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Building a developmental toxicity ontology

Nancy Baker[1] | Alan Boobis[2] | Lyle Burgoon[3] | Edward Carney[4]* | Richard Currie[5] | Ellen Fritsche[6] | Thomas Knudsen[7] | Madeleine Laffont[8] | Aldert H. Piersma[9] | Alan Poole[8] | Steffen Schneider[10] | George Daston[11]

[1] Lockheed Martin, Research Triangle Park, Piedmont, North Carolina

[2] Department of Medicine, Imperial College London, London, United Kingdom

[3] U.S. Army Engineer Research and Development Center, Raleigh-Durham, North Carolina

[4] Dow, Midland, Michigan

[5] Syngenta, Reading, United Kingdom

[6] IUF – Leibniz Research Institute, Düsseldorf, Germany

[7] U.S. Environmental Protection Agency, Research Triangle Park, Piedmont, North Carolina

[8] European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Brussels, Belgium

[9] Center for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, and Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands

[10] BASF SE, GB/T-Z470, Ludwigshafen, Germany

[11] Central Product Safety Department, The Procter & Gamble Company, Mason, Ohio

Correspondence
G. Daston, Procter & Gamble, 8700 Mason Montgomery Road, Mason, OH USA 45040.
Email: Daston.gp@pg.com

Background: As more information is generated about modes of action for developmental toxicity and more data are generated using high-throughput and high-content technologies, it is becoming necessary to organize that information. This report discussed the need for a systematic representation of knowledge about developmental toxicity (i.e., an ontology) and proposes a method to build one based on knowledge of developmental biology and mode of action/ adverse outcome pathways in developmental toxicity.

Methods: This report is the result of a consensus working group developing a plan to create an ontology for developmental toxicity that spans multiple levels of biological organization.

Results: This report provide a description of some of the challenges in building a developmental toxicity ontology and outlines a proposed methodology to meet those challenges. As the ontology is built on currently available web-based resources, a review of these resources is provided. Case studies on one of the most well-understood morphogens and developmental toxicants, retinoic acid, are presented as examples of how such an ontology might be developed.

*Died on 12 January, 2015.

Discussion: This report outlines an approach to construct a developmental toxicity ontology. Such an ontology will facilitate computer-based prediction of substances likely to induce human developmental toxicity.

KEYWORDS

development, framework, ontology, retinoic acid, toxicity

1 | INTRODUCTION

As toxicology moves toward high-throughput and high-content screening, scientists are becoming overwhelmed with data on the effects of chemicals at a molecular/mode-of-action level. In order to efficiently use such data to predict adverse effects at an organismal level, it is first necessary to extract biologically meaningful information (knowledge) from the data. A systematic organization of data by presumed mode of action (MOA) is then required. Ontologies provide one means to deal with such knowledge in a structured manner by (1) linking molecular information to traditional toxicology study outputs and to human disease states, (2) providing clarity on whether existing high-throughput or high-content approaches are sufficiently inclusive of the universe of MOAs for toxicity, and (3) serving as an organizing structure for constructing adverse outcome pathways (AOPs).

Ontologies are often used when there is a need to integrate information from disparate sources allowing investigators to ask questions of the data encoded by the ontology. As developmental biology is characterized by a complex interplay between a multitude of processes at the molecular, cellular, tissue, and organism level, the development of a formal system (i.e., ontology) organizing knowledge of chemical structure, developmental biology, and developmental toxicology has potential value to:

- Predict and explain which chemicals are likely to induce human developmental toxicity;
- 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;
- Enable the design of more informative and predictive models and assessment strategies for developmental toxicity of chemicals;
- Improve public health protection through increased relevance and accuracy of testing;
- Facilitate the design of pharmaceuticals and other chemicals so that they are unlikely to have the potential for developmental toxicity in humans; and
- Save resources (time, animals).

The purpose of this report is to develop organizational principles and frameworks that can be used to build a developmental toxicity ontology (DTO). Initially, the scope of the ontology will focus on MOA. The ultimate goal is to expand the ontology to help create AOPs and integrated approaches to testing and assessment (IATA) that enable prediction of developmental toxicity. While the ultimate goal is to produce an ontology that encompasses quantitative AOPs, this report proposes that the starting point has to be a state-of-the-science MOA ontology. It is recognized that MOA and AOP have different analytical constructs, but from the computer science perspective, the structure of an AOP ontology will be similar to an MOA ontology. However, moving from an MOA approach describing qualitative molecular initiating events (MIEs) and qualitative key events (KEs) to a quantitative AOP approach will be challenging. To achieve this will require consideration of the nonlinearity of dynamic biological systems and critical periods in development as the same MIE at different time points in development might produce different outcomes. By starting with an MOA containing as much knowledge of biology and signaling mechanism as currently available allows movement toward building a quantitative AOP ontology.

This report explores how relevant qualitative and quantitative information from structured data (formal data sets) and unstructured data (from literature) can be organized into a logical ontology framework. Relevant information will include existing knowledge and inter-relationships 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 is to make the ontology user-friendly and understandable to health scientists. Case studies on one of the best understood morphogens and developmental toxicants, retinoic acid (RA), are presented as examples of how such an ontology can be built.

2 | THE AOP/MOA ONTOLOGY CONCEPT

Ontologies are used in biology as a way to classify terms, how they relate to broader concepts, and their interrelationships.

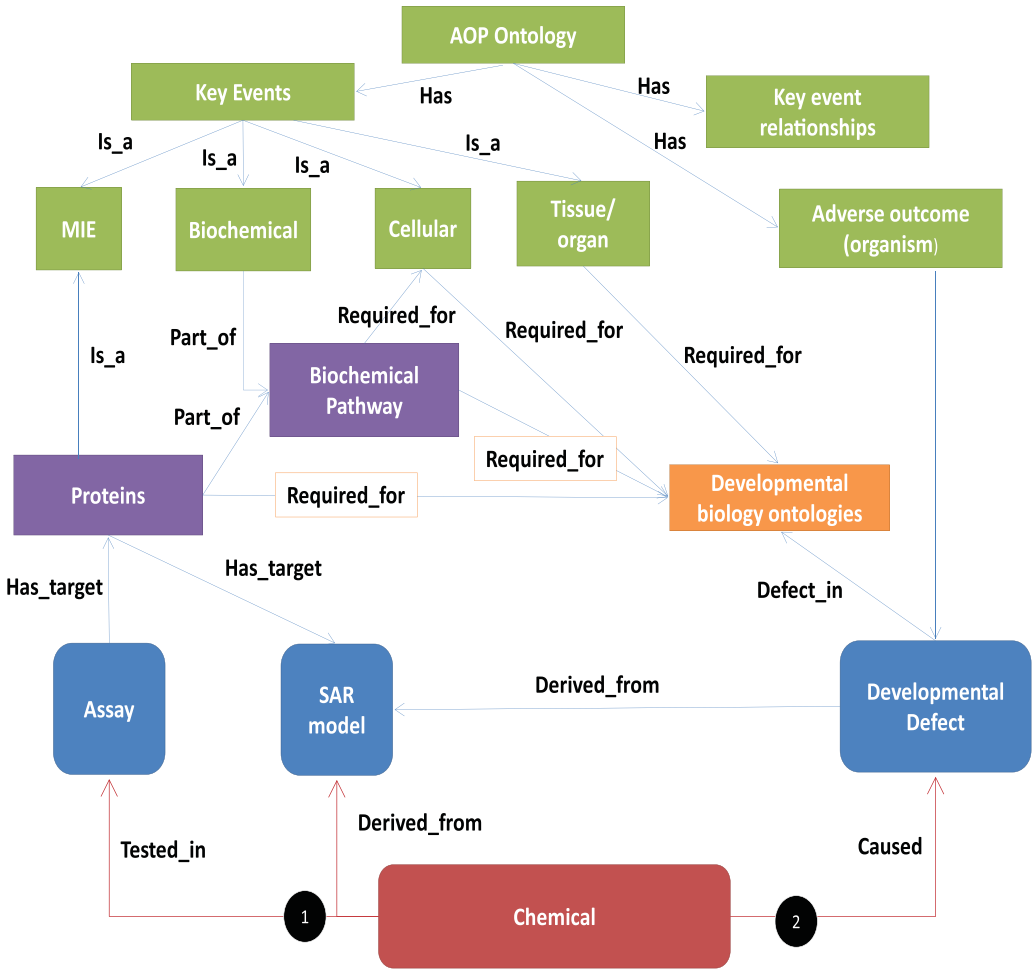

FIGURE 1 Interrelationships between the building of AOPs, developmental ontologies, and potential screening assays

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 concept becomes possible. Formally, concepts are generally called "classes"; relationships are called "relationships." Generally, ontologies operate as a system of triples consisting of a subject–predicate–object. The subject and objects are classes, while the predicate is the relationship that connects them. An example of a developmental biology triple would be an increase in RA level (subject) enhances (predicate) cell differentiation (object). To use terms common to AOP construction, KEx (subject) leads to (predicate) change in KEx + 1 (object).

Having the data encoded in an ontology makes it easy to store and manage. Data can be obtained from various sources, including those already encoded in other ontologies, and easily encoded into the DTO. Once the data are encoded, they can be queried and analyzed using a number of freely available or commercial tools. For example, once assays associated with the minimal suite of KEs within an AOP that are sufficient to infer an adverse outcome with high confidence have been identified, it becomes possible to 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.

The ontology can be stored in an resource description framework (RDF) 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. For example, if a developmental ontology links palate growth to RA signaling, the RDF triple store will contain the connections between palate growth and RA and between the RA receptor (RAR) and levels of RA. If the RDF has assay information showing that an environmental chemical also binds and blocks the RAR with high affinity, it becomes possible to use query tools to ask if the environmental chemical might also disrupt palate growth.

The RDF format also facilitates merging and integrating data and concepts. An example might be employing a chemical structure ontology to question if certain chemical structures are linked to the same developmental perturbation.

The potential contribution of building AOP developmental ontologies and the identification of appropriate

TABLE 1 Summary of some publicly available resources for terminology, concepts, or relationships important to ontology development

Ontologies for human development include:	
• Uberon (Mungall, Torniai, Gkoutos, Lewis, & Haendel, 2012)	Primarily based on anatomical relationships
• EHDAA2, Edinburgh Human Developmental Anatomy Abstract Version 2 (Human Developmental Anatomy Ontology; http://purl.bioontology.org/ontology/EHDAA) (J. Bard, 2012)	
• Gene Ontology (GO; http://geneontology.org/)	
• AmiGO 2 from University of Berkeley (http://amigo2.berkeleybop.org/amigo)	
• National Library of Medicine (2015), MeSH Terms	
• HPO (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:	
• Online Mendelian Inheritance in Man (OMIM; 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 (http://bioportal.bioontology.org/)	
• AOPO (https://github.com/DataSci/Burgoon/aop-ontology)	
• BAO (https://bioassayontology.org/)	
• HPO (https://bioportal.bioontology.org/ontologies/HP)	
• Chemical Entities of Biological Interest Ontology (ChEBI) (https://www.ebi.ac.uk/chebi/)	
• International Classification of Diseases (http://www.cdc.gov/nchs/icd.htm)	Currently in transition to the tenth revision
Ontologies based on the developmental effects of specific chemicals	
• U.S. 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/DTO, http://www.qsartoolbox.org/ontologies)	
Efforts are underway to construct a comprehensive toxicological ontology (Open Toxipedia Ontology Browser, 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 (among others)	
• RepDose (http://fraunhofer-repdose.de/repdose/)	

(Continues)

TABLE 1 (Continued)

• ACToR (http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=D37C5BDFE4B361E108FD2BD56FE48770),
• 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 nonanimal methods is being compiled into publicly accessible databases
• U.S. 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/)

high-throughput assays and in silico models for prediction of developmental toxicants is shown in Figure 1.

3 | AVAILABLE RESOURCES FOR BUILDING A DTO

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. Additionally there have been 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).

Following work by the WHO International Programme on Chemical Safety on MOA (WHO, 2007), the Organisation for Economic Co-operation and Development (OECD) started 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 was 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 nonanimal 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 currently underway to integrate nonanimal-derived 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) utilized data from high-throughput screening to develop predictive algorithms for a number of adverse effects on prenatal development. Others such as Robinson, Port, Yu, and Faustman (2010), Robinson and Piersma (2013), and van Dartel, Pennings, Robinson, Kleinjans, and Piersma (2011) investigated the use of toxicogenomics data for this purpose. Bal-Price et al. (2015) have reported on putative AOPs for developmental neurotoxicity. While there are few instances where AOPs, as defined by the OECD, have been elaborated using toxicogenomics, the

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/

utility of this technology in developing AOPs can be found in the work of Zhang et al. (2014), who examined molecular signaling networks as a mechanistic basis to describe threshold effects.

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

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. Approaches to building such an ontology are described below.

4 | APPROACHES TO BUILDING AN AOP/MOA DTO

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

Another approach is to take advantage of multiscale modeling approaches, especially AOPs that define the KEs from MIE to ultimate outcome, as a starting point for such an ontology. Unfortunately, as AOPs rely on mechanistic data that require considerable effort to construct and validate, there are currently still too few examples to begin constructing an ontology. It is, however, impractical to wait for a critical mass of relevant AOPs before embarking on a DTO, particularly given that the latter can inform and expedite AOP development. Hence, alternative strategies are required.

For most chemicals or small molecules, the chemical structure is known, and 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). 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 on this basis. It is supported by the first approach (mechanistic studies from the literature) to add strength to conclusions about MOA.

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

5 | TOOLS AND INFORMATION FRAMEWORKS AVAILABLE TO HELP DEVELOP AN MOA/AOP DTO

For all database operations, there is a need for a harmonized and internationally accepted nomenclature. In the case of developmental toxicity, there has been an effort to achieve this through a series of "Workshops on the Terminology in Developmental Toxicology." The purpose is to eliminate ambiguities and inconsistencies within the terminology and to establish working definitions for malformations (Chahoud et al., 1999; Makris et al., 2009; Solecki et al., 2001, 2003, 2013, 2015; Wise et al., 1997). Adaptations to facilitate use in computerized systems were made by dividing a teratological diagnosis into a *localization* term and an *observation* term, by:

- Eliminating topographical descriptions from the apical endpoints; and
- Adding a hierarchical structure for the anatomical localizations, based on observational *modes* (External, Skeletal, SoftTissue).

TABLE 3 Literature and data sources

Scientific literature sources	
Medline	
Pubmed	
Databases	
Gene Ontology (GO) project	www.geneontology.org/
EMAGE database	www.emouseatlas.org/emage/
MPO Browser	www.informatics.jax.org/
Zebrafish Model Organism Database	http://zfin.org/cgi-bin
OMIM database	www.ncbi.nlm.nih.gov/omim/
Potential sources of terminology and data to address developmental toxicology	
DevTox—a public website for internationally harmonized terms	www.DevTox.org
Licensed database	www.LeadScope.com
USEPA Toxicology Reference Database housing reference in vivo animal toxicology data for the ToxCast research program	ToxRef DB

The United States Environmental Protection Agency's (U.S. EPA) toxicity reference database (ToxRefDB) slightly enhanced the annotation system by joining 895 terms from the harmonized nomenclature (version 1) with standardized terms from the OECD-Office of Prevention, Pesticides, and Toxic Substances vocabulary to generate a thesaurus of 982 nonredundant terms (Knudsen et al., 2009). The website for this developmental toxicology (DevTox) nomenclature, together with other potential sources of terminology for developmental toxicology, is listed in Table 3.

While the DevTox vocabulary is valuable, it does have limitations for an AOP-based approach. One is that it is observational rather than embryological. For example, hypospadias is mapped to "trunk." While the perineum is part of the trunk, it would perhaps be more informative to annotate hypospadias as genitourinary (system), urethra (tissue), and penis (location). The latter triad maps informative relationship between embryology and defect.

A second limitation 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 localized to the iris, retina, or choroid. Since DevTox adaptations made for computability divide a diagnosis into localization and observation terms, while eliminating topographical descriptions, "retinal coloboma" does not appear in the lexicon despite being the most common coloboma. In addition, the DevTox terminology does not consider larger syndromes (e.g., the 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 [CHARGE] syndrome). The hierarchical relationship of these malformations in the DevTox vocabulary shows a need for a stronger developmental ontology linking observational descriptions of related defects to the embryology of the target system. In this way, the view of DevTox as an observation-based ontology system would be extended with new concepts and relations derived from an embryology-based ontology.

Classification systems for human birth defects such as the National Birth Defects Prevention Network, based on The Metropolitan Atlanta Congenital Defects Program (Correa-Villaseñor et al., 2003) and the Medical Dictionary for Regulatory Activities (MedDRA), are available. However, while these meet the needs of large, population-based birth defects surveillance programs and grouping human birth defects by anatomical location or clinical condition in smaller databases, respectively, they are basically anatomy-based observational systems that do not systematically address embryology. An example of what can be achieved is the work of Georgas et al. (2015) who 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 sex.

Other useful tools and information databases to help develop an ontology include gene networks and ToxCast information. Regarding gene networks as mutations in gene regulatory networks underlie many human congenital

anomalies (J. Bard, 2007), it follows that developmental toxicants may also produce adverse effects by altering these same developmental networks. Mouse is the commonly used mammalian model for understanding the connectivity between genes and human disease and as such there is a goal to construct genetic and physical maps for the mouse genome within the Human Genome Project. Online encyclopedias are available to support this knowledge exchange including the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/), providing integrated access to data on the genetics, genomics, and biology of the laboratory mouse. Users can 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 subterm. The MPO is a structured vocabulary aimed at standardizing annotations and describing unambiguous clinical phenotypes in mice using terms derived from ~100 physiological systems, behaviors, developmental phenotypes, and survival/aging conditions (C. L. Smith, Goldsmith, & Eppig, 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 Edinburgh Mouse Atlas Gene Expression (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. A possible way to filter linkages that are biologically meaningful is to specify threshold occurrences or use strings that reliably extract developmentally relevant grammar. For example, CoPub (Frijters, Verhoeven, Alkema, van Schaik, & Polman, 2007; Frijters et al., 2008) can be used to calculate keyword over-representations from text-mining of the literature, based on gene–gene co-occurrences. There is a good deal of ontology information already available for this purpose (Baldock, Bard, Kaufman, & Davidson, 1992; J. 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 catalog gene expression domains.

An example is an ontology for early eye development that can be perturbed by genetic mutations and environmental exposures. This can result in malformations such as anophthalmia, microphthalmia, and coloboma. Such defects occur in more than a million children worldwide. An OVID search of the Medline database reveals that there are specific references to ocular malformations in 2% of the teratology literature in general and about 25% of the mouse teratology literature in particular. This implies broad susceptibility of the eye to diverse agents. Modeling eye development provides a first step in laying out its normal pattern graphically and provides an understanding and importance of reciprocal tissue development over time. This can then be associated with gene-expression information associated with eye development

The U.S. EPA's ToxCast program (Kavlock et al., 2012) and cross-agency Tox21 program (Tice, Austin, Kavlock, & Bucher, 2013) are building large collections of in vitro data on diverse sets of chemicals to which humans are potentially exposed, including pesticides, food additives, cosmetics and personal care ingredients, pharmaceuticals, and industrial chemicals. Chemicals are being tested for bioactivity at various levels of biological organization 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 dashboard (http://actor.epa.gov/dashboard/).

The utility of ToxCast data in AOPs for developmental toxicity was demonstrated by Sipes et al. (2011). They built a predictive model in which the in vitro high-throughput screening data (ToxCastDB) were anchored to in vivo adverse outcomes from prenatal developmental toxicity studies (ToxRefDB). This early model utilized 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.

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 assays for diverse cellular behaviors 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 (Hutson et al., 2017), and digital defects (Ahir et al., 2014). Since RA signaling mediates correct 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 signaling pathways.

Analysis of ToxCast data allows a provisional catalog of MIEs to be built that mechanistically invoke AOPs associated with RA signaling and homeostasis pathways. An ontology for developmental toxicity is necessary to put this complexity into a computable and integrated form.

5.1 | Ontology development

It is possible to build either formal or informal ontologies.

Formal ontologies link facts as a triad of related terms that can be integrated with other data using common controlled vocabularies (B. Smith et al., 2007). This can be done using web-accessible resources such as Common Anatomy Reference Ontology, Cell Type, Zebrafish Anatomy and Development, and EMAP (Mouse Gross Anatomy and Development), which can be found at the Open Biomedical Ontology (OBO) and Web Ontology Language (OWL) hotlinks 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 (J. B. Bard, 2005). To bring together the vertical observational series (e.g., phenotype ontology) with a longitudinal/chronological 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 taxonomizes 982 terms (Level-5) into 51 embryological targets (Level-4), 24 embryological systems (Level-3), 141 tissue localizations (Level-2), and 3 observational modes (Level-1 modes), combining DevTox ontology (Levels-1, −2, and −5) with developmental ontology from EMAP (Level-3, embryological system; Level-4, embryological target). The OBO website in OWL format has also been used to write developmental ontologies for Theiler stages (see J. Bard, 2007) and Carnegie stages (see Hunter et al., 2003), describing mouse and human development, respectively. (OWL is a language of the semantic web to express natural language [used on the worldwide web] in machine-readable form. It uses a triad structure to define classes and interrelationships to annotate taxonomic hierarchy <classes><properties><individuals>.)

Having a sound DTO will codify the organization 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/MPO resource using OBO is being developed for the MPO browser as part of the MGI project at The Jackson Laboratory (http://www.informatics.jax.org/).

Informal ontologies that include less explicit information can make a useful contribution when the end-user is somewhat knowledgeable about the field (J. B. 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 (J. B. Bard, 2005). Interoperability can be built with ontology tools such as Protégé.

Literature text-mining is an important for developing informal ontologies. Whereas many database projects are underway to manually curate data from developmental endpoints, unstructured data present 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 more structured data.

6 | CHALLENGES TO BUILDING AND APPLYING AN AOP/MOA DTO

While as described above there are many tools and information frameworks that could help build a DTO, there are also a number of challenges including:

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.

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, at least not at the granularity that is needed to support a relatively complete ontology.

Translation of an AOP/MOA ontology into a testing strategy comprising assays covering the KEs (qualitatively and quantitatively) is necessary to enable 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, helping to provide the knowledge for a mechanistically informed IATA. 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. Agreement on the level of detail considered sufficient and comprehensive to identify developmental toxicants is beyond the scope of the current manuscript but will depend to some extent on the lines of evidence deemed to be helpful or indeed essential to reach a decision. Lines of evidence will include human data, animal test data, in vitro data, and absorption, distribution, metabolism, and elimination (ADME)/kinetic data. This would be facilitated by complete and open sharing of all toxicology data to ensure the comprehensiveness of the DTO.

A further aspect that needs consideration is the interaction between the dam 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, that is, 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, determining aspects such as bioavailability, metabolism and placental transfer. Moreover, some additional factors, such as the availability of essential nutrients from the dam necessary for development may also be influenced, leading to toxicity, which can only be identified in vivo or by using a complex integrated model.

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, ADME of the chemical in the mother and in the placenta determine and control the exposure of the conceptus—when it is exposed, how long it is exposed, and to how much 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. Consideration of maternal toxicity and possible effects on the conceptus should be integrated into the DTO and while this might add another layer of complexity to the process it is important to consider within an ontology framework.

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 of including these in a more complex model. Physiologically based pharmacokinetic (PBPK) modeling should be an essential part of the final risk assessment. However, by itself, PBPK modeling 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.

6.1 | Potential MIEs and KEs for building AOPs and IATA for developmental toxicity

Simply defining the level of biological organization at which the initiating event for toxicity occurs can be a challenge. As indicated below, toxicity might result from an exogenous chemical interacting with a specific biomolecule, such as a receptor or enzyme if there is sufficient occupancy of the receptor, or inhibition of the enzyme 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 2.

7 | CASE STUDIES FOR DEVELOPMENT TOXICITY ONTOLOGY

7.1 | Role of RA during embryogenesis

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 (RADH) family of enzymes. Acting primarily as a differentiation inducer, RA competes with

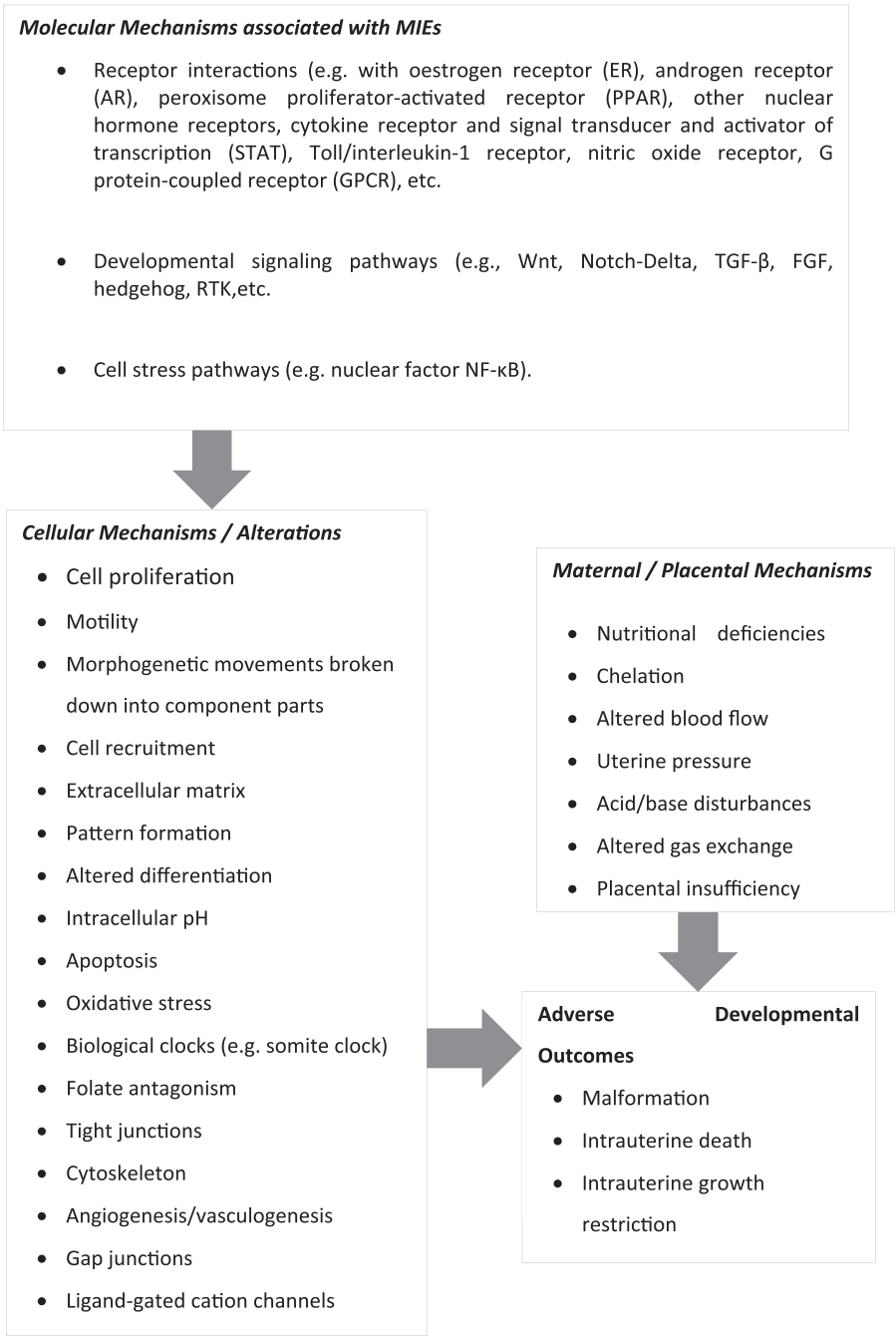

FIGURE 2 Examples of molecular, cellular, and maternal/placental mechanisms that may be involved in MIEs and KEs in AOPs for developmental toxicity

growth stimulating factors, such as those of the fibroblast growth factor (FGF) family, and with other developmental regulators, such as those belonging to the Wnt and Hox families, to exert its effects.

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 into RAR and retinoid X receptor (RXR) families. While RAR seem ubiquitous throughout the embryo, individual representatives of these receptor families have specific spatial distributions within embryonic tissues (Elmazar, Reichert, Shroot, & Nau, 1996; Mandal et al., 2013; Romand, Sapin, & Dollé, 1998; Rowe, Richman, & Brickell, 1992; Viallet & Dhouailly,

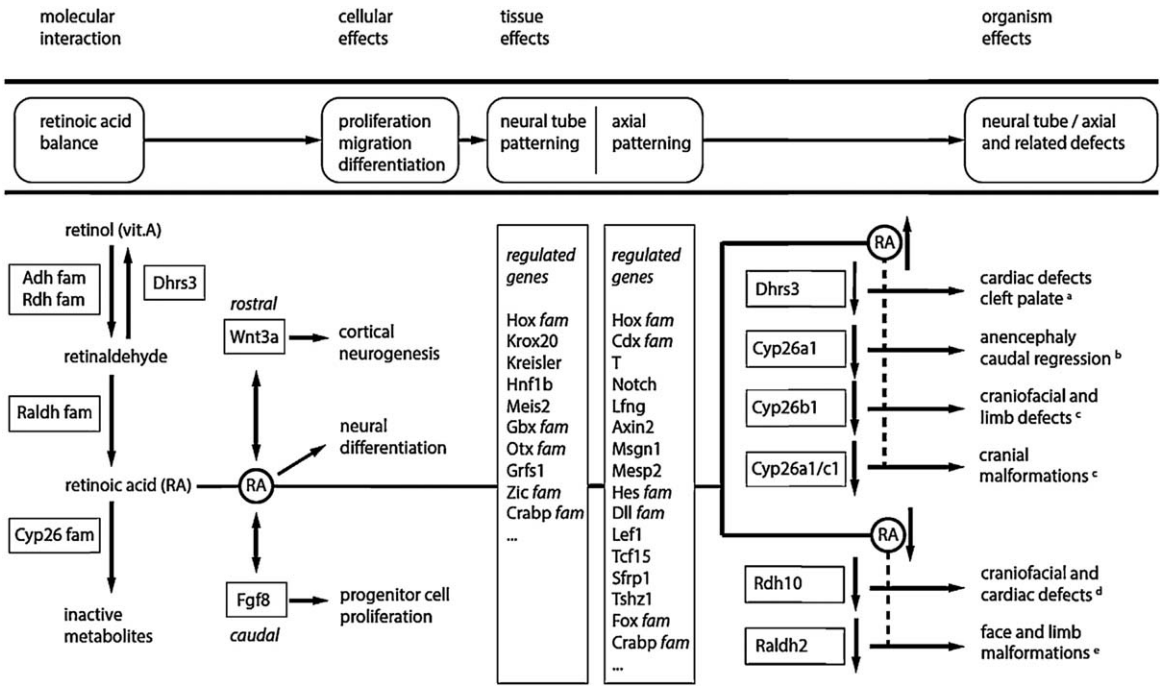

FIGURE 3 Proposed AOP framework for RA-neural tube/axial patterning pathway

Note. Reproduced with permission from Tonk et al. (2015). Reviewed in [a]Billings et al. (2013), [b]Abu-Abed et al. (2001), [c]Pennimpede et al. (2010), [d]Sandell, Lynn, Inman, McDowell, and Trainor (2012), and [e] Rhinn and Dollé (2012).

1994). This distribution pattern may explain differences in embryotoxic characteristics among various toxicants that all interfere with RA homeostasis.

In addition, RA is metabolized through members of the CYP26 family of P450 enzymes that 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 craniofacial morphogenesis, including palatogenesis. Overexpression of RA at specific fetal 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 the developing head, would result in increasing RA levels (Chambers et al., 2014). Thus, a strictly programed multifactorial interplay between RA-producing and RA-metabolizing enzymes, competing growth and development stimulating factors, and retinoid receptors and their time- and location-specific expression leads vertebrate embryogenesis from a fertilized egg to a morphologically recognizable 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 retinoid effects, 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 toward such a framework was published by Tonk, Pennings, and Piersma (2015) and is shown in Figure 3. This study also reviews data showing that RADH, 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.

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 humans. 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. In comparison synthetic retinoids used as pharmaceuticals against persistent acne caused severe facial, limb, and heart malformations (Lammer et al., 1985) while oral human exposure, during pregnancy, to RA via multivitamin preparations has resulted in children with similar abnormalities (Rothman et al., 1995; Werler, Lammer, Rosenberg, & Mitchell, 1990). RADH

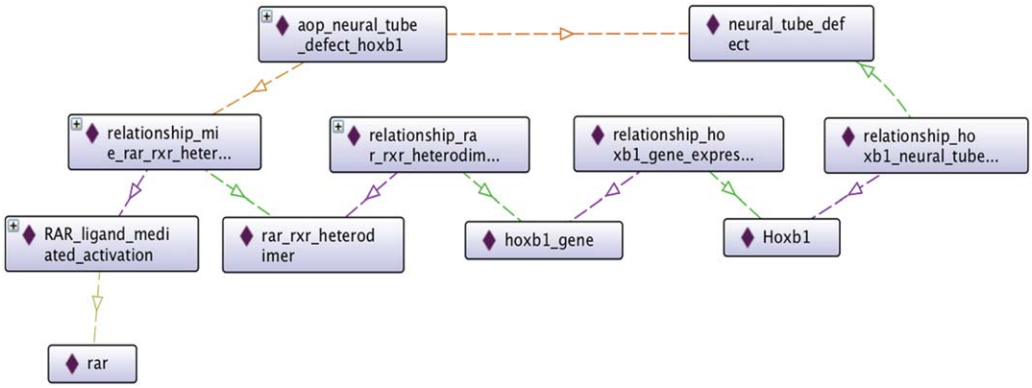

FIGURE 4 A representation of the RAR-mediated neural tube defect AOP

Note. The boxes represent individuals or instances of a class within the ontology. For example, neural_tube_defect is an individual of the adverse outcome 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"; and the golden line represents the "has_activated_key_event" relationship

deficient mice show uncontrolled growth of undifferentiated tissue in the facial area (Rhinn & Dollé, 2012), while CYP26-deficient mice showed caudal regression syndrome due to precocious cell differentiation limiting caudal growth (Rhinn & 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 & West, 2014).

Investigating all areas of chemical space for their interactions with the retinoid system during embryogenesis has the potential to define sensitive biomarkers for abnormal development in alternative test systems to animal testing. 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 pharmaceuticals and other chemicals 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 RADH (Hermsen, Pronk, van den Brandhof, van der Ven, & Piersma, 2012). The use of azole compounds as fungicides is based on inhibiting fungal sterol 14α-demethylase (CYP51) preventing the synthesis of the essential membrane component ergosterol. Azole compounds are not specific Inhibitors of CYP51 and other CYPs including CYP19 (the aromatase) and CYP26, that metabolizes RA can be affected. Consequently application of RA or ketoconazole to pregnant rats (Mineshima et al., 2012) or itraconazole to pregnant mice (Tiboni, Marotta, Del Corso, & Giampietro, 2006) induced cleft palates and other skeletal effects. Inhibition of aromatase by azole compounds leads to postimplantation loss due to inhibition of 17β-oestradiol synthesis.

7.2 | RA and neural tube defects

A single AOP for neural tube defects has been described by Tonk et al. (2015) starting with an MIE of chemical binding to and activating of the RAR followed by RAR and RXR heterodimerization, leading to upregulation of Hoxb1 gene expression, Hoxb1 protein translation, and finally neural tube defects. This AOP was modeled in the DTO 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 Human Phenotype Ontology (HPO) class "Abnormality of Neural Tube Closure." This allows us to more easily connect/link the AOP Ontology (AOPO) to other ontologies that use definitions based on the HPO.

Because of the interconnectedness of the AOPO with the Bioassay Ontology (BAO), in vitro assay data and toxicogenomic data can be overlaid on the AOP for RA-mediated neural tube defects. Thus, when assays detect, or transcriptomic experiments suggest, activation of RAR and Hoxb1 protein translation, it can be inferred that these chemicals may cause neural tube defects through this MOA. A representation of the RAR-mediated neural tube defect AOP is shown in Figure 4.

7.3 | RA and (hind)brain development

During neurodevelopment, the spinal cord contains the highest RA levels, while forebrain, midbrain, and hindbrain contain very little RA (Horton & Maden, 1995). As RA cannot be synthesized de novo by embryonic or adult organisms, developmental RA supply is produced in the target tissue from maternal dietary retinol uptake. Retinol dehydrogenases

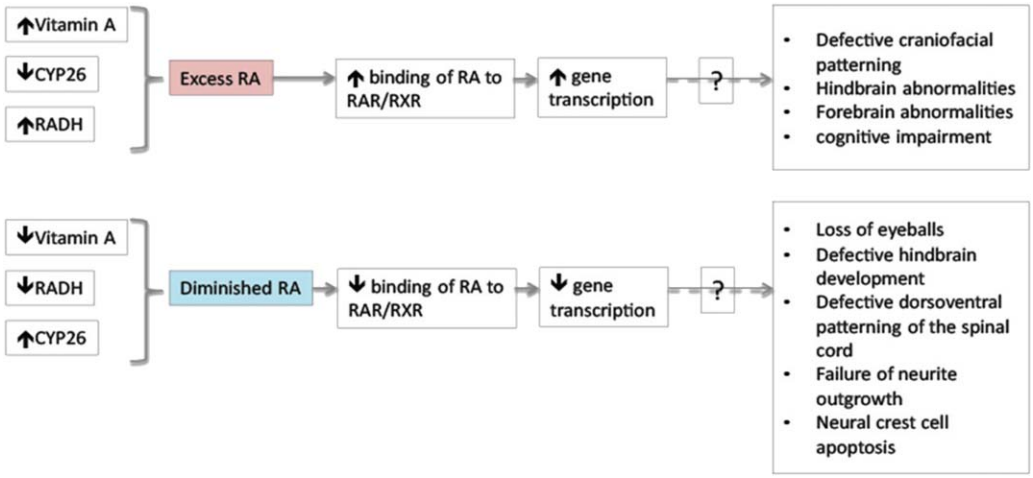

FIGURE 5 Retinoids and brain development

produce retinaldehyde from retinol, which is further metabolized by RADH to RA. Due to lack of RADH2 expression, the embryonic brain tissue does not produce RA from retinaldehyde. However, mesodermal somites flanking the neural tube do produce RA that diffuses into areas of neuroectodermal tissue, which will form segmental units for future hindbrain, midbrain, and forebrain development. In the cranial part of the neural tube, RA-metabolizing CYP26A1 converts RA to 4-hydroxy-RA and 4-oxo-RA that are glucuronidated and excreted. 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, Gale, Kostetskii, & Zile, 1996; McCaffery, Adams, Maden, & Rosa-Molinar, 2003; White, Highland, Kaiser, & Clagett-Dame, 2000). During hindbrain development, seven to eight rhombomeres form that correspond to later defined hindbrain areas. Individual rhombomeres contain specific expression patterns for transcription factors including *Wnt* family members (reviewed by Marshall, Morrison, Studer, Popperl, & Krumlauf, 1996; Rijli, Gavalas, & Chambon, 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 5.

A clinical hypothesis has been proposed that RA deficiency also causes underdevelopment of the hindbrain in humans (Emmett & 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, Subbarayan, Dollé, & Chambon, 1999). These abnormalities are likely due to the loss of RA-dependent regulation of hindbrain development and the otic morphogenic process (Maden et al., 1996; White et al., 2000). To test the hypothesis that this mechanism contributes to hearing loss in the human population, a vitamin A-supplemented population study is planned (Emmett & West, 2014).

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

- 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).

7.4 | Conclusions on case studies on RA

The above shows the 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 behavior of KEs in a compound concentration-related way, should, in due course, allow quantitative inferences about potency and risk.

8 | DISCUSSION

Chemical risk assessment is at a crossroads as it moves from classical animal studies identifying and recording adverse health effects, mainly in experimental rodents, toward 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 biology in general and toxicity in particular, the availability of an abundant array of animal-free test methods, and the expanding work on the description of MOA, 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 noted, such as the U.S. EPA Virtual Embryo project, the ToxCast database of alternative assays, and the RA Pathway of (dys)morphogenesis.

Combining all existing knowledge into a single developmental ontology will allow the derivation of novel AOPs. In addition, it will allow the selection of prioritized 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. A grant from CEFIC LRI has been provided to researchers to use the concepts described in this manuscript to build a prenatal DTO. Work began in 2016 with expected completion in 2018.

ORCID

Alan Poole http://orcid.org/0000-0002-8645-6257

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