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Building knowledge maps to support NAM development

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Physiological maps (PMs) are comprehensive, machine-readable graphical representations of organ-specific biological mechanisms. These standardized frameworks integrate extensive knowledge from scientific literature to depict undisturbed physiology, creating valuable platforms for studying chemical-induced perturbations and their mechanisms. PMs streamline the integration, organization, and visualization of diverse biological, toxicological, chemical, and kinetic data to support New Approach Methodologies (NAMs).

Within ONTOX, five organ-specific PMs were developed: bile secretion & lipid metabolism (liver), nephron physiology (kidney), neural tube closure, and cognitive function development (brain). Their creation involved manual literature curation, ontological term annotation, and expert revision. PM development requires collaboration between domain experts and biocurators, guided by specific planning documents and curation guidelines with detailed metadata. This ensures compliance with FAIR principles (Findability, Accessibility, Interoperability, Reusability), facilitating use across projects and platforms. The modular structure simplifies review processes, enhances curation engagement, and enables complex data visualization for stakeholders.

Each map uses Systems Biology Graphical Notation (SBGN) for human interpretation, stored in machine-readable SBML format, and built using CellDesigner software following a workflow inspired by the Disease Maps Project. The MINERVA platform enables interactive exploration with a Google Maps-like interface, making complex biological information accessible.

These maps form the foundation for computational logic models that simulate system behaviors, contributing to ONTOX's *in silico* NAM toolbox. Future development will incorporate AI-assisted text-mining and automatic model updating to maintain current, human-relevant, and animal-free tools that serve as knowledge repositories. The PMs advance next-generation risk assessment by promoting collaboration between toxicology and systems biology communities, benchmarking adverse outcome pathways, identifying therapeutic targets, and visualizing omics data. Continuous refinement and collaborative development will enhance their utility in developing personalized medicine approaches and human-relevant risk assessment methodologies.

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Next generation risk assessment (NGRA) of hair dye HC Yellow No. 13: Ensuring protection from liver steatogenic effects

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This study employs animal-free NGRA principles to evaluate the safety of repeated dermal exposure to 2.5% (w/w) of the hair dye HC Yellow No. 13 (HCY13). As multiple *in silico* tools consistently flagged hepatotoxic potential, likely due to HCY13's trifluoromethyl group, which is known to interfere with hepatic lipid metabolism, liver steatosis was chosen as the primary mode of action for evaluation. Adverse outcome pathway (AOP)-guided *in vitro* tests were conducted, exposing human stem cell-derived hepatic cells to varying HCY13 concentrations over 72 hours. The expression of 11 lipid metabolism-related marker genes (AHR, PPARA, LXRA, APOB, ACOX1, CPT1A, FASN, SCD1, DGAT2, CD36, and PPARG) and triglyceride accumulation, a phenotypic hallmark of steatosis, were measured. PROAST software was used to calculate *in vitro* Points of Departure (PoDNAM) for each biomarker. Using GastroPlus 9.9, physiologically-based pharmacokinetic (PBPK) models estimated internal liver concentrations (Cmax liver) of HCY13, ranging from 4 to 20 pM. All PoDNAM values significantly exceeded the predicted Cmax liver, indicating that HCY13 at 2.5% (w/w) is unlikely to induce liver steatosis under the assumed conditions. This research demonstrates the utility of NGRA, integrating AOP-based *in vitro* assays and computational models to protect human health and support regulatory decision-making without animal testing.

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