interfere with RA homeostasis.

In addition, RA is metabolised through CYP26 isoforms, which also show a subtype, time- and location-specific expression during embryogenesis. Other mechanisms such as sequestering to RA-binding protein 1 and 2 may also contribute to this regulation. For example, RA plays a crucial age- and cell-specific role in cranio-facial morphogenesis, including palatogenesis. Over-expression of RA at specific foetal ages can disrupt these processes and cause teratogenic effects, including the induction of cleft palate. Since catabolism by CYP26 is the most important pathway, inhibition of this enzyme in a particular tissue, such as

[12] AOPO: https://github.com/DataSciBurgoon/aop-ontology

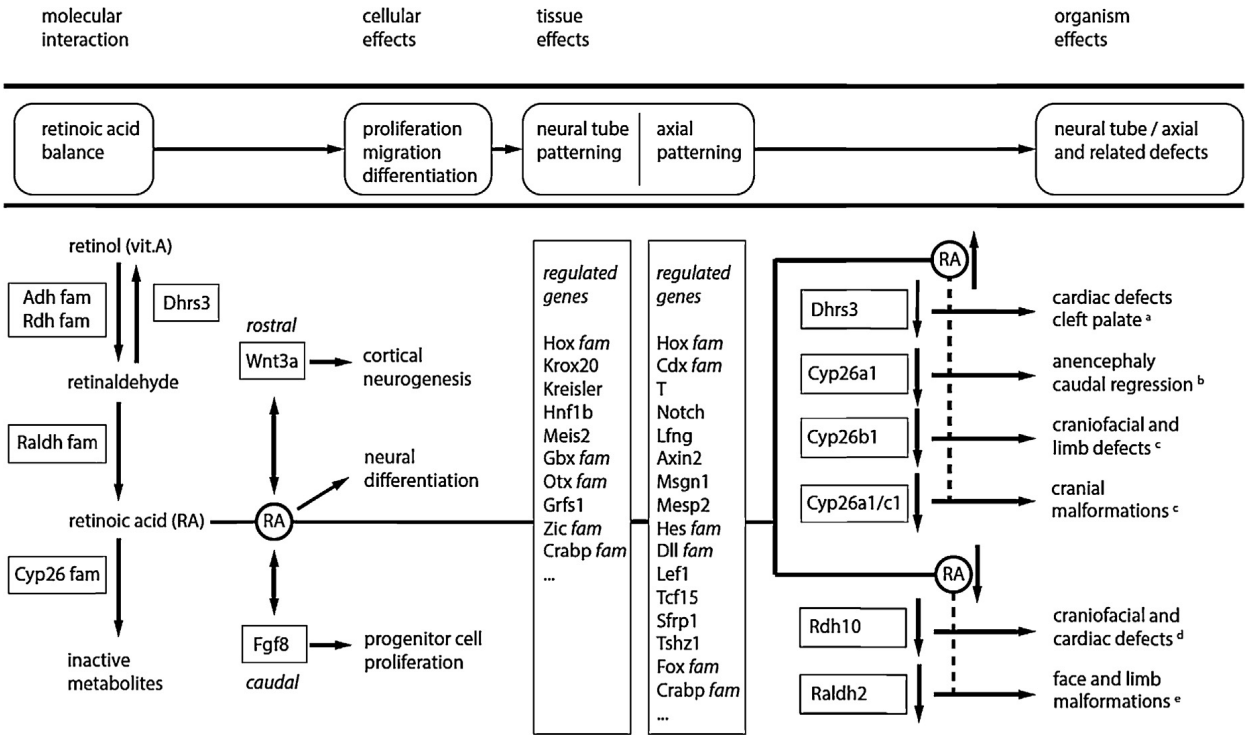
[13] BAO: http://bioassayontology.org/

[14] HPO: https://bioportal.bioontology.org/ontologies/HP

[15] ChEBI: https://www.ebi.ac.uk/chebi/

the developing head, would result in increasing RA levels (Chambers *et al*., 2014). Thus, a strictly programmed multifactorial interplay between RA-producing and RA-metabolising enzymes, competing growth and development stimulating factors, and retinoid receptors and their time- and location-specific expression leads vertebrate embryogenesis from a fertilised egg to a morphologically recognisable vertebrate embryo. The central role of retinoid function in vertebrate embryogenesis provides opportunities for identifying biomarkers of abnormal development that may allow detection of a large proportion of developmental toxicants. Many teratogens and embryotoxicants may be assumed to interfere at some level with retinoid homeostasis, be it through direct interaction with, for example, its production, metabolism or receptor binding, or as a secondary consequence of initiating events occurring in pathways that interact with the retinoid effect, such as the expression of Hox genes or FGF. An AOP framework describing RA homeostasis and its functional interactions with other morphogenetic factors in embryogenesis could help identify such biomarkers. A first attempt towards such a framework was published recently (Tonk *et al*., 2015) and is depicted in Figure 5 below. This study also reviews data showing that retinaldehyde dehydrogenases, CYP26 members, and a host of RA-regulated patterning genes can be readily detected and shown to be regulated in alternative assays such as whole embryo culture, zebrafish embryo test and embryonic stem cell tests. Furthermore, *in silico* developmental models (Knudsen *et al*., 2015), such as exist for eye and limb development, also show direct connections with retinoid regulation.

Figure 5: Proposed AOP framework for RA-neural tube/axial patterning pathway



Reproduced with permission from Tonk et al. (2015).

The importance of RA homeostasis is exemplified by human teratogens as well as by knockout mouse studies. The production of RA from beta-carotene is an important rate-limiting mechanism for systemic

exposure in man. It is well known that pregnant women who consume high amounts of carrots during pregnancy may acquire an orange skin through extensive beta-carotene deposition, but this does not affect their babies due to limited metabolism to the active form of vitamin A, which is RA. Synthetic retinoids used as pharmaceuticals against persistent acne caused severe facial, limb and heart malformations (Lammer *et al*., 1985). However, oral human exposure in pregnancy to RA via multivitamin preparations marketed in the 1980s resulted in children with similar abnormalities (Werler *et al*., 1990; Rothman *et al*., 1995) Retinaldehyde dehydrogenase deficient mice show uncontrolled growth of undifferentiated tissue in the facial area (Rhinn and Dollé, 2012). CYP26-deficient mice show caudal regression syndrome due to precocious cell differentiation limiting caudal growth (Rhinn and Dollé, 2012). Because of the regional specification of CYP26 subtype expression, the specificity of malformations in CYP26-deficient mice depends on the CYP26 subtype being knocked out (Pennimpede *et al*., 2010). In humans, vitamin A deficiency has recently been related to ear malformations (Emmett and West, 2014).

It will be of great interest to investigate all areas of chemical space for their interactions with the retinoid system during embryogenesis in order to determine its predictive value and to define sensitive biomarkers for abnormal development in alternative test systems. Existing databases can be searched specifically for retinoid-related mediators of development, be it at the level of gene expression, proteomics, metabolomics, or whatever level of biology that provides practical tools for monitoring possible adverse effects of chemicals and drugs on vertebrate (and especially human) development. As an example, in the zebrafish embryo model, developmentally toxic triazole antifungals have been shown to upregulate CYP26 enzymes and downregulate retinaldehyde dehydrogenase (Hermsen *et al*., 2012). The use of azole compounds as fungicides is based on their greater affinity for the fungal sterol 14α-demethylase (CYP51) than for the mammalian or plant enzymes. In fungi they block the synthesis of the essential membrane component ergosterol. However, inhibition of CYP51 is not specific and other CYPs can also be affected, including CYP19 (the aromatase) and CYP26, which metabolises RA. Consequently application of RA or ketoconazole to pregnant rats (Mineshima *et al*., 2012) or itraconazole to pregnant mice (Tiboni *et al*., 2006) induced cleft palates and other skeletal effects. Inhibition of aromatase by azole compounds leads to post-implantation loss due to inhibition of 17β-oestradiol synthesis. Multiple additional examples of retinoid pathway modulation by embryotoxicants have been identified.

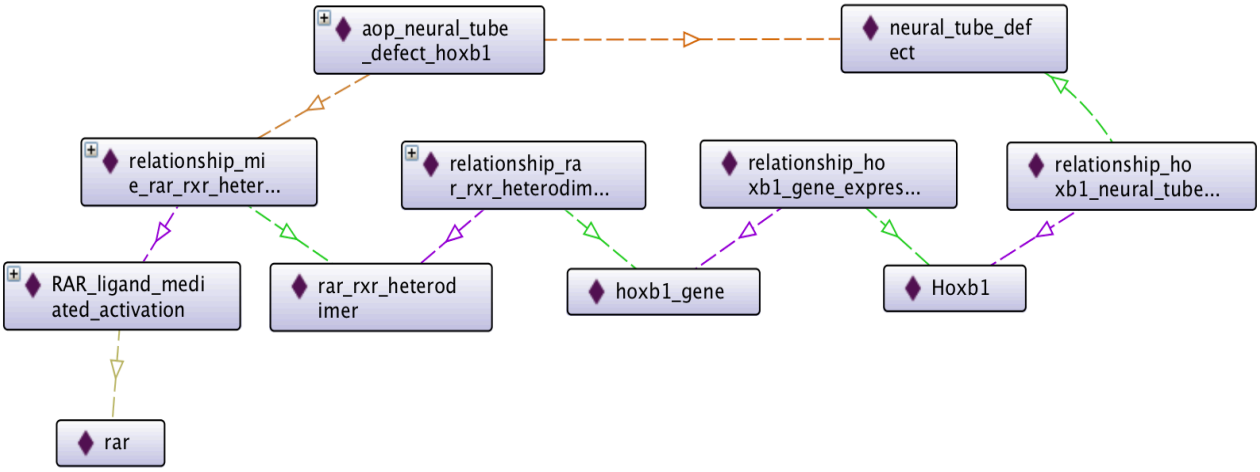
8.2 Retinoic Acid and Neural Tube Defects

A single AOP for neural tube defects from Tonk *et al*. (2015) was modelled in the AOPO. This AOP starts with an MIE of chemical binding to and activating of the retinoic acid receptor (RAR). This MIE is followed by RAR and retinoid X receptor (RXR) heterodimerisation, leading to upregulation of Hoxb1 gene expression, Hoxb1 protein translation, and finally neural tube defects.

This AOP was modelled in the AOPO by creating a class for the adverse outcome (AO-NeuralTubeDefect), and an individual derived from this class (neural_tube_defect). Note that individuals are actual instantiation of a class, meaning that an individual is tangible, whereas a class is a description of the traits that individuals within a class must have. In addition, we have defined the individual neural_tube_defect to also be an instantiation of the HPO class “Abnormality of Neural Tube Closure.” This allows us to more easily connect/link the AOPO to other ontologies that use definitions based on the HPO.

Because of the interconnectedness of the AOPO with the BAO, *in vitro* assay data and toxicogenomic data can be overlaid on the AOP for RA-mediated neural tube defects. When assays detect, or transcriptomic experiments suggest, activation of RAR and Hoxb1 protein translation, we can infer that these chemicals may cause neural tube defects through this MOA. A representation of the retinoic acid receptor-mediated neural tube defect AOP is shown in Figure 6 below.

Figure 6: A representation of the retinoic acid receptor-mediated neural tube defect AOP



The boxes represent individuals or instances of a class within the ontology. For example, neural_tube_defect is an individual of the AdverseOutcome class. The lines are semantic relationships connecting two boxes, as follows:

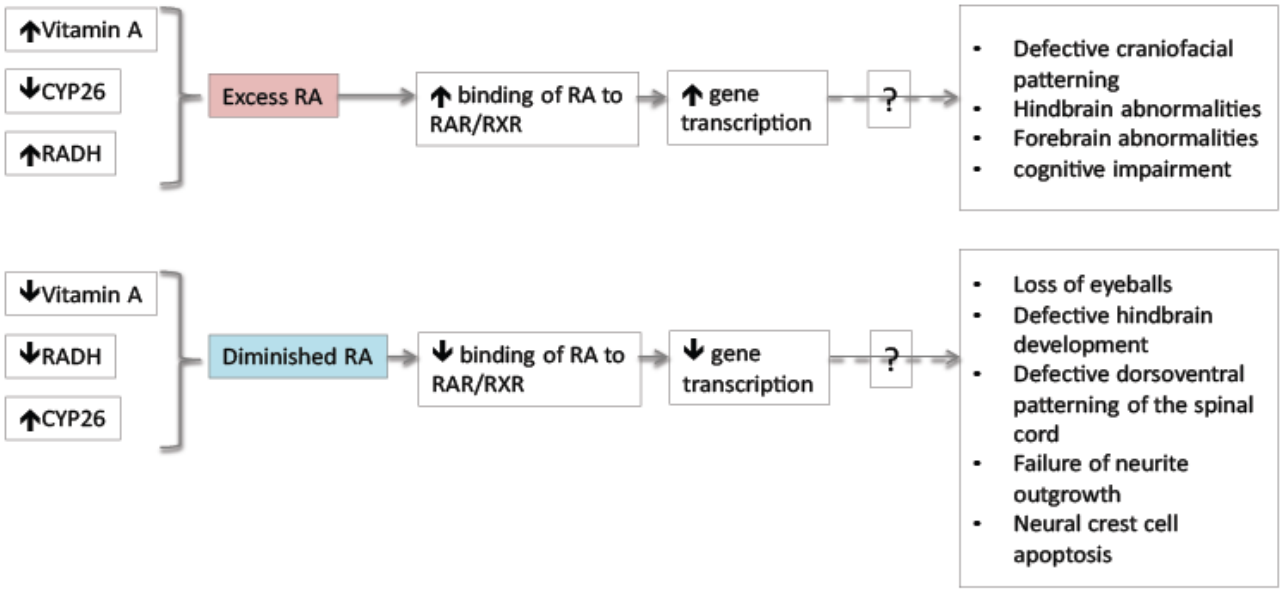
- green lines are “has_downstream_key_event” relationships;
- purple lines are “has_upstream_key_event” relationships;
- the brown line between aop_neural_tube_defect_hoxb1 and neural_tube_defect represents the “has_adverse_outcome” relationship;
- the darker brown line from aop_neural_tube_defect_hoxb1 represents the “has_mie_relationship”;
- the golden line represents the “has_activated_key_event” relationship.

8.3 Retinoic Acid and (hind) brain development

During neurodevelopment, the spinal cord contains the highest RA levels, while forebrain, midbrain and hindbrain contain very little RA (Horton and Maden, 1995). As RA cannot be synthesised de novo by embryonic or adult organisms, developmental RA supply is produced in the target tissue from maternal dietary retinol uptake. Retinol dehydrogenases (RDH) produce retinaldehyde from retinol, which is further metabolised by retinaldehyde dehydrogenases (RADH) to RA. However, due to lack of RADH2 expression, embryonic brain tissue does not produce RA from retinaldehyde itself, but mesodermal somites flanking the neural tube produce RA. This diffuses into areas of neuroectodermal tissue, which will form segmental units for future hind-, mid- and forebrain development. In the cranial part of the neural tube, RA-metabolising CYP26A1 is substantially expressed converting RA to 4-hydroxy-RA and 4-oxo-RA, the substrates for

glucuronidation and excretion. Due to RADH2-dependent RA formation in more caudal areas and CYP26A1-reliant RA metabolism a RA gradient spans across the future hindbrain. This gradient is thought to determine hindbrain formation as Vitamin A-deficient embryos display a complete lack of the caudal hindbrain (Maden *et al*., 1996; White *et al*., 2000; McCaffery *et al*., 2003). During hindbrain development seven to eight rhombomeres form that relate to later defined hindbrain areas. Individual rhombomeres contain specific expression patterns for transcription factors including *Wnt* family members (reviewed by Marshall *et al*., 1996; Rijli *et al*., 1998), which facilitate the identification of the missing rhombomeres numbers four to seven in experimentally-induced RA-deficiency (McCaffery *et al*., 2003). Thus, caudal hindbrain development is dependent on RA homeostasis. Both RA deficiency and RA excess can produce developmental abnormalities, as shown in Figure 7 below.

Figure 7: Retinoids and brain development



Recently, a clinical hypothesis has been proposed that RA deficiency also causes underdevelopment of the hindbrain in humans (Emmett and West, 2014). This hypothesis was based on the observations that hearing loss is a global public health problem, mainly in low- and middle-income countries, paralleled by Vitamin A deficiency in such developing areas (WHO 2009, 2013). While it is now well established that other reasons, like ear infections triggered by lack of RA, contribute to hearing loss, Emmett and West (2014) proposed the scientifically plausible, but virtually unexplored causal relationship between hearing loss due to RA-deficiency-dependent underdevelopment of the inner ear in humans. There seems to be a critical threshold for proper inner ear development in mice with low retinoid intake causing immature and/or ectopic otic vesicles (Niederreither *et al*., 1999). These abnormalities are likely due to the loss of RA-dependent regulation of hindbrain development and the otic morphogenic process (White *et al*., 2000; Maden *et al*., 1996). To test the hypothesis that this mechanism contributes to hearing loss in the human population, a Vitamin A-supplemented population study is to be planned by Emmett and West (2014).

Besides hindbrain development, additional processes of neurodevelopment are affected by RA-deficiency:

- Decreased neurite outgrowth (reviewed in McCaffery *et al*., 2003).
- Neural crest cell apoptosis (reviewed in McCaffery *et al*., 2003).
- Abnormal dorsoventral patterning of the anterior spinal cord (reviewed in McCaffery *et al*., 2003).
- Anterior-posterior patterning of the forebrain (reviewed in Rhinn & Dollé, 2012).
- Cell survival in the telencephalon (reviewed in Rhinn & Dollé, 2012).

That different processes of neurodevelopment can be targets for disturbed RA homeostasis is important for the human relevance of RA-dependent signalling pathways for brain development. Targeted genetic manipulation or experimental pharmacological interference causes defined neurodevelopmental, histopathological phenotypes in quail and rodent embryos. In humans, such brain developmental phenotypes cannot easily be studied. However, human mutations in the *Stra6* gene, a RA-inducible gene that regulates cellular retinol uptake, causes – amongst other severe developmental defects – mental retardation across all living cases (Chassaing *et al*., 2009) implying necessity of RA also for human neurodevelopment beyond inner ear development. Precise pathophysiological processes underlying human mental retardation due to intracellular RA-deficiency are not known.

8.4 Conclusions on Case Studies on Retinoic Acid

The above has shown that we now have extensive mechanistic knowledge on the central role of RA in vertebrate embryo development. Therefore, this theme provides a good starting point for deriving a DTO that will inform the construction of a developmental AOP network. KEs in the network can be defined which allow the collection of relevant assays in an IATA to detect a major part of developmental toxicants. Key ontological terms will have to be defined at the molecular, cellular, tissue, organ, and organism level in a hierarchical connectivity construct. Testing KE modulation in dedicated *in vitro* assays will allow the projection of compound effects upon the network, resulting in prediction of developmental toxicity hazard potential. The addition of kinetic models, especially those addressing the behaviour of KEs in a compound concentration-related way, should, in due course, allow quantitative inferences about potency and risk.

9. USING TOXCAST TO DEVELOP AOPS

The U.S. EPA's ToxCast program (Kavlock *et al*., 2012) and cross-agency Tox21 program (Tice *et al*., 2013) are building large collections of *in vitro* data on diverse sets of chemicals to which humans are potentially exposed, including pesticides, food, cosmetics and personal care ingredients, pharmaceuticals, and industrial chemicals. Chemicals are being tested for bioactivity at various levels of biological organisation in a broad battery of *in vitro* assays that include cell-free systems, cell lines and primary cells from multiple tissue types, complex culture systems, embryonic stem cells and zebrafish embryos. The ToxCast database can be found at http://epa.gov/ncct/toxcast/data.html (release date December 2014) and explored by chemical or assay using the Interactive Chemical Safety for Sustainability (iCSS) dashboard (http://actor.epa.gov/dashboard/).

The utility of ToxCast data in AOPs for developmental toxicity was demonstrated by Sipes *et al*. (2011), who built a predictive model in which the *in vitro* high-throughput screening data (ToxCastDB) was anchored to *in vivo* adverse outcomes from prenatal developmental toxicity studies (ToxRefDB). This early model utilised the first phase (Phase-I) of ToxCast, which consisted of 309 chemicals, mostly pesticide compounds, and a range of over 600 high-throughput screening assays. In the analysis, univariate associations (one assay to one endpoint) were used to identify significant *in vitro* to *in vivo* correlations. Multivariate predictive models (multiple assays to multiple endpoints) were then built from these identified assays using linear discriminant analysis with five-fold cross validation. The rat developmental toxicity multivariate model had a predictive accuracy of 71% (sensitivity 0.72, specificity 0.70; P-value = 7.5E-11). Among the positive predictors composing this model, the RAR assay set was the strongest weighted variable (weight factor 0.58) followed by G protein-coupled receptors (weight factor 0.55), TGF-beta (weight factor 0.38), microtubule organisation (weight factor 0.30), and other lesser weighted features (Sipes *et al*., 2011).

Since the Sipes *et al*. (2011) study, ToxCastDB has expanded to include *in vitro* results for 1,858 chemicals and up to 821 assay features. The latter derives from 541 unique high-throughput screening assays that can be mapped to 293 molecular targets and high-throughput screening assays for diverse cellular behaviours and responses, including 37 different assays for cytotoxicity (Judson *et al*., 2016). Several recent analyses of the ToxCastDB (*in vitro*) and ToxRefDB (*in vivo*) data identified the retinoid pathway as a major component in models for male reproductive developmental defects (Leung *et al*., 2015), cleft palate (Baker *et al*. in preparation), and digital defects (Ahir *et al*. 2014, and in preparation). Since RA signalling mediates proper growth and differentiation of the embryo, a potential application for ToxCast is to identify possible targets that could, in the context of AOPs, define MIEs for critical alterations to RA homeostasis or signalling pathways. Across the 293 molecular targets, gene ontology (GO) annotations[16] produced 18,367 records of which at least 52 could be mapped to a molecular target in the retinoid system. This includes reporter assays for transactivation of retinoid receptors (RARs and RXRs), and cis-activation of the retinoic acid response element (RARE) by RAR/RXR heterodimers. A detailed analysis of chemical-bioactivity profiles for *in vitro* targets in the retinoid signalling system is presently underway; however, for the purposes of this illustration, we simply mined for coherent linkages to MIEs affecting the aforementioned assay targets. All-trans retinoic

[16] GO: http://amigo.geneontology.org

acid (ATRA) was a reference compound used to benchmark the AC_{50} (concentration at 50% of maximum activity) for each particular assay.

A total of 879 ToxCast AC_{50}s were mapped to a molecular target in the retinoid system (Baker *et al*., 2016). In total, 97 of 1,858 chemicals (5.2%) hit one or more assays in the retinoid system at an AC_{50} below 2 uM. With regards to retinoid metabolism (KEGG[17] pathway hsa00830: Retinol metabolism), the ToxCast dataset presently lacks information on retinal dehydrogenase (EC: 1.2.1.36; RALDH), the enzyme that generates RA from retinol, and on cytochrome-P450 family 26 (EC: 1.14.-.-; CYP26), the enzyme specific to its breakdown. However, the dataset does have results on the biochemical activity of cytochrome-P450 family 1, subfamily A, polypeptide 1 (EC:1.14.14.1; CYP1A1), another enzyme capable of ATRA breakdown. ATRA competed with the substrate of the CYP1A1 assay to inhibit its biochemical activity with an AC_{50} of 1.32 uM, whereas retinol was inactive. Flusilazole, an antifungal known to disrupt RA homeostasis and invoke dysmorphogenesis (Tonk *et al*., 2015), registered an AC_{50} of 3.7 uM on CYP1A1 activity, and, in all, 11 ToxCast chemicals, mostly pesticides, inhibited CYP1A1 activity at AC_{50}s below ATRA. This supports other evidence that disruption of RA homeostasis is a possible MIE for developmental AOPs and that some environmental chemicals may disrupt normal development through this mechanism.

ToxCast has reporter gene assays for three distinct RAR subtypes (RARα, RARβ, RARγ) and three distinct RXR subtypes (RXRα, RXRβ, RXRγ). The main assay platform utilises a HepG2 cell line engineered for transactivation of reporter genes and fold-induction in response to chemical exposure. ATRA was the most potent of all chemicals tested in the RARα and RXRα transactivation assays (subnanomolar AC_{50} values of 0.429 nM and 0.309 nM, respectively). Retinol had weaker AC_{50} values of 69 nM (RARα) and 1.54 µM (RXRα). RXR/RAR heterodimers bind to RAREs composed of tandem 5'-AGGTCA-3' sites known as DR1-DR5; ATRA activated the DR5 cis-reporter assay with an AC_{50} value of 6.26 nM whereas retinol had a moderate AC_{50} of 147 nM. Across the entire ToxCast inventory the numbers of chemicals registering an $AC_{50} < 2$ µM were: 9 (RARα), 4 (RARβ), 6 (RARγ), 9 (RXRα), 23 (RXRβ), 0 (RXRγ), and 51 (DR5).

Some classes of persistent organic pollutants (POPs) preferentially activated the RARs at AC_{50}s below 2 uM (e.g., organochlorines). In contrast, at least two classes of environmental chemicals preferentially activated RXRs with AC_{50}s below 2 µM (e.g., tert-butyl compounds) or 0.2 uM (e.g., organotins). Those compounds displayed similar activity on the DR5 assay as well as distinct retinoid receptors. A number of pesticides that disrupt mitochondrial respiration displayed activity on DR5 responses with AC_{50}s below 2 uM (e.g., strobins, rotenone). Thus, *in vitro* profiling of the retinoid signalling system in ToxCast identified approximately 5% chemicals with a potential for direct disruption of RA signalling through transactivation of RAR or RXR systems at submicromolar concentrations. Given the potential for these receptors to heterodimerise with different nuclear receptor subtypes (e.g. RARα with RXRα; RXRα with PPARγ, LXRβ, VDR, TRβ, or FXR), the analysis of ToxCast data allows a provisional catalogue of MIEs to be built that mechanistically invoke AOPs

[17] KEGG: Kyoto Encyclopedia of Genes and Genomes. KEGG PATHWAY mapping is the process to map molecular datasets, especially large-scale datasets in genomics, transcriptomics, proteomics, and metabolomics, to the KEGG pathway maps for biological interpretation of higher-level systemic functions.

associated with RA signalling and homeostasis pathways. An ontology for developmental toxicity is necessary to put this complexity into a computable and integrated form.

Conclusions

Chemical risk assessment is at a crossroads, moving from classical animal studies looking for adverse health effects towards mechanistic approaches based on human relevant scientific knowledge involving molecular to organism targets and all intermediate levels of complexity. This change of perspective is supported by increased knowledge of molecular mechanisms underlying toxicity, the availability of an abundant array of animal-free test methods, and the expanding work on the description of AOPs, integrated toxicity testing strategies and integrated approaches to toxicity testing and assessment.

The application of these innovative approaches is especially challenging in the area of developmental toxicity, with the developing embryo as its moving target, changing its form, its physiology and its susceptibility to exposures continuously as morphogenesis progresses. The complexity of embryogenesis and its time- and location-specific changes in susceptibility require an integral approach to mechanistic developmental toxicology.

Thus, there is a need for an ontology specific to developmental toxicity that would enable computer-based prediction of which chemicals are likely to induce human developmental toxicity. The ontology should be built by developmental toxicity experts in collaboration with ontology experts. The AOP concept plays a critical role in the ontology by facilitating connections between the chemicals, biological processes, and adverse outcomes.

This report has described some of the principles and approaches feeding into the definition and derivation of a developmental ontology, which could serve as a tool for an integrated assessment of developmental toxicity. Several examples of activities feeding into the development of such an ontology are mentioned, such as the US EPA Virtual Embryo project, the ToxCast database of alternative assays, and the Retinoic Acid Pathway of (dys)morphogenesis.

Combining all existing knowledge into a single developmental ontology will allow the derivation of novel adverse outcome pathways. In addition, it will allow the selection of prioritised biomarkers of adversity throughout the ontology that may be used in efficient integrated approaches of developmental toxicity assessment. More broadly, such an ontology could provide a template for the development of an ontology covering all of toxicity.

ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
AO	Adverse outcome
AOP	Adverse outcome pathway
AOPO	Adverse outcome pathway ontology
AR	Androgen receptor
BAO	Bioassay ontology
CARO	Common anatomy reference ontology
ChEBI	Chemical Entities of Biological Interest Ontology
CL	Cell Type
cpAOP	Computationally predicted AOP
DTO	Developmental toxicity ontology
EMAGE	Mouse embryo gene expression database
EMAP	Edinburgh Mouse Atlas Project
ER	Oestrogen receptor
GeneIDs	Gene expression identifiers
GO	Gene ontology database
GPCR	G protein-coupled receptor
GXD	Mouse gene expression database
HPO	Human phenotype ontology
IATA	Integrated approaches to testing and assessment
iCSS	Interactive Chemical Safety for Sustainability
KE	Key event
KER	Key Event Relationship
MACDP	Metropolitan Atlanta Congenital Defects Program
MGI	Mouse Genome Informatics database
MIE	Molecular initiating event
MOA	Mode of action

MPO	Mammalian Phenotype Ontology database
NLP	Natural Language Processing
OMIM	Online Mendelian inheritance in man database
OWL	Web ontology language
PBPK	Physiologically-based pharmacokinetic
PMID	PubMed identifier
POP	Persistent organic pollutant
PPAR	Peroxisome proliferator-activated receptor
QA/QC	Quality assurance/quality control
RA	Retinoic acid
RADH	Retinaldehyde dehydrogenases
RAR	Retinoic acid receptor
RDF	Resource description framework
RDH	Retinol dehydrogenases
RXR	Retinoid X receptor
SPARQL	SPARQL Protocol and RDF Query Language
STAT	Signal transducer and activator of transcription
ToxRefDB	(USEPA's) Toxicity reference database
USEPA	US Environmental Protection Agency
WHO	World Health Organization
XML	Extensible Markup Language
ZFA	Zebrafish Anatomy and Development
ZFIN	zebrafish model organism database

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