trimethoprim and sulfadiazine for the intravenous, intramuscular, and subcutaneous administration routes and validated with plasma and milk concentrations from the literature. Overall, model simulations were within a factor of two for the included administration routes of sulfadiazine and the iv administration of trimethoprim. However, it was not possible to reproduce trimethoprim plasma and milk concentration from literature after intramuscular and subcutaneous administration. A possible explanation may be the infection after administration leading to a prolonged release of both pharmaceuticals into the plasma. Nevertheless, the model performed well when administering sulfadiazine and trimethoprim (intravenous administration) and could be extended to other chemicals.

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P05-42
Comparison of Acceptable Daily Intake (ADI) and *in vivo* doses extrapolated from *in vitro* point of departure obtained with cell painting on U2OS

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The Acceptable Daily Intake (ADI) is the dose of a chemical compound that can be ingested daily by a human without eliciting any adverse effect. The ADI is derived from *in vivo* experiments. The highest dose without any adverse effect from the most sensitive species, for any type of study, is used with typically a safety factor of 100 (10 for intra specie variability and 10 for inter specie variability).

In the context of Next Generation Risk Assessment (NGRA), there is a pressing need to employ new alternative methods (NAMs), such as *in vitro* assays, to reduce or even replace animal studies. One such assay, is Cell Painting, an *in vitro* assay developed by the Broad institute, which generates morphological profiles of cells perturbated by chemicals. It uses 6 dyes to reveal 8 cell compartments, to form after image analysis a robust and unbiased morphological profile describing the morphology of cells.

Inspired by results from the US EPA, where Cell Painting was used with reverse dosimetry to extrapolate an *in vivo* dose and to prioritize risky compound testing, we ran a Cell Painting campaign on chemical compounds with known ADIs to compare ADIs with doses extrapolated from *in vitro* point of departure (PODs) obtained with the Cell Painting assay.

We performed Cell Painting on U2OS (human osteoblast cell line) on 71 compounds at 8 concentrations (from 0.03 µM to 100 µM). We determined their *in vitro* Point of Departure (POD), the concentration for which the cell morphology started to defer from the negative controls. Out of the 71 compounds, 49 had a POD.

For the reverse dosimetry, we used the US EPA R httk package. The parametrization was done using ADME *in vitro* measures: Human Clint, fraction unbound and human blood to plasma ratio, along with unspecific binding ratio on plastic and media for the 384-w plate, to estimate the free concentration and refine the POD.

We computed the Administered Equivalent Dose (AED) from the *in vitro* POD using httk.

We obtained AED ranges: the 5th, 50th and 95th quantiles. The results of this comparative analysis will be presented and discussed in the context of the development of NGRA.

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P05-43
What does it take to make a PBK for a pesticide in a NGRA framework? Evaluation of the kinetic space of pesticides and how to model it using open-source tools

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Physiologically-based kinetic models (PBK) represent a mechanistic modeling approach to predict the systemic availability and organ concentrations over time for chemicals through external exposure. These model can be used to link *in vitro* hazard characterization data and external dose estimations hence, constituting a cornerstone in Next Generation Risk Assessment (NGRA). To be amenable for NGRA framework, PBK models have to be more bottom-up and mechanistic. While there is a common generic structure and set of input parameters for PBK models, special characteristics of the test chemical might require the inclusion of specific processes in the PBK models. In the absence of new *in vivo* data to understand whether such specific processes are needed, we rely on read-across of PBK models of similar chemicals.

PBK (Physiologically Based Kinetic) models have found widespread utility in the safety evaluation of therapeutic agents. Conversely, the application of PBK models to pesticides poses unique challenges due to the broader spectrum of physicochemical properties associated with pesticides, coupled with the scarcity of comprehensive ADME (Absorption, Distribution, Metabolism, and Excretion) and *in vivo* kinetic data. Thus, modelling pesticides kinetics requires more tailored approaches. The primary objective of this study was to assess the feasibility of leveraging existing knowledge and conducting systematic PBK model read-across within the domain of pesticide chemistry.

We reviewed literature on PBK models for organic pesticides, focusing on phenoxy herbicides, organochlorine insecticides, and pyrethroids. Then we identified key aspects of the compounds kinetics and how they are modelled in the PBK models, including parameterization and evaluation approaches. Finally, we assessed the suitability of open PBK software (e.g. PK-sim and TKplate) to integrate some of these kinetic specificities but also the capacity of algorithms of chemical grouping (e.g. KWAAS) to pair target chemical with relevant analogues Certain pesticides exhibit high lipophilicity, challenging standard PBK assumptions. To address this, some models incorporate a separate blood compartment for lipoproteins, and may include deep liver compartments or lymphatic routes of oral absorption. The low solubility of some pesticides leads to uncertainty in *in vitro*-derived ADME parameters, which could be mitigated by integrating quantitative structure-property relationships to correct for the fraction unbound. Some pesticides also exhibit bile excretion, formation of bioactive metabolites, and toxicodynamic effects, which should be considered in modeling.

This study contributes to the broader goal of analyzing how kinetic information is reported in academic literature and regulatory documents and explores its integration into systematic, automated PBK read-across methodologies, setting the stage to integrate AI tools in the near future.

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