Mapping physiology: a systems biology approach for the development of alternative methods in toxicology

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Summary

Chemical safety assessment still heavily relies on animal testing, presenting ethical dilemmas and limited human predictive value. New approach methodologies (NAMs), including *in vitro* and *in silico* techniques, offer alternative solutions. *In silico* toxicology has made progress in predicting chemical effects but frequently lacks biological mechanistic foundations. Recent developments focus on mechanistic understanding of adverse effects inflicted by chemicals, as embedded in (quantitative) adverse outcome pathways (AOPs). However, there is a demand for more detailed mechanistic insights at the gene and cell levels, encompassing both pathology and physiology. Drawing inspiration from the Disease Maps Project, this paper introduces Physiological Maps (PMs) as comprehensive graphical representations of biochemical processes related to specific organ functions. PMs are standardized using Systems Biology Graphical Notation and controlled vocabularies and annotations. Curation guidelines have been developed to ensure reproducibility and usability. This paper presents the methodology used to build PMs, emphasizing the essential collaboration between domain experts and curators. PMs offer user-friendly, standardized visualization for data analysis and educational purposes. Enabling a better understanding of (patho)physiology, they also complement and support the development of AOPs by providing detailed mechanistic information at the gene and cell level. Furthermore, PMs contribute to developing *in vitro* test batteries and to building (dynamic) *in silico* models aiming to predict the toxicity of chemicals. Collaborative efforts between the toxicology and systems biology communities are crucial for creating standardized and comprehensive PMs, supporting and accelerating the development of human-relevant NAMs for next-generation risk assessment.

Keywords

physiological map, standardized graphical representation, curated biochemical networks, conceptual *in silico* models, new approach methodology.

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1. Introduction

Currently, the **safety assessment of chemicals** heavily relies on animal testing, which raises serious ethical concerns and is poorly predictive of human safety, as the effects observed in animals may not necessarily reflect the responses seen in humans (Vinken et al., 2021). Consequently, there is a pressing need for alternative methods to reduce animal testing and improve chemical risk assessment. **New approach methodologies (NAMs)** include *in vitro* (experimental) approaches, *in silico* (computational) approaches and combinations thereof. In terms of ***in silico* toxicology**, a great deal of effort has gone into developing methods for predicting the activity of a chemical in the body based on its structural properties (e.g., read-across, QSAR models) (Luechtefeld and Hartung, 2017; Luechtefeld et al., 2018; Punt et al., 2020; Schmeisser et al., 2023). However, these predictive models are not based on a solid mechanistic foundation using knowledge of biological processes (Wittwehr et al., 2017).

Nowadays, the focus of (*in silico*) toxicology has shifted from simply determining a compound's hazard to providing a mechanistic explanation, particularly with the development of **adverse outcome pathways (AOPs)** (Hemmerich and Ecker, 2020; Schmeisser et al., 2023). An AOP is an analytical construct capturing existing knowledge and describing a sequential chain of causally linked events at different levels of biological organization (e.g., cell, tissue, organ) that lead to an adverse health effect in an organism following exposure to a stressor [1] (Ankley et al., 2010). AOPs, therefore, bring some mechanistic explanation to allow for interpretation of hazards and to support chemical risk assessment. Recent years have seen the development of quantitative AOPs (qAOPs), from simple dose–response modeling to more complicated Bayesian network modeling and differential equation based systems biology approaches (Ito et al., 2024). Although AOPs and qAOPs are able to provide more mechanistic information, chemical risk assessment would benefit from: (1) a more detailed representation of biological mechanisms at the gene and cell levels, (2) a description of these mechanisms not only in the context of the pathology/adverse outcome but for the underlying physiology in general.

In recent years, the Disease Maps Project Community has brought the concept of **disease maps** (DM) to the forefront, building a bridge between the domains of biological and computational research (Ostaszewski et al., 2019). By fostering the development of DMs, this open community effort aims to graphically and comprehensively represent biological mechanisms, such as interconnected signaling, metabolic and gene regulatory pathways, in

[1] OECD website: https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/adverse-outcome-pathways.html , accessed August 19, 2024

standardized and machine-readable formats for various human disorders. While several projects are currently mapping a wide range of diseases, a similar effort has not been made to map the undisturbed underlying physiology. Drawing inspiration from the DM project, we believe that **physiological maps** (PMs), *i.e.* mapping the physiology of specific biological processes**,** can be a cornerstone for the development and refinement of alternative methods in toxicology, including *in vitro* test batteries, AOPs and *in silico* predictive models.

2. Physiological maps: concept and methodology

By integrating biological knowledge from existing databases and scientific literature, the PMs developed as part of the European Commission funded Horizon 2020 ONTOX project[2] can be described as graphical representations of cellular and molecular processes associated with specific organ functions (Vinken et al., 2021). They encapsulate comprehensive physiological knowledge in a standardized graphical notation that is designed for human comprehension and is also machine readable. In ONTOX, organ-specific PMs are currently being developed: bile secretion and lipid metabolism (liver), nephron physiology (kidney), neural tube closure (updated version of Heusinkveld et al., 2021), and cognitive function development (brain).

Main workflow

The workflow used to build and exploit PMs in ONTOX (Figure 1) is strongly inspired by that of the DM Project and comprises 4 main phases: (1) planning, (2) curation, (3) updating, and (4) application (Mazein, Acencio, et al., 2023). During the **planning phase**, domain experts and curators define the purpose and scope of the PM, identify the biological mechanisms to be mapped, determine granularity (e.g., the architecture of the map, including the presence of submaps, and the level of details for representing the molecular interactions) and list the key components in terms of cell types, molecules and pathways. The **curation phase** involves retrieving available knowledge and data from literature and databases, which are then integrated into a PM using CellDesigner, a structured diagram editor (Funahashi et al., 2008). For the **updating phase**, the PM is uploaded to the Molecular Interaction Networks Visualization (MINERVA) platform, a navigable tool (Hoksza et al., 2020), enabling better visualization and facilitating feedback from domain experts. In the **application phase**, PMs can be exploited in many possible ways, such as (omics) data visualization, benchmarking and filling gaps in AOPs, identifying new *in vitro* assays, exploring drug targets and developing *in silico* models.

Human resources: domain experts, curators, data scientists

Every phase of this workflow requires strong collaboration between curators and domain experts who are identified in the planning phase. While curators drive PM development (design, documentation, curation, visualization, updates and user support), domain experts also play key roles throughout the process by: (i) helping to define the goals and scope of the PM, particularly in terms of the applications required; (ii) identifying the relevant pathways and hallmarks to be mapped; (iii) screening different data resources and providing relevant knowledge to be integrated; (iv) providing continuous feedback for updating the maps; and (v) guiding the application phase.

[2] ONTOX website : https://ontox-project.eu/

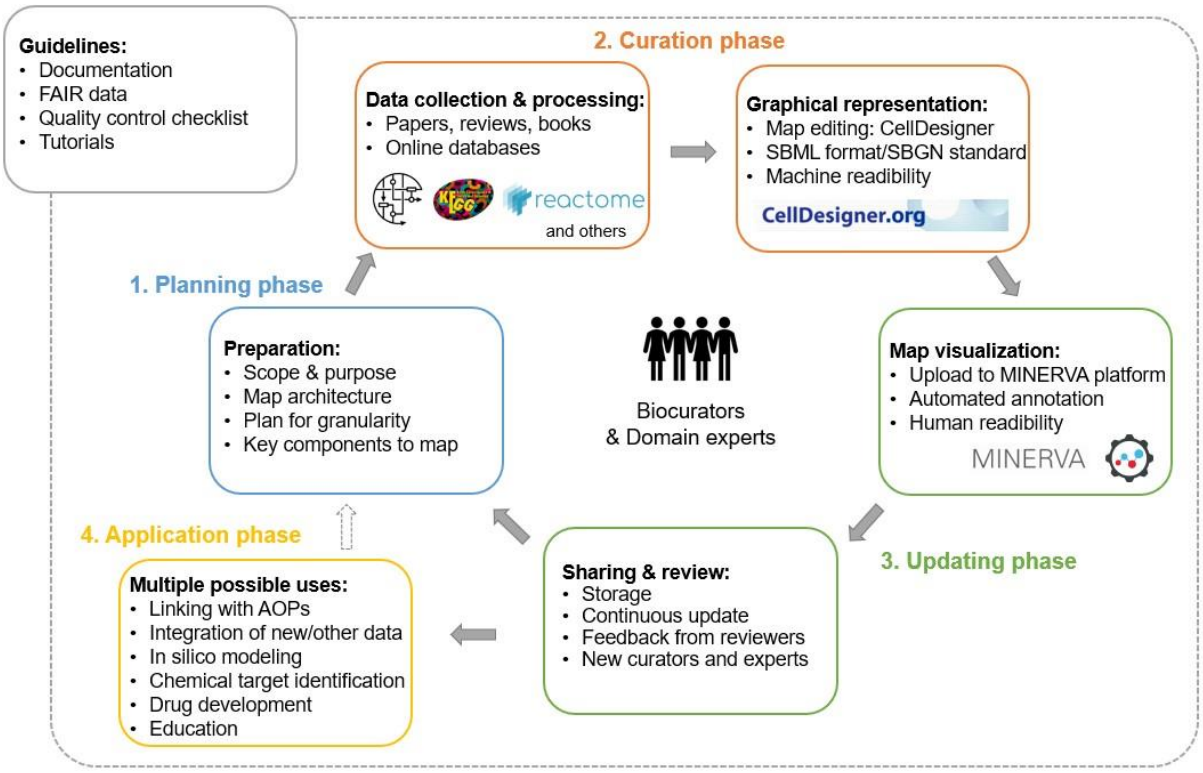

Figure 1. Workflow to construct ONTOX physiological maps. The workflow consists of 4 main phases: (1) Planning, (2) Curation, (3) Update and (4) Application. Inspired by Mazein, Acencio, *et al*., 2023.

Data resources

The knowledge integrated in the PMs comes from various sources including original research papers, review articles and books covering the physiology of the processes concerned. In addition, the following databases and platforms are used: WikiPathways (Agrawal et al., 2024), Reactome (Gillespie et al., 2022), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2023), among other pathway resources. Parts of maps and/or submaps from other DM projects are adapted and integrated in the PMs (Fujita et al., 2014; Mazein et al., 2018; Ostaszewski et al., 2019, 2021; Serhan et al., 2020). Manual data extraction is complemented by AI-based tools, using natural language processing in particular, to integrate human-readable data into semi-automated data extraction projects (Bozada et al., 2021; Corradi et al., 2022). The ONTOX project aims to further automate the process from data extraction to graphical representation by integrating tools for data extraction, data conversion and diagram editing.

Graphical standard

The **Systems Biology Graphical Notation** (SBGN) is a visual language used to represent biochemical interaction networks in a standardized and unambiguous way (Novère et al., 2009). This language has been developed by a community including biologists, biochemists, mathematicians, and computer scientists, with the aim of standardizing the graphical notation used in maps of biological processes (such as gene regulation, metabolism and cell signaling). PMs are designed to comply as closely as possible with SBGN, in particular the Process Description (PD) language, one of the three SBGN languages. PD depicts how entities, represented by nodes in the network, change from one form to another and influence each other (mechanistic, directed and sequential descriptions) (Mazein et al., 2018; Rougny et al.,

2019). In some modules, such as gene regulatory networks, the SBGN Activity Flow language is used as a method to prevent visual overload on the map. This approach ensures a clean design that remains adaptable for modeling purposes and that can be detailed in the PD language on request.

Editor and visualization software

PMs have been drawn based on SBGN using the **CellDesigner** structured diagram editor (Funahashi et al., 2008). CellDesigner is used to graphically represent SBGN-compatible networks, storing the maps in the **Systems Biology Markup Language** (SBML), a free and open software data format for describing models in systems biology (Keating et al., 2020). CellDesigner does not fully comply with SBGN (e.g. for the shape of the nodes), but we have ensured that the representation of the network is as close as possible to the standard. PMs can then be imported onto the MINERVA platform, enabling annotation, data cross-linking and improved visualization (Gawron et al., 2016). **MINERVA** integrates automated tools which annotate nodes in the PMs using the name of an element (e.g. HGNC approved symbol) or its MIRIAM identifier such as ChEBI, Ensembl, Uniprot and Gene Ontology. MINERVA also enables an interactive visualization for both elements and interactions, provides functionalities for content exploration (such as searching for drug targets), allows for the overlay of experimental datasets and facilitates feedback from reviewers. Altogether, the CellDesigner/MINERVA platform offers: (i) the ability to draw biochemical networks, integrate and annotate data from a variety of sources; (ii) easy-to-interpret visual representation; and (iii) compatibility with other platforms and machine readability, allowing for sharing data and building *in silico* models. Figure 2 shows a submap of the liver metabolism PM (hepatocyte-specific cholesterol metabolism) as designed on CellDesigner.

Sustainability

PMs are developed on the basis of current knowledge and are intended to be continuously updated as new data becomes available. The possibility to have a **modular structure** (dividing the main map into submaps) offered by the CellDesigner/MINERVA platform facilitates complex data visualization and thereby strongly simplifies the review and curation processes. Smaller modules can then be submitted to WikiPathways (Pico et al., 2008; Agrawal et al., 2024) to accelerate curation via a community effort. To ensure the development, curation and update of maps that adhere to the FAIR principles (Findability, Accessibility, Interoperability, and Reusability) (Wilkinson et al., 2016), we developed **curation guidelines** for PMs, providing recommendations in terms of design, annotation, documentation (e.g., planning document, issue report), quality control, and storage (Ladeira et al., 2024). These guidelines are expected to enhance scientific reproducibility, facilitate the usage of the maps and the development of new modules. When PMs are ready for publication, they can be made publicly available online on MINERVA, which is a stable infrastructure hosted by ELIXIR Luxembourg[3]. As PMs require **continuous updating**, review and feedback from domain experts and curators will continue after publication. In terms of traceability, successive versions of PMs are stored in the BioStudies database (part of the ELIXIR infrastructure) and can be tracked by persistent identifiers, while all associated data and metadata are stored on GitHub[4].

[3] https://elixir-luxembourg.org/services/catalog/minerva
[4] https://github.com/ontox-maps

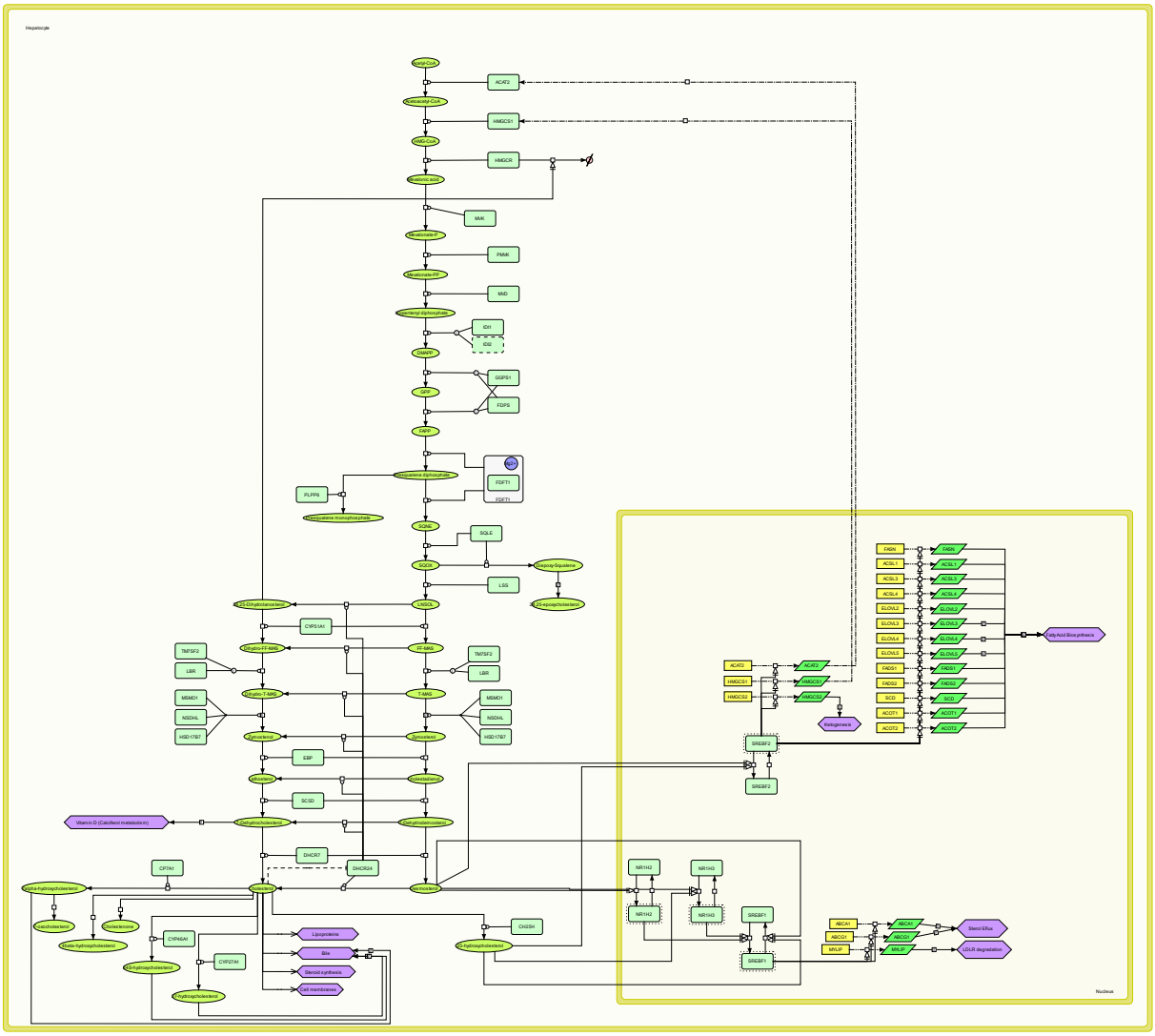

Figure 2. Example of a PM. Hepatocyte-specific cholesterol metabolism submap designed on CellDesigner, and accessible through MINERVA.

3. Applications

The workflow used to build PMs is largely inspired by the DM Project, using a diagram editor (CellDesigner) to develop and store maps in SBML and a user-friendly platform for annotation and visualization (MINERVA). Unlike DMs, the foundation of PMs is, as their name suggests, the unperturbed physiology of the mechanisms involved. We believe that the mapping of physiology is highly complementary to the representation of disease mechanisms, enabling a better understanding of both physiological and pathological processes, improvements in target identification and the personalization of therapies. In addition, PM-derived applications should benefit from the existing pipelines developed for successful DM applications (Highlight Box).

Assessment of AOPs

Not only are PMs complementary to DMs, but they can also be seen as a supporting tool for developing AOPs. In addition to the mechanistic information contained in AOPs, PMs add another level of detail in terms of gene regulation, biochemical networks and cell-cell interactions. As a benchmarking tool, PMs can help fill gaps in AOPs, identify new key events

and molecular initiating events, and build more comprehensive AOP networks. Recently, the SBGN-based approach used for building PMs and DMs has been applied to transform an AOP into a standardized, structured digital diagram, using controlled vocabulary and automated annotations (Mazein, Shoaib, et al., 2023; Mazein et al., 2024). This paves the way for the integration of PMs and AOPs in a multi-layer structure, linking data from one layer to another.

Building on the success of disease maps

- The **Parkinson's DM** was the first map published in the context of the DM Project. It resulted from the manual curation of more than 1000 research articles and public databases from which the information was integrated in a molecular interaction map.
- The **Atlas of Cancer Signalling Network** (ACSN) has been successfully exploited. Applications: (1) an example is the analysis of omics data from breast cancer cell lines using the ACSN identified different molecular portraits of sensitivity to Dbait and PARP inhibitors and therefore explained the synergistic therapeutic effect of these two drugs; (2) from the several applications of ACSN in preclinical studies, a more general workflow has been proposed, starting with the construction of a comprehensive network and aiming to understand the molecular mechanisms in different human diseases (not only in cancer), to predict drug sensitivity and to identify optimal therapeutic approaches.
- The **Covid-19 DM** is the result of a remarkable community effort that brought together 277 scientists from 130 institutions around the world.
  Applications: using several systems biology tools and omics data, Niarakis et al. leveraged the Covid-19 DM to unravel certain biological mechanisms and signaling events following infection by SARS-CoV-2. Their pipelines make it possible to test combinatorial therapies and evaluate the repurposing of existing drugs to combat new waves of Covid-19 or other pandemics.

Highlight Box. The DM community is working together on several projects for which some maps have already been successfully exploited: the Parkinson's DM (Fujita et al., 2014); the Atlas of Cancer Signalling Network and applications (Kuperstein et al., 2015; Jdey et al., 2017; Monraz Gomez et al., 2019); the Covid-19 DM and applications (Ostaszewski et al., 2021; Niarakis et al., 2023).

Foundation of multi-layered data structures

In ONTOX, PMs will constitute the foundation of multi-layer frameworks integrating physiology, AOPs and AOP networks (e.g., van Ertvelde et al., 2023; Barnes et al., 2024; Verhoeven et al., 2024), chemical and kinetic data. These frameworks will address the following adverse outcomes: liver steatosis, cholestasis, tubular necrosis, crystallopathy, neural tube closure and cognitive function defects. Enabling integration, annotation, and visualization of diverse types of data, these multi-layered structures will enhance understanding of organ- and disease-specific pathways in response to chemical perturbations.

Visualizing and analyzing your own data

PMs consist of a user-friendly and standardized visualization that allows for analyzing your own datasets (e.g., omics). Using PMs, you can, for example, identify biological mechanisms driving a pathology and uncover potential drug targets. Indeed, using the features of the MINERVA platform, you can query external ontologies to identify chemicals and drugs that interact with the molecular targets within the maps. More information on the current capabilities of the platform for data visualization and analysis can be found in Mazein *et al.*, 2023.

Improving *in vitro* and *in silico* risk assessment approaches

While PMs will be integrated with AOPs and will be used to identify key molecules and pathways involved in specific functions or diseases, they will support the development of *in vitro* test batteries, advancing animal-free approaches for next-generation risk assessment. Furthermore, PMs will aid in the development of quantitative methods for computational disease modeling and for assessing chemical safety from perturbation of the biology. Standardized PMs, AOPs and multi-layer frameworks can indeed be leveraged to predict the toxicity of chemicals using data-driven (machine learning) and knowledge-driven *in silico* models, with the goal of developing probabilistic risk assessment approaches that account for the complex interactions between different physiological systems in the body.

Education material

PMs provide a visual representation of biological pathways and interactions that can serve as educational material for training in physiology, toxicology and related disciplines. The user-friendly interface of the MINERVA platform, the structure co-designed with domain experts, the graphical standards and the comprehensive annotations undoubtedly facilitate the exploration and understanding of the biological processes.

4. Perspectives

PMs can be defined as comprehensive graphical representations of biological processes and interactions using the SBGN. The PMs developed within the ONTOX project are designed to map the functional processes in the liver, kidney, and developing brain. This workflow, however, can be adapted to map any physiological process. While the structure and annotation scheme of PMs are expected to benefit the scientific community in terms of data management, accessibility and interoperability, curation guidelines for PMs have been developed, including a comprehensive description of the mandatory and recommended annotations for each map feature in ONTOX. The capability of creating submaps (modules of the full map) facilitates the integration of new data and updates, as well as the identification of data gaps, prioritizing research needs, and enhancing the PMs' usability and scalability. The modularized architecture of PMs also enables a more detailed and comprehensive mapping of AOPs and key events, which can foster the development of novel *in vitro* assays for assessing chemical toxicity. In addition, the use of annotations and controlled vocabularies to establish linkages allows for connections between different communities and initiatives. PMs are tools that need to be constantly updated, which is only possible through a collaborative, community-based effort, drawing on the expertise and knowledge of biocurators and experts from different fields. In particular, the ONTOX project aims to bring the toxicology and systems biology communities closer together to create standardized and comprehensive PMs that will play a crucial role in the development of NAMs for toxicological mechanistic next-generation risk assessment.

Data availability

All the maps are available on our GitHub page[5], on the BioStudies ONTOX database[6], and on the ONTOX MINERVA platform[7], under the License Creative Commons Attribution 4.0 International (CC BY 4.0) License[8].

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Competing interests

The authors declare that they have no conflict of interest.

References

Agrawal, A., Balcı, H., Hanspers, K. et al. (2024). WikiPathways 2024: next generation pathway database. *Nucleic Acids Res 52*, D679–D689. doi:10.1093/nar/gkad960.

Ankley, G. T., Bennett, R. S., Erickson, R. J. et al. (2010). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem 29*, 730–741. doi:10.1002/etc.34.

Barnes, D. A., Firman, J. W., Belfield, S. J. et al. (2024). Development of an adverse outcome pathway network for nephrotoxicity. *Arch Toxicol 98*, 929–942. doi:10.1007/s00204-023-03637-7.

Bozada, T., Borden, J., Workman, J. et al. (2021). Sysrev: A FAIR Platform for Data Curation and Systematic Evidence Review. *Front Artif Intell 4*, 685298. doi:10.3389/frai.2021.685298.

Corradi, M. P. F., de Haan, A. M., Staumont, B. et al. (2022). Natural language processing in toxicology: Delineating adverse outcome pathways and guiding the application of new approach methodologies. *Biomater Biosyst 7*. doi:10.1016/j.bbiosy.2022.100061.

van Ertvelde, J., Verhoeven, A., Maerten, A. et al. (2023). Optimization of an adverse outcome pathway network on chemical-induced cholestasis using an artificial intelligence-assisted data collection and confidence level quantification approach. *J Biomed Inform 145*. doi:10.1016/j.jbi.2023.104465.

Fujita, K. A., Ostaszewski, M., Matsuoka, Y. et al. (2014). Integrating pathways of parkinson's disease in a molecular interaction map. *Mol Neurobiol 49*, 88–102. doi:10.1007/s12035-013-8489-4.

Funahashi, A., Matsuoka, Y., Jouraku, A. et al. (2008). CellDesigner 3.5: A versatile modeling tool for biochemical networks. *Proc IEEE 96*, 1254–1265. doi:10.1109/JPROC.2008.925458.

[5] https://github.com/ontox-maps
[6] https://www.ebi.ac.uk/biostudies/ONTOX/studies
[7] https://ontox.elixir-luxembourg.org/minerva/
[8] https://creativecommons.org/licenses/by/4.0/
[9] https://minerva.pages.uni.lu/doc/
[10] https://elixir-luxembourg.org

Gawron, P., Ostaszewski, M., Satagopam, V. et al. (2016). MINERVA—A platform for visualization and curation of molecular interaction networks. *npj Syst Biol Appl 2*, 1–6. doi:10.1038/npjsba.2016.20.

Gillespie, M., Jassal, B., Stephan, R. et al. (2022). The reactome pathway knowledgebase 2022. *Nucleic Acids Res 50*, D687–D692. doi:10.1093/nar/gkab1028.

Hemmerich, J. and Ecker, G. F. (2020). In silico toxicology: From structure–activity relationships towards deep learning and adverse outcome pathways. *WIREs Comput Mol Sci 10*, e1475. doi:10.1002/wcms.1475.

Heusinkveld, H. J., Staal, Y. C. M., Baker, N. C. et al. (2021). An ontology for developmental processes and toxicities of neural tube closure. *Reprod Toxicol 99*, 160–167. doi:10.1016/j.reprotox.2020.09.002.

Hoksza, D., Gawron, P., Ostaszewski, M. et al. (2020). Closing the gap between formats for storing layout information in systems biology. *Brief Bioinform 21*, 1249–1260. doi:10.1093/bib/bbz067.

Ito, S., Mukherjee, S., Erami, K. et al. (2024). Proof of concept for quantitative adverse outcome pathway modeling of chronic toxicity in repeated exposure. *Sci Rep 14*, 4741. doi:10.1038/s41598-024-55220-4.

Jdey, W., Thierry, S., Russo, C. et al. (2017). Drug-driven synthetic lethality: Bypassing tumor cell genetics with a combination of AsiDNA and PARP inhibitors. *Clin Cancer Res 23*, 1001–1011. doi:10.1158/1078-0432.CCR-16-1193.

Kanehisa, M., Furumichi, M., Sato, Y. et al. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res 51*, D587–D592. doi:10.1093/nar/gkac963.

Keating, S. M., Waltemath, D., König, M. et al. (2020). SBML Level 3: an extensible format for the exchange and reuse of biological models . *Mol Syst Biol 16*, e9110. doi:10.15252/msb.20199110.

Kuperstein, I., Bonnet, E., Nguyen, H. A. et al. (2015). Atlas of Cancer Signalling Network: A systems biology resource for integrative analysis of cancer data with Google Maps. *Oncogenesis 4*, e160–e160. doi:10.1038/oncsis.2015.19.

Ladeira, L., Staumont, B., Gamba, A. et al. (2024). *ONTOX's Physiological Maps Curation Guidelines (1.0)*. doi:10.5281/ZENODO.13239453.

Luechtefeld, T. and Hartung, T. (2017). Computational approaches to chemical hazard assessment. *ALTEX 34*, 459–478. doi:10.14573/altex.1710141.

Luechtefeld, T., Marsh, D., Rowlands, C. et al. (2018). Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility. *Toxicol Sci 165*, 198–212. doi:10.1093/toxsci/kfy152.

Mazein, A., Acencio, M. L., Balaur, I. et al. (2023). A guide for developing comprehensive systems biology maps of disease mechanisms: planning, construction and maintenance. *Front Bioinforma 3*, 1197310. doi:10.3389/fbinf.2023.1197310.

Mazein, A., Lopata, O., Reiche, K. et al. (2024). An explorable model of an adverse outcome pathway of cytokine release syndrome related to the administration of immunomodulatory biotherapeutics and cellular therapies. doi:10.21203/rs.3.rs-5163108/v1.

Mazein, A., Ostaszewski, M., Kuperstein, I. et al. (2018). Systems medicine disease maps: community-driven comprehensive representation of disease mechanisms. *npj Syst Biol Appl 4*, 21. doi:10.1038/s41540-018-0059-y.

Mazein, A., Shoaib, M., Alb, M. et al. (2023). Using interactive platforms to encode, manage and explore immune-related adverse outcome pathways ORCID. *bioRxiv*, 2023.03.21.533620. doi:10.1101/2023.03.21.533620.

Monraz Gomez, L. C., Kondratova, M., Ravel, J.-M. et al. (2019). Application of Atlas of Cancer Signalling Network in preclinical studies. *Brief Bioinform 20*, 701–716. doi:10.1093/bib/bby031.

Niarakis, A., Ostaszewski, M., Mazein, A. et al. (2023). Drug-target identification in COVID-19 disease mechanisms using computational systems biology approaches. *Front Immunol 14*. doi:10.3389/fimmu.2023.1282859.

Novère, N. Le, Hucka, M., Mi, H. et al. (2009). The Systems Biology Graphical Notation. *Nat Biotechnol 27*, 735–741. doi:10.1038/nbt.1558.

Ostaszewski, M., Gebel, S., Kuperstein, I. et al. (2019). Community-driven roadmap for integrated disease maps. *Brief Bioinform 20*, 659–670. doi:10.1093/bib/bby024.

Ostaszewski, M., Niarakis, A., Mazein, A. et al. (2021). COVID19 Disease Map, a computational knowledge repository of virus–host interaction mechanisms. *Mol Syst Biol 17*. doi:10.15252/msb.202110387.

Pico, A. R., Kelder, T., van Iersel, M. P. et al. (2008). WikiPathways: Pathway Editing for the People. *PLoS Biol 6*, e184. doi:10.1371/journal.pbio.0060184.

Punt, A., Bouwmeester, H., Blaauboer, B. J. et al. (2020). New Approach Methodologies (NAMs) for Human-Relevant Biokinetics Predictions: Meeting the Paradigm Shift in Toxicology Towards an Animal-Free Chemical Risk Assessment. *ALTEX 37*, 607–622. doi:10.14573/altex.2003242.

Rougny, A., Touré, V., Moodie, S. et al. (2019). Systems Biology Graphical Notation: Process Description language Level 1 Version 2.0. *J Integr Bioinform 16*. doi:10.1515/jib-2019-0022.

Schmeisser, S., Miccoli, A., von Bergen, M. et al. (2023). New approach methodologies in human regulatory toxicology – Not if, but how and when! *Environ Int 178*. doi:10.1016/j.envint.2023.108082.

Serhan, C. N., Gupta, S. K., Perretti, M. et al. (2020). The Atlas of Inflammation Resolution (AIR). *Mol Aspects Med 74*. doi:10.1016/j.mam.2020.100894.

Verhoeven, A., van Ertvelde, J., Boeckmans, J. et al. (2024). A quantitative weight-of-evidence method for confidence assessment of adverse outcome pathway networks: A case study on chemical-induced liver steatosis. *Toxicology 505*. doi:10.1016/j.tox.2024.153814.

Vinken, M., Benfenati, E., Busquet, F. et al. (2021). Safer chemicals using less animals: kick-off of the European ONTOX project. *Toxicology 458*, 152846. doi:10.1016/j.tox.2021.152846.

Wilkinson, M. D., Dumontier, M., Aalbersberg, Ij. J. et al. (2016). Comment: The FAIR Guiding Principles for scientific data management and stewardship. *Sci Data 3*, 1–9. doi:10.1038/sdata.2016.18.

Wittwehr, C., Aladjov, H., Ankley, G. et al. (2017). How Adverse Outcome Pathways Can Aid the Development and Use of Computational Prediction Models for Regulatory Toxicology. *Toxicol Sci 155*, 326–336. doi:10.1093/toxsci/kfw207.