Developmental Toxicity NAMs – 2023 Update & 2024 Literature Rescreening Establishing the State of the Science Using Systematic Review Tools

August 2024

Contents

1. Executive Summary

The dynamics and complexities of in utero fetal development create significant challenges in transitioning from lab animal-centric developmental toxicity testing methods to assessment strategies based on new approach methodologies (NAMs). Nevertheless, considerable progress is being made, stimulated by increased research investments and scientific advances, such as induced pluripotent stem cell-derived models. To help identify developmental toxicity NAMs for toxicity screening and potential funding through the American Chemistry Council's (ACC) Long-Range Research Initiative (LRI), a scoping literature review was conducted to better understand the current landscape of developmental toxicity NAMs.

Scoping review tools were used to systematically survey the literature, results and metadata were then extracted, and the user-friendly interactive DevTox NAMs Tableau Dashboard was created and made publicly available. The first scoping review, covering publications indexed up August 15, 2021, was published as an open access article in 2022, *Identifying the Landscape of Developmental Toxicity New Approach Methodologies*.[1] Thereafter, annual updates were conducted through June, 2023.

The data visualization dashboard, DevTox NAMs Tableau Dashboard, developed using Tableau® software, is provided as a free, open-access web tool at https://public.tableau.com/app/profile/acc.vizzes5590/viz/DevelopmentalToxicityNAMsSRResults/Dashboard. This dashboard enables straightforward interactive queries and visualizations to identify trends and to distinguish and understand areas or NAMs where research has been most, or least focused.

This report describes the scoping review purpose, the approach and methods used, and highlights the functionality of the dashboard that can be used to explore the many different facets of developmental toxicity NAMs. For this project, the ACC LRI committed to updating the literature review every year through June 2023 to ensure that the DevTox NAMs Tableau Dashboard is representative of current developmental toxicity NAMs research. This report summarizes the final updates to the literature scoping review and analysis and the resulting updates to the DevTox NAMs Tableau Dashboard that were conducted; these activities conclude this research project.

Below are several example questions or queries that users may wish to pose to become familiar with the utility of the DevTox NAMs Tableau Dashboard.

- What types of assays or topics are heavily researched? What areas need more attention?
- Is there a specific endpoint or organ system that requires more attention and review?
- Are the topics/areas of research reviewed in the most recent publications similar or unique?
- Does a particular chemical class require a closer look?
- What are the current trends?
- What biological space is currently being covered, and what gaps are there?

[1] Becker RA, Bianchi E, LaRocca J, Marty MS, Mehta V. Identifying the landscape of developmental toxicity new approach methodologies. Birth Defects Res. 2022 Oct 15;114(17):1123-1137. doi: 10.1002/bdr2.2075. Epub 2022 Aug 12. PMID: 36205106; PMCID: PMC9804744.

2. Background

ICF supports the ACC LRI to establish the current state of the science regarding the development and use of New Approach/Alternative Methods (NAMs) for characterizing developmental toxicity (DevTox). This project was initiated in October 2020 and has received annual updates. This bulletin provides a review of the methodology used for the 2023 update of the literature search and the rescreening endeavor of all previously captured literature identified between the 2020-2022 updates.

This project aims to provide the ACC LRI the ability to:

1) identify the potential research opportunities in an area with known research gaps in a systematic way *and*
2) learn and test systematic review tools to utilize in future RfP processes.

For the initial scoping report, conducted in 2020, ICF performed an extensive literature search and selected a targeted search strategy that identified references considered representative or potentially most relevant to this effort. ICF employed topic extraction and supervised clustering technology to identify the most potentially relevant literature within that reference set. ICF screened these studies for relevancy and extracted data from the relevant references to determine what information is represented in the papers that met the original requirements for DevTox NAMs scope. All extracted information was presented in a Tableau dashboard for use by the ACC LRI to explore the state of science in DevTox NAMs. This process was repeated in 2021 and 2022, and the results were published.

In June 2023, ICF utilized the same methodology as outlined above, to perform a third update of the scoping report literature search. Initial literature searches identified 36,100 references considered potentially relevant to the proposed scope. Prioritization methodology identified 1,205 potentially highly relevant references. These studies were screened for relevancy, data pertinent to the scope of the review was extracted, and all information was presented in the Tableau dashboard. [1]

The 2023 literature search strategy was updated to be more inclusive of and/or incorporate the following topics:

- Developmental Neurotoxicity + respective batteries
- Embryonic stem cells
- Thyroid (general) + respective batteries
- Zebrafish + respective batteries
- Induced pluripotent stem cells utilized within Developmental Toxicity assays

In 2024, ICF added all relevant literature identified during the 2023 Update into the scoping review seed study list. The seed study is utilized within the categorization modelling algorithm, see Supervised Clustering for details. All previously captured literature from the 2020-2022 scoping review updates was reprocessed through the algorithm. This process ensured that (i) literature previously excluded under a prior scope or (ii) literature previously categorized within a lower priority cluster was evaluated for relevancy. Studies in the highest priority cluster were reviewed under the 2023 Scoping Review Update inclusion criteria, as shown in Title and Abstract Screening.

3. Project Monitoring Team Meetings

The Developmental Tox for NAMs Project Monitoring Team (PMT) of the LRI, comprised of the members listed in Table 1, held routine check-in meetings over the span of the five years the scoping review and subsequent updates occurred. The PMT provided topic expertise throughout the process to ensure deliverables met the project goals. PMT members assisted with the following tasks:

- Provided "on-topic" publications to assist librarian in determining terms and how papers were indexed to ensure the search strategy results met project goal.
- Approved search terms/key words and final search strings based on search results.
- Approved key studies for use as seeds in reference prioritization.
- Provided expert advice/guidance on screening criteria of search results.
- Reviewed results.
- Provided guidance on process to rescreen previously captured literature under new scope and the incorporation of newly identified relevant literature into the Tableau dashboard.

Table 1. PMT Membership

Name	Title	Organization
Rick Becker, PhD, DABT	Senior Toxicologist & Senior Director, ACC LRI	American Chemistry Council
Sue Marty, PhD, DABT	Toxicology and Environmental Research and Consulting Science Director	The Dow Chemical Company
Vatsal Mehta, PhD	Senior Scientist - Toxicologist	The Procter & Gamble Company
Catherine Mahony	Principal Scientist - Central Product Safety	The Procter & Gamble Company
Paige Mundy, PhD	Investigative Toxicologist	Corteva Agriscience
Enrica Bianchi, PhD	Senior Mammalian Toxicologist	Corteva Agriscience

4. Scoping Report Process for the 2023 Literature Search Update

Table 2 and Figure 1 details the process used to identify and extract the relevant references during the 2023 scoping review update. The 2023 literature search update identified 36,100 studies. A total of 1,205 potentially relevant studies were identified following supervised clustering of the initial literature pool. Of those, 178 studies (~15%) presented developmental or reproductive toxicity research using a new approach methodology. One hundred studies (~8%) were categorized as supplemental and 927 studies (~77%) were found not relevant. Further details on study categorization can be found in Table 6.

Table 2. Scoping Report Process

Step Name	Description	Tool(s) Used	References Moving to Next Step
Literature Search	1. Determined keywords/search strings in coordination with the ICF Librarians and Project Monitoring Team (PMT). 2. Assessed multiple keyword combinations and gathered literature PubMed and the Keyword Analysis Tool (KAT). 3. Deduplicated the literature found using Deduper.	**PubMed** **Keyword Analysis Tool (KAT)** **Deduper**	**36,100**
Prioritization and Scoping	1. Prioritized studies by clustering by topic, using ICF's automated DoCTER software. 2. Grouped references into 10 clusters based on shared terms in the title and abstract. ICF selected 6 clusters that contained references most likely to be relevant based on the terms identified in their titles and abstracts. 3. Identified "seed" studies, including all previously relevant literature, to use in supervised clustering (ICF and PMT). 4. Supervised clustering was implemented to identify the references from the 6 clusters most likely to be relevant based on the seed studies. 5. At the request of ACC LRI, performed a targeted priority author search (G. Daston, T. Knudsen, A. H. Piersma) for additional references that were not captured previously (N=22).	**DoCTER**	**1,205**

Step Name	Description	Tool(s) Used	References Moving to Next Step
Title and Abstract Screening	1. Determined Inclusion/Exclusion Criteria and developed instructions. 2. Applied criteria to pilot study using Litstream®. The pilot study consisted of a representative sample of 10 studies from Supervised Cluster 6 ('most relevant'). Pilot studies were screened at the Title and Abstract level. Two screeners reviewed each pilot reference. 3. Performed QA/QC (by ICF senior technical specialist) to resolve differences. 4. Completed screening of the remaining references resulting in 1,205 total references screened. 5. Results of screening are shown in Figure 1 below.	**Litstream**	**1,205**
Data Extraction	1. Extracted data/information from studies that were identified as relevant during title and abstract screening (N=178) using Litstream. 2. Performed QA/QC (by ICF senior technical specialist) on data extracted from all studies.	**Litstream**	**178**
Data Analysis and Visualization	1. ICF developmental toxicology experts reviewed the extracted data and prepared it for visualization. 2. ICF Data Visualization experts produced an interactive tool using Tableau, to easily navigate extracted data to identify gaps and trends.	**Tableau**	**178**

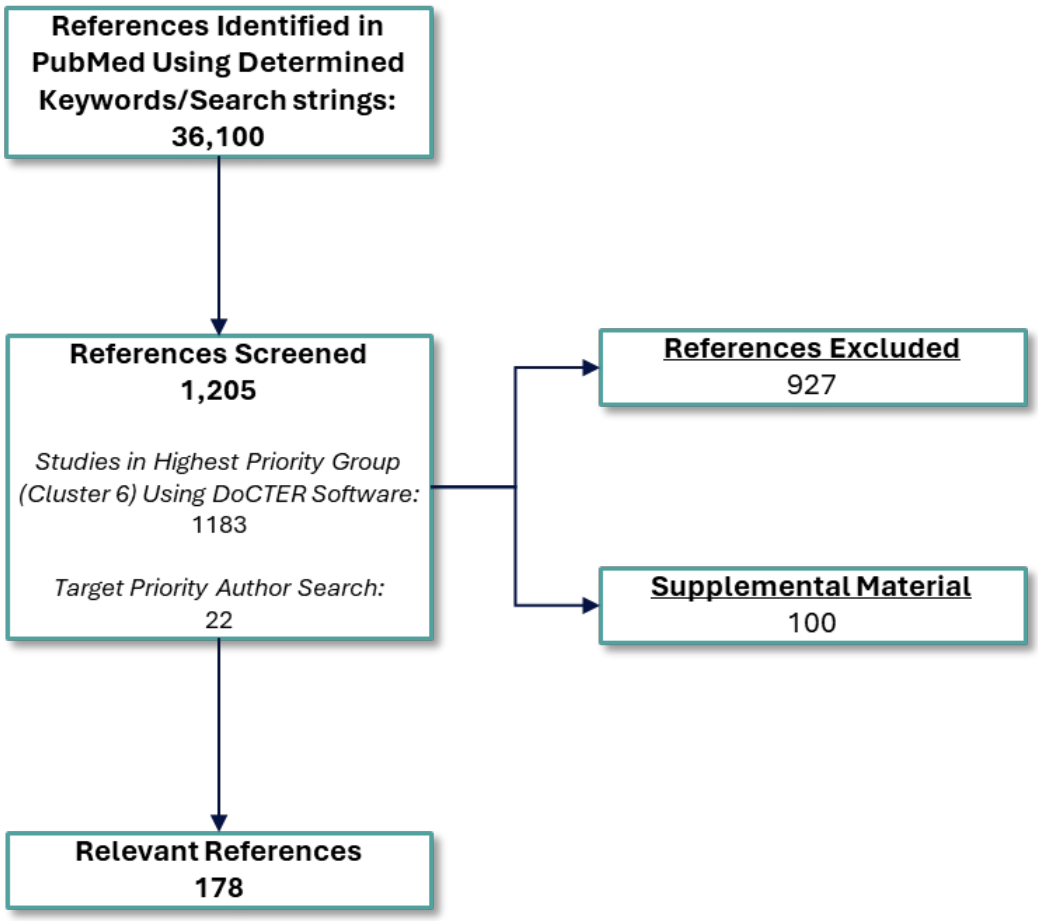

Figure 1. Summary of literature search results and disposition of references of 2023 Update.

Literature Search

Using various combinations of key words established in coordination with the ACC LRI and the PMT, ICF librarians conducted multiple literature searches to determine what combination of search terms resulted in the most useful set of references. Four "Categories" of search terms were created in coordination with the PMT, and combinations of these groups were assessed as shown in Table 3:

1. "Dev Tox": General health effects and endpoint terms associated with Developmental Toxicology.
2. "NAMs": Terms that are associated with already accepted NAMs.
3. "Models": Terms associated with species and other models used in Developmental Toxicology.
4. "Pathways": Specific pathways associated with developmental toxicology of known interest.

Following several trial literature searches, a search strategy encapsulating all four "Categories" (Dev Tox, NAMs, Pathways, and Models) was selected to be the most likely to be inclusive of the most relevant studies while maximizing the time and budget available. This strategy was utilized within the initial scoping review (2020) and in all subsequent updates (2021-2023).

As the state of science has evolved, modifications have been made to the search strategy to ensure the capture of publications on new approach methodologies and developmental toxicity research concepts. Details of the initial search, subsequent updates, and their modifications can be found in Table 3.

Table 3. Literature Search Results

Search Date	Strategy Description	Modifications to Search Strategy	PubMed	Total Unique
May 15, 2023	Dev Tox-new AND NAMs-new AND Pathways-new AND Models-new No date limit	• Added general battery terms to NAMs • Added embryonic stem cells and thyroid (general) to NAMs and Models set • No date limit for search	36,100	All studies reviewed in KAT, studies with KAT terms moved to Supervised Clustering
November 16, 2022*	Dev Tox-new AND NAMs-new AND Pathways-new AND Models	• Added cell viability terms to Pathways set • Added Embryonic Stem Cell terms to NAMs set • No date limit for search • Results deduplicated against previous results	21,782	2,230 (not captured in previous searches)
August 10, 2022*	Dev Tox AND NAMs AND Pathways AND Models		1,618	1,071
August 15, 2021	Dev Tox AND NAMs AND Pathways AND Models		1,555	861
November 12, 2020	Dev Tox AND NAMs AND Pathways AND Models		17,192	

*ICF performed two searches in 2022. The August 2022 search utilized an identical search strategy as the 2021 scoping review update. The November 2022 search contained updated term lists. Results of both August 2022 and November 2022 searches were combined, and duplicates were removed. The resulting literature pool was processed through supervised clustering. Prioritized clusters were screened, and relevant data were extracted.

Prioritization Methods

The goal of this project was to identify a representative sample of studies that could inform the state of the science on developmental toxicity NAMs. Throughout this process decisions were made (during the search strategy and the clustering process) regarding the types of references to be prioritized to achieve the project goals. These decisions were made to accurately identify a representative sample of studies while remaining efficient with time and budgetary resources.

Specific examples are detailed below, as well as in the Screening Criteria section.

1. PMT requested to exclude references that only measured a developmental endpoint through gene expression as these types of methods were considered to likely focus on basic discovery science related to ontogenesis and not likely to be NAM assays suitable for application to testing and assessment to replace intact animal developmental toxicity tests for regulatory use.[a]
2. PMT requested a targeted search of three priority authors (G. Daston, T. Knudsen, A. H. Piersma) for any references that may be relevant to this project. These authors have been considered experts in this area and their references were of particular interest for this project.[b]
3. ICF employed supervised clustering technology and seed studies (studies previously categorized as relevant) to identify the most potentially relevant literature with that reference set.[b]

[a] Occurred prior to Tableau Dashboard development.

[b] Occurred prior to title and abstract screening.

Supervised Clustering

All previously relevant literature (N=402), identified and confirmed through title and abstract screening, were utilized as 'seed studies'. Supervised Clustering used seed studies to identify the publications most likely to be relevant through a set of six algorithms. The results of Supervised Clustering results are represented in an "Ensemble Score" which is a score (1-6) of how many of the six algorithms in DoCTER included the reference as relevant. ICF and the PMT agreed to screen only those references that all six models included as relevant, resulting in 1,205 references.

5. Process for the Rescreening of All Previously Captured Literature

The 2024 rescreening effort utilized the same process as prior years. The process is outlined in Table 4 below. All studies captured within the literature searches conducted from 2020 to 2022 were included for potential rescreening.

Table 4. Scoping Report Process: Rescreening of Previously Captured Literature

Step Name	Description	Tool(s) Used	References Moving to Next Step
Literature Search	A literature search update was not performed for the rescreening task.	–	**NA**
Prioritization and Scoping	1. Prioritized studies by clustering by topic, using ICF's automated DoCTER software. 2. Grouped references into 10 clusters based on shared terms in the title and abstract. ICF selected 6 clusters that contained references most likely to be relevant based on the terms identified in their titles and abstracts. 3. Identified "seed" studies, including all previously relevant literature, to use in supervised clustering (ICF and PMT). 4. Supervised clustering was implemented to identify the references from the 6 clusters most likely to be relevant based on the seed studies.	**DoCTER**	**568**
Title and Abstract Screening	1. Applied criteria to pilot study using Litstream. The pilot study consisted of a representative sample of 10 studies from Supervised Cluster 6 ('most relevant'). Pilot studies were screened at the Title and Abstract level. Two screeners reviewed each pilot reference. 2. Performed QA/QC (by ICF senior technical specialist) to resolve differences. 3. Completed screening of the remaining references resulting in 568 total references screened.	**Litstream**	**568**
Data Extraction	1. Extracted data/information from studies that were identified as relevant during title and abstract screening (N=59) using Litstream.	**Litstream**	**59**

Step Name	Description	Tool(s) Used	References Moving to Next Step
	2. Performed QA/QC (by ICF senior technical specialist) on data extracted from all studies.		
Data Analysis and Visualization	1. ICF developmental toxicology expert reviewed the extracted data and prepared it for visualization. 2. ICF Data Visualization expert produced an interactive tool using Tableau, to easily navigate extracted data to identify gaps and trends.	**Tableau**	**59**

Literature Search

All previously captured literature, from the 2020-2022 literature searches, were used for the rescreening effort. Thus, an additional search was not a part of the rescreening task.

Prioritization Methods

The goal of rescreening previously captured literature from the 2020-2022 literature searches:

(i) to ensure studies previously excluded were re-evaluated with the revised inclusion and exclusion criteria used in the 2023 Update *and*

(ii) to utilize an updated seed study list to ensure relevant literature was evaluated into higher priority clusters for evaluation.

These efforts ensured the DevTox NAMs Scoping Review Tableau dashboard provided a review on the current state of science for developmental and reproductive toxicological NAMs research.

To determine which previously captured reference required rescreening, ICF employed supervised clustering technology and seed studies (relevant studies identified in the 2020-2023 updates). These tools aided in identifying the most potentially relevant literature within the reference set.

Supervised Clustering

All previously relevant literature (N=499), identified and confirmed through title and abstract screening, were utilized as 'seed studies'. Supervised Clustering used seed studies to identify the publications most likely to be relevant through a set of six algorithms. The results of Supervised Clustering are represented in an "Ensemble Score" which is a score (1-6) of how many of the six algorithms in DoCTER included the reference as relevant. The PMT agreed to screen only those references that all six models included as relevant, resulting in 568 references.

ICF did not re-evaluate references previously categorized as relevant, and focused on screening references that were previously marked as "not relevant", "supplemental", or had not been evaluated in a previous scoping review update due to the lack of priority (as determined by Supervised Clustering) of the reference. Table 5 provides further detail on the delineation of studies within Priority Cluster 6 re-evaluated by ICF in 2024.

Table 5. Categorization of Studies in Priority Cluster 6

Study Type	Number of Studies
New Priority Studies *Indicates studies previously categorized in 'low' priority clusters.*	343
Studies previously considered **'Not Relevant'** under past scoping criteria *Scopes utilized within 2020-2022 searches*	138
Studies previously considered **'Supplemental Material'** under past scoping criteria *Scopes utilized within 2020-2022 searches*	87
Total Studies to Review	**568**

6. Screening and Data Extraction

For the screening of potentially relevant literature, ICF utilized the same methodology for the 2023 Literature Update and the 2024 Rescreening efforts. Steps included:

- Two independent reviewers performed title and abstract screening using structured forms in Litstream, with a process for conflict resolution by a subject matter expert.
- Studies identified as relevant to scope were extracted by a primary independent extractor and quality assured by a subject matter expert.
- References identified as not relevant during the screening process did not move forward into data summarization and visualization.

Key Terminology

To ensure consistency and transparency, key technical concepts were utilized during all screening and extraction efforts.

- DART: Developmental and Reproductive Toxicology
- NAMs: New Approach Methodologies
- Clinically or morphologically relevant endpoint: A measurable or observable adverse outcome. A pathway not linked to a measurable outcome would not be considered relevant.
- Actionable: The method, system, model, etc., could be picked up and assessed right now, as is, with only needing to change test chemicals and models, etc.
- Dose Response: Any form of chemical exposure within a model, system, or assay.
- Systemic health effects: Any endpoint that relates across 3 or more systems or total body effects.

Title and Abstract Screening

Figure 2 provides an example of ICF's Litstream title and abstract screening form. Table 6 defines the screening criteria used to prioritize the references most relevant to this project.

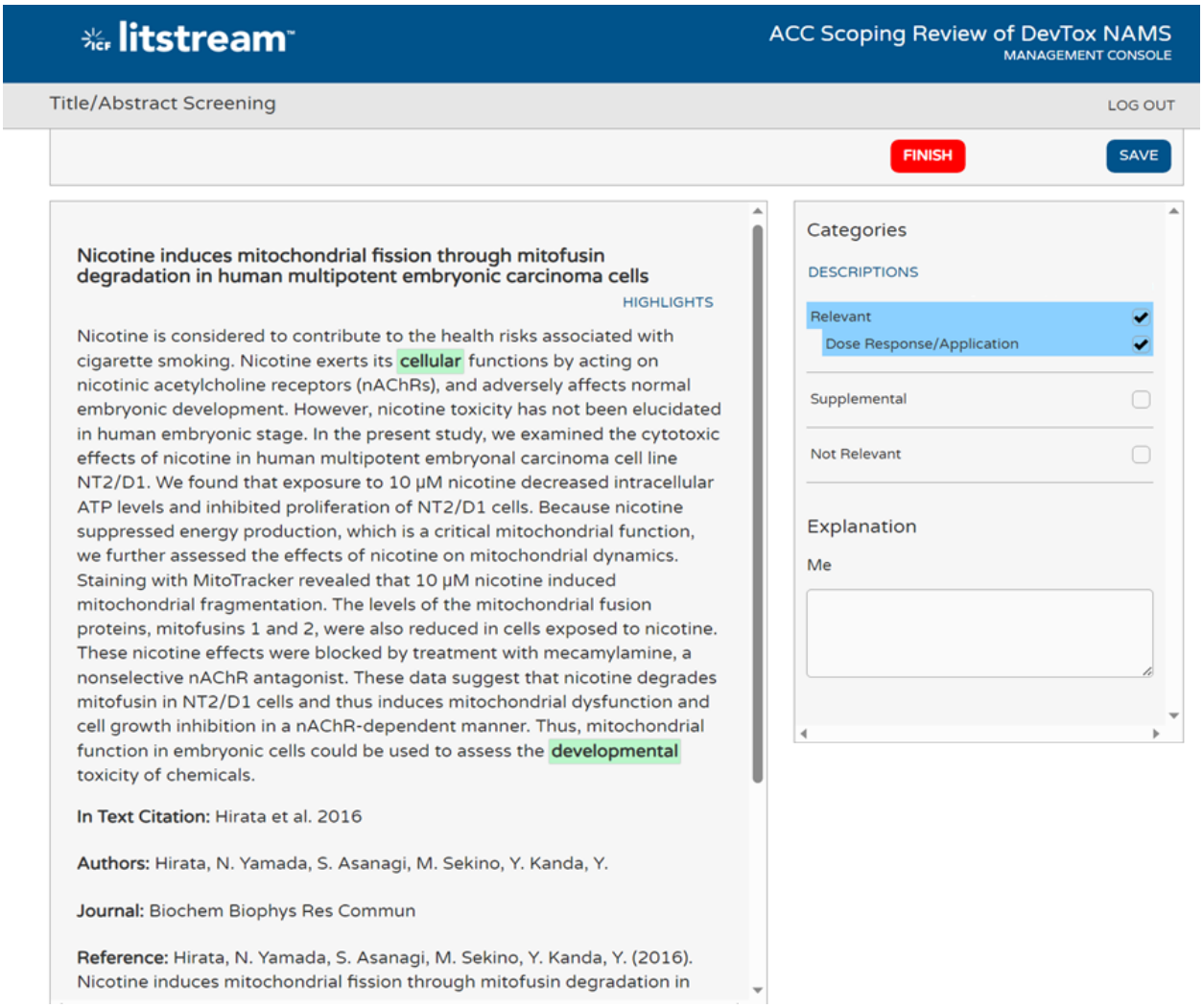

Figure 2. Title and Abstract Screening in Litstream. *The title/abstract screening form utilized to identify and categorize relevant or non-relevant research publications.*

Table 6. Title and Abstract Screening Criteria

Tag Name	Description
Relevant	A reference is "relevant" if it covers a NAM and a DART Endpoint. Other relevancy criteria include: ✓ Does not have to be creating a novel approach, can be an overview on the utilization or improvement of the new approach. ✓ The paper or abstract should be using a methodology to measure a clinically or morphologically relevant endpoint. ✓ Descriptions of pathways or gene sets is not enough. ✓ A report would not be relevant if it uses a developmental model, but the purpose of the paper is not an evaluation of a DART endpoint. ✓ Reviews can be considered relevant if the secondary data presents a strong comparison of a NAM to traditional models of development and reproductive toxicity or presents a strong analysis of how a NAM system, model, or assay has been used in DART applications.
Dose Response	<u>This is a subcategory of Relevant. These references are also considered Relevant</u>. A reference would be tagged to "Dose Response/Application" if it meets the relevancy criteria and provides evidence of the measurement of a dose response or chemical exposure. It is the demonstration of a NAM with specific chemicals or stressors.
Supplemental	Supplemental Material includes studies that are close to being relevant. The report covers a DART outcome and focuses on a NAM, but the NAM may not be fully actionable yet. Examples of supplemental studies are: ✓ A relevant pathway and outcome have been linked, but an assay to assess for this outcome has not been developed, ✓ The NAM presented is: • non-mammalian in vivo model (with the purpose of evaluating systemic effects) • an *in-silico* method • evaluating a pathway (expression, genomics) focused on only systemic effects
Reviews/Secondary Data Sources	For a review/secondary data source to be tagged as 'Relevant,' it must meet the following criteria: ✓ A meta-analysis, systematic literature review, or review article of previously published data that: o Only contains secondary data but presents a strong comparison of a NAM to traditional models of developmental toxicity. o Only contains secondary data but presents a strong analysis of how a NAM system, model, or assay has been used in DART applications.

Tag Name	Description
	For a review to be tagged as 'Supplemental,' it must: ✓ Only present conceptual, high-level/hypothetical (e.g., proposed assay, proposed methodology) ideas or methods that cannot be currently utilized. A review is tagged as 'Not Relevant' if it does not pertain to either NAMs or DART endpoints.
Not Relevant	A reference is considered not relevant if it does not meet any of the criteria of the other categories. × A reference that utilizes a traditional methodology (i.e., uses whole animals of rats, mice, monkeys, other mammals) and does not compare the results to the results from a NAM. × Simply describing the discovery of a new pathway is not considered relevant. × Ecological studies are NOT relevant. Anything noting ecological risk assessments, or ecological endpoints, will not be included. × Endocrine Disrupting assays pertaining to organs not directly related to thyroid outcomes are not of interest. Endocrine studies must be directly related to thyroid to be marked relevant.

Data Extraction

After the title and abstract screening step was completed, ICF used Litstream to extract information from the titles and abstracts of the relevant studies. Figure 3 shows the data extraction form used to capture extracted information. The collected data was imported into a Tableau Dashboard.

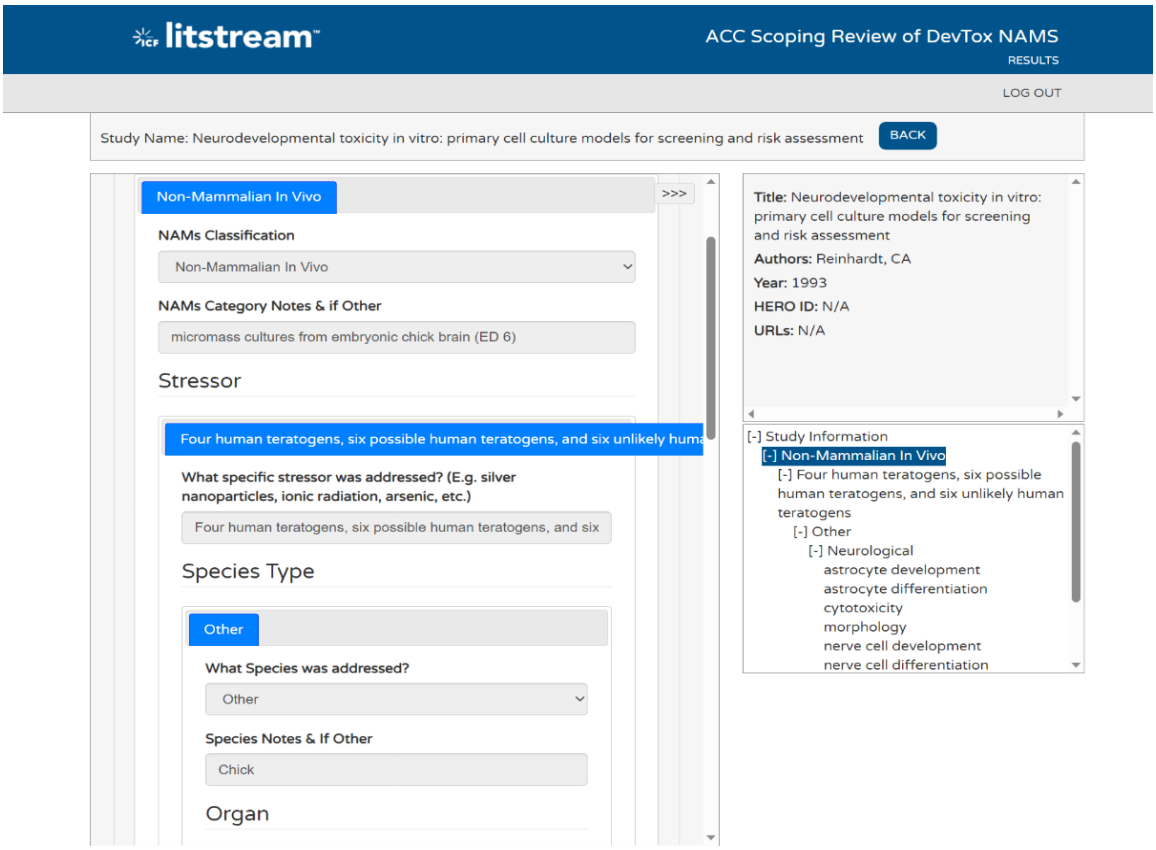

Figure 3. Screen capture of Litstream extraction "FlexForm" used in this scoping report. *The data extraction form is utilized to extract and categorize relevant data to import into the Tableau dashboard.*

7. Tableau® Dashboard

ICF created an interactive exploratory dashboard[2] to allow users to explore and analyze the DevTox NAMs data. The dashboard provides an excellent way to present final systematic-review findings to a larger audience. Figure 4 displays the DevTox NAMs Tableau Dashboard homepage.

Once data are categorized, the user can visualize the counts of references to examine various data categories. Reference counts can be viewed in colored 'heatmaps' using blue shading for smaller counts and red shading for larger counts allowing for instant identification for where there might be 'hotspots' of data – categories with many references examining the variables; or 'gaps' – categories where there might be little data or few references available. The dashboard can be customized with interactive filters for additional variables of interest to allow further exploration of the available data resulting from systematic categorization.

[2] https://public.tableau.com/app/profile/acc.vizzes5590/viz/DevelopmentalToxicityNAMsSRResults/Dashboard

Components of the DevTox NAMs Dashboard

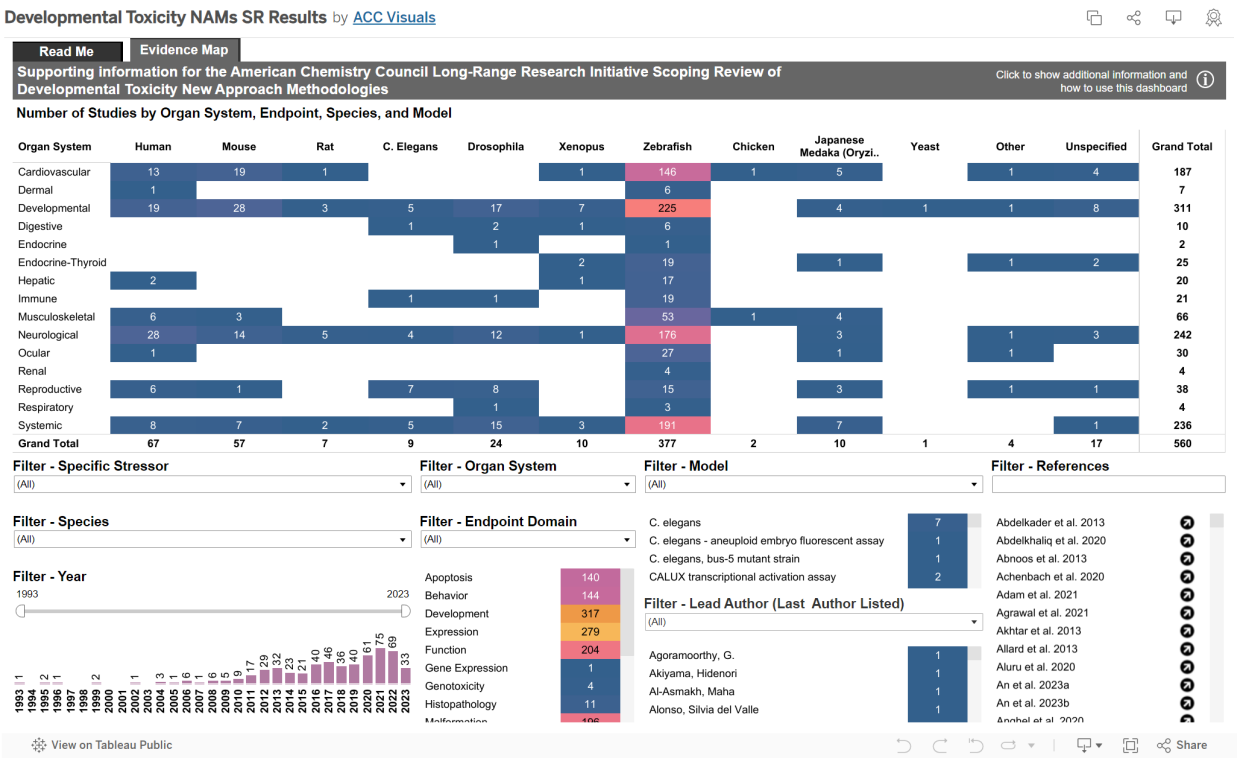

Figure 4. Tableau Dashboard. *The homepage of the DevTox NAMs Tableau Dashboard. References are categorized by model and organ system. Additional filters are available to customize the viewable publications.*

Heatmap: The main heatmap at the top of the page shows numbers of references by Organ System (y-axis) and Species (x-axis). Numbers represent distinct counts of references, and shading indicates least-to-most references in a blue-to-red shaded pattern. The heatmap tooltip shows details about examined and measured endpoints by hovering over each colored cell in the heatmap. Clicking on a cell will filter other visuals on the dashboard to only those relevant references.

References: The references shown in this dashboard are listed in the bottom right of the dashboard by short citation. The user can hover over the arrows to review additional reference information and clickable PubMed URLs. Furthermore, a user may select a reference to filter the dashboard visuals to the reference of interest.

Filters: A variety of filters are available at the bottom left to filter the dashboard by various variables. The drop-down checkbox filters can be used to include or exclude various values, and any bar of the 'year' bar chart or row of the 'Endpoint Domain,' 'Model,' and 'Lead Author' filters can be clicked to filter as well. Once the dashboard has been filtered to the desired references, the information can be downloaded using the download icon in the bottom right of the dashboard and selecting "Data".

Customization: The DevTox NAMs Tableau Dashboard is a customizable, user-friendly platform where viewers can create unique visualizations based on what information is needed. Variables that are available for review (or exclusion) include, but are not limited to, organ system, model type, year of publication, species, and endpoint assessed.

Appendix A. ICF Project Staff

Name	Title
Samantha Goodman, MS*	Senior Toxicologist
Denyse Marquez Sanchez	Health Scientist
Lisa Prince, PhD	Lead Toxicologist
Wren Tracy, MHS	Data Visualization Scientist, Lead
Nicole Vetter, MLS	Information Sciences, Senior Manager
Catherine Smith, MEM	Health Scientist, Lead
Jessica Wignall, MSPH	Health Sciences, Senior Director

*Indicates primary contact for this project.

Appendix B. Literature Search Strategy

Each literature search was completed within PubMed; no other databases were considered. The final literature search was conducted on May 5, 2023. The individual sets of terms can be found below. Please note, the literature search strategy was modified each year to include new area topics of interest. The below search strategy was utilized in the 2023 scoping review update without data restrictions.

Set	Search Strategy for PubMed
Dev Tox-new	((teratogenesis[mh] OR teratogenesis[tiab] OR teratogens[tiab] OR teratogenic[tiab] OR teratology[mh] OR teratology[tiab] OR teratologies[tiab] OR gravidity[mh] OR pregnant[tiab] OR pregnancy[mh] OR pregnancy[tiab] OR gestation[tiab] OR parturition[mh] OR parturition[tiab] OR birth defects[tiab] OR "congenital abnormalities"[mh] OR "congenital abnormalities"[tiab] OR morphology[tiab] OR morphogenesis[mh] OR morphogenesis[tiab] OR dysmorphology[tiab] OR dysmorphologies[tiab] OR dysmorphogenesis[tiab] OR malformation[tiab] OR malformed[tiab] OR "cell differentiation"[mh] OR "cell differentiation"[tiab] OR “neuronal differentiation”[tiab] OR proliferation[tiab] OR fetus[mh] OR fetus[tiab] OR fetal[tiab] OR "embryonic structures"[mh] OR embryo[tiab] OR embryonic[tiab] OR embryotoxicity[tiab] OR conceptus[tiab] OR organogenesis[mh] OR organogenesis[tiab] OR implantation[tiab] OR "embryo implantation"[mh] OR "programmed cell death"[tiab] OR abortion[tiab] OR placenta[mh] OR placenta[tiab] OR "yolk sac"[mh] OR "yolk sac"[tiab] OR ectoderm[mh] OR ectoderm[tiab] OR mesoderm[mh] OR mesoderm[tiab] OR endoderm[mh] OR endoderm[tiab] OR "neural crest"[mh] OR "neural crest"[tiab] OR "neural plate"[mh] OR "neural plate"[tiab] OR notochord[mh] OR notochord[tiab] OR somites[mh] OR somites[tiab] OR "neural tube"[mh] OR "neural tube"[tiab] OR "limb buds"[mh] OR "limb buds"[tiab] OR "limb bud"[tiab] OR larval[tiab] OR nestin[mh] OR nestin[tiab]) AND develop*[tiab]) OR ((developmental[tiab] OR development[tiab] OR endpoints[tiab] OR endpoint[tiab] OR endpoint determination/trends[mh] OR neurodevelopmental[tiab] OR neurodevelopment[tiab] OR effects[tiab]) AND (toxicity[tiab] OR toxicities[tiab] OR toxicity[sh] OR toxicant[tiab] OR toxicants[tiab] OR neurotoxicity[tiab] OR neurotoxic[tiab] OR neurotoxicities[tiab]))
NAMS – new (with Embryonic stem cells)	((“Embryonic stem cells”[tiab] OR “Embryonic stem cell”[tiab] "Embryonic Stem Cells"[mh]) AND (human[tiab] or mouse[tiab] or rat[tiab])) OR assay[tiab] OR assays[tiab] OR "biological assay"[mh] OR pathway[tiab] OR alternative[tiab] OR "Models, Biological"[mh] OR model[tiab] OR approach[tiab] OR assay[tiab] OR "biological assay"[mh] OR profile[tiab] OR predictive[tiab] OR "high-throughput"[tiab] OR "high throughput"[tiab] OR "high content"[tiab] OR "induced pluripotent stem cells"[mh] OR "induced pluripotent"[tiab] OR "pluripotent stem cells"[tiab] OR "pluripotent stem cells"[mh] OR toxicokinetics[tiab] OR transcriptomics[tiab] OR transcriptome[mh] OR HTS[tiab] OR HTTr[tiab] OR "High-Throughput Screening Assays"[mh] OR "new approach methodologies"[tiab] OR "new approach methods"[tiab] OR NAMs[tiab] OR "Computational Biology/trends"[mh] OR "Animal Testing Alternatives"[mh] OR "Animal Use Alternatives/methods"[mh] OR "Animal Use Alternatives/trends"[mh] OR "alternative approach"[tiab] OR “novel approach”[tiab] OR genomic[tiab] OR genomics[tiab] OR "non-mammalian"[tiab] OR "non-animal"[tiab] OR "computational toxicology"[tiab] OR "Adverse outcome

Set	Search Strategy for PubMed
	pathways"[mh] OR "Adverse outcome pathway"[tiab] OR "AOP networks"[tiab] OR biomarkers[mh] OR biomarkers[tiab] OR "in vitro"[tiab] OR "in vitro techniques"[mh] OR "gene expression"[tiab] OR toxicogenomics[tiab] OR in silico[tiab] OR "computer simulation"[mh] OR ((batteries[tiab] OR battery[tiab] OR suite[tiab] OR suites[tiab] OR panel[tiab] OR panels[tiab] OR integrated[tiab]) AND (assays[tiab] OR assay[tiab])) OR "test batteries"[tiab] OR "assessment batteries"[tiab] OR "test battery"[tiab] OR "assessment battery"[tiab] OR "toxicity tests"[mh] OR "toxicity tests"[tiab] OR "integrated testing strategy"[tiab] OR OCT4[tiab]
Pathways - new	("wnt signaling pathway"[mh] OR "wnt signaling"[tiab] OR "beta catenin"[mh] OR "beta catenin"[tiab] OR JNK[tiab] OR "transforming growth factor beta"[mh] OR "serine receptor"[supplementary concept] OR "protein-serine-threonine kinases"[mh] OR "protein-serine-threonine kinases"[tiab] OR "receptors, transforming growth factor beta"[mh] OR "tgf beta"[tiab] OR "serine receptor"[tiab] OR "threonine kinase"[tiab] OR "forkhead box"[tiab] OR "Forkhead Transcription Factors"[mh] OR "transcription factors"[tiab] OR FOXO[tiab] OR hedgehog[tiab] OR hedgehogs[mh] OR hedgehogs[tiab] OR "patched receptors"[mh] OR "patched receptors"[tiab] OR "patched receptor"[tiab] OR "receptor protein-tyrosine kinases"[mh] OR "protein-tyrosine"[tiab] OR "receptor tyrosine kinase"[tiab] OR "monomeric gtp-binding proteins"[mh] OR "gtp-binding"[tiab] OR "small g protein"[tiab] OR RAS[tiab] OR Notch[tiab] OR "Notch-delta"[tiab] OR "delta protein"[supplementary concept] OR "Janus Kinases"[mh] OR "JAK/STAT"[tiab] OR cytoplasm[mh] OR "cytoplasmic tyrosine kinase"[tiab] OR "protein-tyrosine kinases"[mh] OR "tyrosine kinase"[tiab] OR "nf-kappa b"[mh] OR "nf-kappa b"[tiab] OR interleukin-1[mh] OR interleukin-1[tiab] OR "toll-like receptors"[tiab] OR "toll-like receptors"[mh] OR "toll-like receptor"[tiab] OR "receptors, cytoplasmic and nuclear"[mh] OR "nuclear hormone receptor"[tiab] OR apoptosis[mh] OR apoptosis[tiab] OR "cell death"[mh] OR "cell death"[tiab] OR "protein tyrosine phosphatases"[mh] OR "protein tyrosine phosphatases"[tiab] OR "phosphotyrosine phosphatase"[tiab] OR RPTPs[tiab] OR "guanylate cyclase"[tiab] OR "guanylate cyclase"[mh] OR "nitric oxide"[tiab] OR "soluble guanylyl cyclase"[mh] OR "guanylyl cyclase"[tiab] OR "G protein-coupled"[tiab] OR GPCR[tiab] OR "receptors, g protein coupled"[mh] OR "gtp binding"[tiab] OR "g protein"[tiab] OR integrins[mh] OR integrins[tiab] OR integrin[tiab] OR cadherins[tiab] OR cadherins[mh] OR "gap junctions"[tiab] OR "gap junction"[tiab] OR "gap junctions"[mh] OR "ligand-gated"[tiab] OR cations[mh] OR "cation channels"[tiab] OR "unfolded protein response"[tiab] OR UPR[tiab] OR "unfolded protein response"[mh] OR "replication stress"[tiab] OR ("DNA damage"[tiab] AND (checkpoint[tiab] OR checkpoints[tiab] OR stress[tiab])) OR "cell cycle checkpoints"[mh] OR "cell cycle checkpoints"[tiab] OR "dna damage"[mh] OR "dna replication"[mh] OR stemina[tiab] OR signaling[tiab] OR receptor[tiab] OR “cell viability”[tiab] OR "Cell Survival"[mh] OR cytotoxicity[tiab] OR “toxicity assay”[tiab]) OR "thyroid gland"[mh] OR thyroid[tiab] OR "PAX6"[tiab] OR "FOXG1"[tiab] OR "CCND1"[tiab] OR "TBR2"[tiab] OR "NEUROD4"[tiab] OR "NEUROG1"[tiab] OR Receptors, Thyroid Hormone[mh] OR thyroid hormone[mh] OR "thyroid hormone"[tiab] OR "thyroid gland"[mh] OR thyroid[tiab] OR bone morphogenetic protein receptors[mh] OR "bone morphogenetic protein"[tiab] OR "BMP"[tiab] OR "FGF"[tiab] OR "fibroblast growth factor"[tiab] OR receptors, fibroblast growth factor[mh] OR EGF[tiab] OR "epidermal

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	growth factor"[tiab] OR epidermal growth factor[mh] OR "general growth factor"[tiab] OR "growth factor"[tiab] OR "TPO inhibition"[tiab] OR endocrine[tiab] OR "nuclear receptors"[tiab] OR transcription factors[tiab] OR hippo[tiab] OR receptors, Cytoplasmic and Nuclear[mh]
Model - new	zebrafish[tiab] OR zebrafish[mh] OR medaka[tiab] OR medakas[tiab] OR xenopus[mh] OR xenopus[tiab] OR "caenorhabditis elegans"[mh] OR "caenorhabditis elegans"[tiab] OR "C. elegans"[tiab] OR Drosophila[tiab] OR Drosophila[mh] OR iPS[tiab] OR "pluripotent stem cells"[tiab] OR "pluripotent stem cells"[mh] OR micromass[tiab] OR "human embryonic stem cells"[mh] OR "human embryonic stem cell"[tiab] OR "human embryonic stem cells"[tiab] OR hESC[tiab] OR “embryonic stem cells”[tiab] OR “embryonic stem cell”[tiab] OR "thyroid gland"[mh] OR thyroid[tiab] OR "induced pluripotent stem cells"[mh] OR "induced pluripotent"[tiab] OR Receptors, Thyroid Hormone[mh] OR thyroid hormone[mh] OR "thyroid hormone"[tiab]