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Next generation risk assessment: An integrated transcriptomic, phenotypic profiling and IIVIVE approach for plant protection product mixture effects

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The pursuit of eliminating animal testing in human health risk assessment has driven a transformative shift towards next-generation risk assessment (NGRA) across multiple sectors and regulatory bodies. NGRA is a human-relevant, exposure-led, hypothesis-driven risk assessment approach that employs new approach methodologies (NAMs). Currently, plant protection product (PPP) risk assessment relies on evaluating individual substances based on animal testing. This innovative framework enables a comprehensive assessment of whole mixtures, overcoming the limitations of traditional compound-based PPP risk assessment, which often overlooks interactions that may increase toxicity. This study aims to evaluate the feasibility of a NGRA approach that integrates systems toxicology-based NAMs, including cell painting and transcriptomics with toxicokinetic models, to determine PPP-based human equivalent doses (HED) and threshold values corresponding to *in vitro* concentration-response data. Notably, toxicological threshold values were derived from cell painting data, and transcriptomics analysis using two concentrations was included as an additional component to further investigate mechanisms associated with steatosis. To this end, transcriptomics analysis and *in vitro* phenotypic profiling using the cell painting plus (CPP) method were performed on a PPP containing two metabolically interacting active substances. This study investigated the effect of individual active substances, PPP-equivalent mixture, and the PPP on HepaRG cells at the transcriptomic level and on HepG2 cells through phenotypic profiling. Transcriptomics data were analyzed to identify the affected biological pathways and benchmark concentrations (BMCs) were derived from CPP data. *In vitro-in vivo* extrapolation (IIVIVE) was performed using the htk package, with relevant parameters and model assumptions for the PPP, to estimate HEDs and toxicological threshold values. Although the BMCs of individual substances exceeded those of the PPP, the affected cellular organelles remained consistent across all scenarios. The predicted PPP-based toxicological threshold value was below the acceptable operator exposure level (AOEL). This study indicates that risk assessment based solely on active substances may not sufficiently capture complex interactions within PPPs. These findings underscore the importance of integrating NAMs to provide valuable insights into PPP interactions for regulatory purposes and advance NGRA.

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A Shiny-based framework for aggregating repeated-dose toxicology and organ-specific findings: A case study across eighteen alpha-chloroacetamide herbicides

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Regulatory dossiers contain extensive but dispersed toxicity information. We developed an application that aggregates study-level histopathology data and harmonized metadata from repeated-dose rat and dog studies. The app incorporates four standardized templates: study-design metadata, histopathology findings, chemical identifiers, and historical-control baselines for simulation. It enables reproducible visualization (user-defined seed/template) via class or substance filters, organ-agnostic and specific heatmaps (legend: finding/no finding/no data), and dot plots displaying all doses and the no observed adverse effect level (NOAEL) (mg/kg bw/day). Lowest observed adverse effect level (LOAEL) findings are shown as text when units are not comparable (e.g., ppm). The app supports data aggregation across studies and compounds. To explore biological correlations or map data to adverse outcome pathways.

We present a case study investigating the role of hepatic enzyme induction in correlation to anti-androgenicity. Molecular initiating event information was derived from ToxCast and CoMPARA (AR agonism/antagonism, AUC_{0.1}), AR binding from CoMPARA, and cytochrome P450 enzyme (CYP) surrogate data for hepatic enzyme induction from ToxCasts (CYP1A1/3A4/2B6). *In vivo* repeated dose data for 18 α -chloroacetamide herbicides were extracted from regulatory documents and transferred to in standard templates. Ten substances yielded evaluable data (89 studies), eight had none. Across the 89 studies, organs coverage was 52.8% for testis (47/89), 41.6% for epididymis (37/89), 43.8% for prostate (39/89), and 36.0% for seminal vesicles (32/89). Author-designated treatment-related findings were reported for testis (n=6), epididymis (n=1), and prostate (n=4), but none for seminal vesicles. Treatment-related findings occurred in long-term rat, dog 90-day, and dog 1–2-year studies, but not short-term studies (rat 28/90-day or dog 28-day).

The application consolidates repeated-dose and long-term data in a transparent, reproducible framework that highlights coverage and data gaps, enabling class- and substance-level comparisons while preserving authors' treatment-related/adverse designations flags. Limitations include reliance on author classifications, variable historical organ coverage (notably seminal vesicles), and heterogeneous LOAEL units (text-only). The combination of AOPs with data visualization across studies will allow for an in-depth WoE evaluation across compounds.

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Physiologically-based toxicokinetic model of the transfer of branched and linear perfluoroalkyl acids in dairy goats

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Introduction Perfluoroalkyl acids (PFAAs) are a subgroup of per- and polyfluoroalkyl substances (PFAS) that are extremely persistent and linked to adverse health effects in humans and animals. The European Union has set maximum levels for four PFAAs in foods of animal origin to limit dietary exposure. Predicting PFAA accumulation kinetics in livestock is therefore crucial for assessing their transfer into food products. This study aims to develop a physiologically based toxicokinetic (PBTK) model for branched (br-) and linear (n-) PFAAs in dairy goats. The model covers several perfluorocarboxylates and perfluorosulfonates with chain lengths from 4 to 13 carbons and quantifies their distribution across tissues and excretory routes.

Materials and methods The dynamic compartmental model represents twelve physiological compartments (blood plasma, liver, kidney, spleen, stomachs, intestines, mammary gland, heart, brain, muscles, lungs, and rest) and three excretion pathways (urine, feces, milk). It was parameterized using literature data and results from an in-house *in vivo* feeding study, in which eight goats received PFAS-contaminated hay for eight weeks followed by a twelve-week depuration phase. PFAA concentrations were measured in serum, milk, and excreta over time, and in body tissues at slaughter (end of depuration). Key parameters were optimized to minimize prediction error, and model uncertainty was quantified using jackknife and Monte Carlo analyses. The model, implemented in Python (v3.14), applies an analytical matrix solution enabling efficient computation.

Results and discussion Preliminary results show that the model achieved high predictive performance across 17 PFAAs ($R^2 = 0.96$; mean absolute error [MAE] = 0.18) with 86% confidence interval coverage, indicating robust uncertainty characterization. Predicted mean residence times ranged from 23.3 h (half-life = 0.7 days) for branched perfluorotridecanesulfonic acid (br-PFTrDS) to 427.9 h (half-life = 12.4 days) for non-branched perfluorooctanesulfonic acid (n-PFOS), reflecting the influence of molecular structure on the kinetics of different PFAAs.

Conclusion and implications This model enables the simulation of accumulation and depuration under various exposure scenarios. It may support quantitative risk assessment by predicting transfer into edible tissues and milk and can address questions related to animal health by estimating internal exposure in livestock.

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Quantitative *in vitro-in vivo* relationship of hepatotoxic concentrations: A case study for food-relevant compounds in the rat

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Question:

How closely are toxic concentrations *in vitro* related to toxic concentrations *in vivo*?

Humans are exposed to a broad variety of substances through food and drinking water, including natural constituents, additives and contaminants. Potential toxicity – for example hepatotoxicity- of these compounds is a significant hazard to public health. The evaluation of human toxicity for the large number of relevant compounds poses a challenge for regulatory toxicology. A high-throughput screening system would therefore be desirable. For the development of such a test system a quantitative comparison of the derived toxic *in vitro* concentrations such as the EC10 to toxic *in vivo* concentrations after administration of known toxic dosages is necessary.

Methods:

As proof-of concept a substance set of 40 food relevant compounds with conventionally derived lowest observed adverse effect levels (LOAEL) for hepatotoxicity was tested with an *in vitro* test battery comprising cytotoxicity and lipid accumulation assays in the rat hepatoma cell lines H4IIE and Zajdela as well as cultured primary rat hepatocytes. In addition, physiologically based pharmacokinetic models were established for all compounds and utilized to obtain *in vivo* concentrations after oral application of the compounds at the LOAEL. To evaluate the *in vitro-in vivo* relationship a quality criterion (Toxicity Iso-concentration Index (TII)) was introduced. A TII of 1 hereby indicates a 100% agreement of all *in vitro* and *in vivo* concentrations.

Results:

The best agreement of the *in vitro* and *in vivo* concentrations was observed with the EC10 for cytotoxicity in H4IIE cells and the peak plasma concentrations in the portal vein. In detail a TII of 0.85 was obtained and 75% of the compounds were within a 10- fold deviation range.