Developing Toxicity and Pharmacokinetic Models for an *in vitro* Integrated Organ Platform (HuDMOP™).

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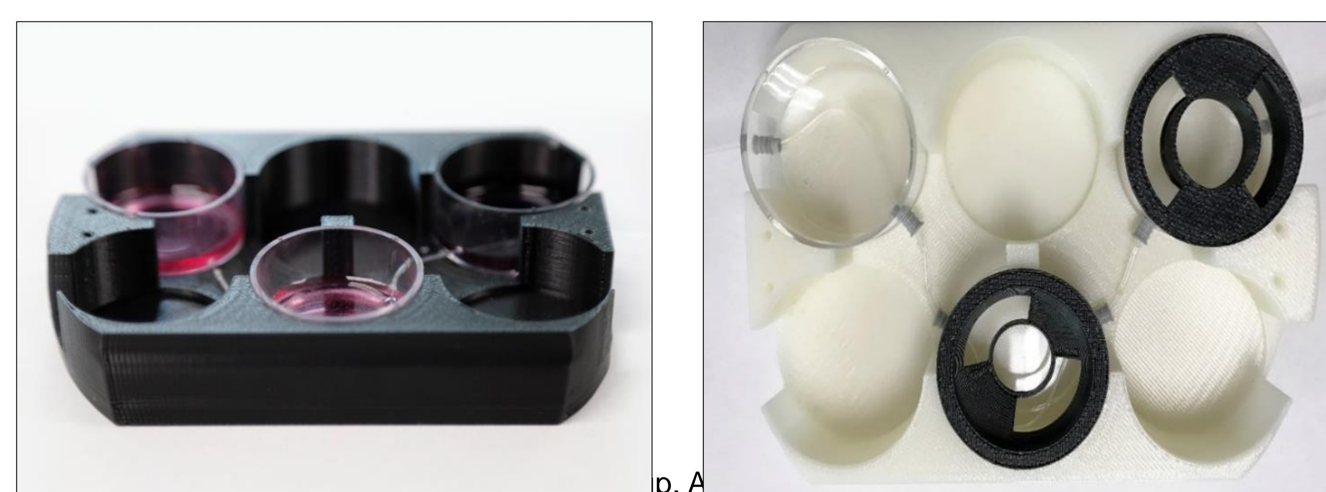
ABSTRACT

In vitro methods capable of describing systemic effects of chemicals require use of multiple tissue types connected with a common perfusate. This arrangement allows integration of absorption, metabolism and toxicity data over extended times *in vitro* and provides a novel, animal-free tool for chemical, cosmetic, and pharmaceutical testing. In order to test this, a study on the uptake and distribution of acetaminophen (APAP) in a human dynamic multi-organ plate (HuDMOP™) with three tissue surrogates arranged in series: first absorption across a human 3D intestine (EpiIntestinal, Mattek Corp), then on to a liver surrogate with human primary hepatocytes in sandwich culture and then to a kidney preparation (human renal proximal tubule cells) was developed. A common perfusate with human albumin connected the three compartments. APAP was placed on the apical side of the intestinal surrogate at 0 and 24 hr. Samples were collected from all three compartments over time and analyzed for APAP by LC/MS/MS and cytotoxicity by LDH leakage. The APAP in the uptake reservoir peaked to 60.7μM at around 4 hours with a total uptake of 72% of the applied dose entering the first reservoir. A simple PK model was developed to describe the three cellular platforms and their physical arrangement. Mass balance equations were fit to experimental data to estimate uptake and transport characteristics. The inter-chamber flow rates and fitted experimental absorption rate constant, 0.79/hr, were consistent with a C_{max} of 62.0 μM and time of maximum concentration between 3 and 4 hr in the intestine compartment. With the current platform flow rates, much lower concentrations were present in the subsequent two compartments (liver and kidney) with maximum observed concentrations of 4.5 and 2.5 versus 3.1 and 0.9 uM predicted. The interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to expected in-life behavior of various chemicals.

METHODS – IN VITRO STUDIES



Preparation of Plates. Hμ-DMOP™ custom designed plates (Figure 1) were used and equipped with a simulated blood system. The simulated vascular system consisted of tubing connected to a semipermeable membrane. The section of semipermeable membrane was 3 cm in length. The tubing was custom fit into the plate, such that only the semi permeable membrane was in contact with each organ compartment. A perfusion rate of 5 μl/min was used in each experiment.

Cell Culture

Intestinal Compartment. The EpiIntestinal™ 3D human tissue from MatTek, Corp. was used for the intestine chamber. Tissues were cultured under standard conditions on transwell inserts. Tight junctions were assessed by transepithelial electrical resistance (TEER). The EpiIntestinal™ tissues were placed into the Hμ-DMOP™ plates (Figure 1) and connected to the liver compartment via simulated blood system (Figure X).

Liver Compartment. The liver compartment was simulated with Transporter Certified™ human primary hepatocytes from BIOIVT in sandwich culture. The cells were added to the Hμ-DMOP™ cup in culture media at a density of 500,000 cells/well and incubated at 37oC, 5% CO2 for 48 hr prior to beginning the experiments.

Kidney Compartment: To simulate a kidney human renal proximal tubule cells from Lonza were used. The cells were added to the Hμ-DMOP cup in culture media at a density of 1.1 x 106 cells/well and incubated at 37oC, 5% CO2 for ~5 days prior to beginning the experiments.

Dosing Regimen: After equilibration, the test material was added to the apical side of the intestinal chamber to simulate an oral exposure at time 0 and 24 hr. For acetaminophen (APAP) the dose was 100 μL from a 2500 μM stock, while for cycloheximide (CyHex) the dose applied was 100 μL from a 100 μM stock.

Analytical Procedures. APAP and CyHex were measured by LC-MS/MS. Standard curves and QC samples were prepared in PBS and compared to standard curves and QC samples in media with and without serum.

METHODS – MODEL DEVELOPMENTS

In order to better understand the in vitro system a pharmacokinetic model was developed. Absorption across the HIE into the intestine compartment was simulated as a first-order absorption process. Intestine, kidney, and liver compartments were described by a volume, flow rate, and clearance rate. The final collection compartment was simulated as a sink accumulating any compound not retained or removed by the previous compartments. Compartment volumes were from 2.5 to 3 mL, and the flow through the system was 5 uL/min. The compartments are assumed to be well-mixed and in equilibrium with the semipermeable tubing perfusing the system. APAP and cycloheximide experiments were used to fit the absorption and clearance rates:

1. Concentration in compartment 1 (Intestine) → First-order absorption rate constant (ka)
2. Concentration in compartment 2 (Liver) → Michaelis-Menten metabolism (Vmax and Km), Partition between media and tissue (PA)
3. Concentration in compartment 3 (Kidney) → First order elimination constant in the kidney (ku)

The model outputs for all compartments and the model output in the collection were exported and plotted using Microsoft Excel.

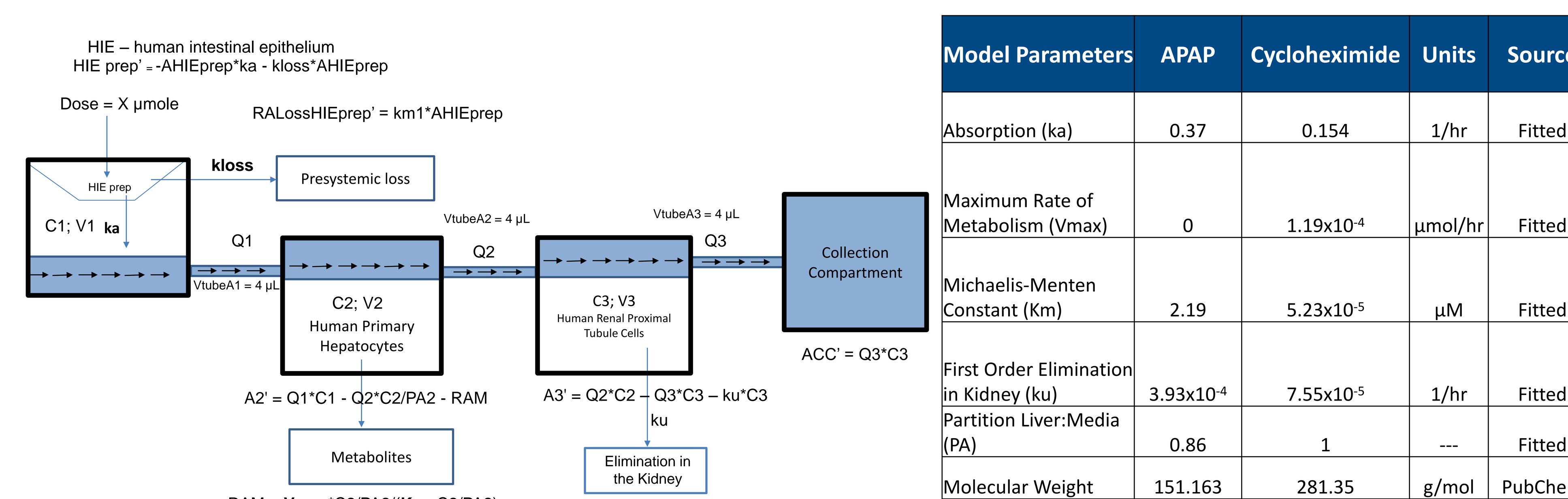


Figure 2: Schematic of the Model with Equation

Model Parameters	APAP	Cycloheximide	Units	Source
Absorption (ka)	0.37	0.154	1/hr	Fitted
Maximum Rate of Metabolism (Vmax)	0	1.19×10^{-4}	μmol/hr	Fitted
Michaelis-Menten Constant (Km)	2.19	5.23×10^{-5}	μM	Fitted
First Order Elimination in Kidney (ku)	3.93×10^{-4}	7.55×10^{-5}	1/hr	Fitted
Partition Liver:Media (PA)	0.86	1	---	Fitted
Molecular Weight	151.163	281.35	g/mol	PubChem

Table 1: Chemical parameters fitted or used in the model.

RESULTS

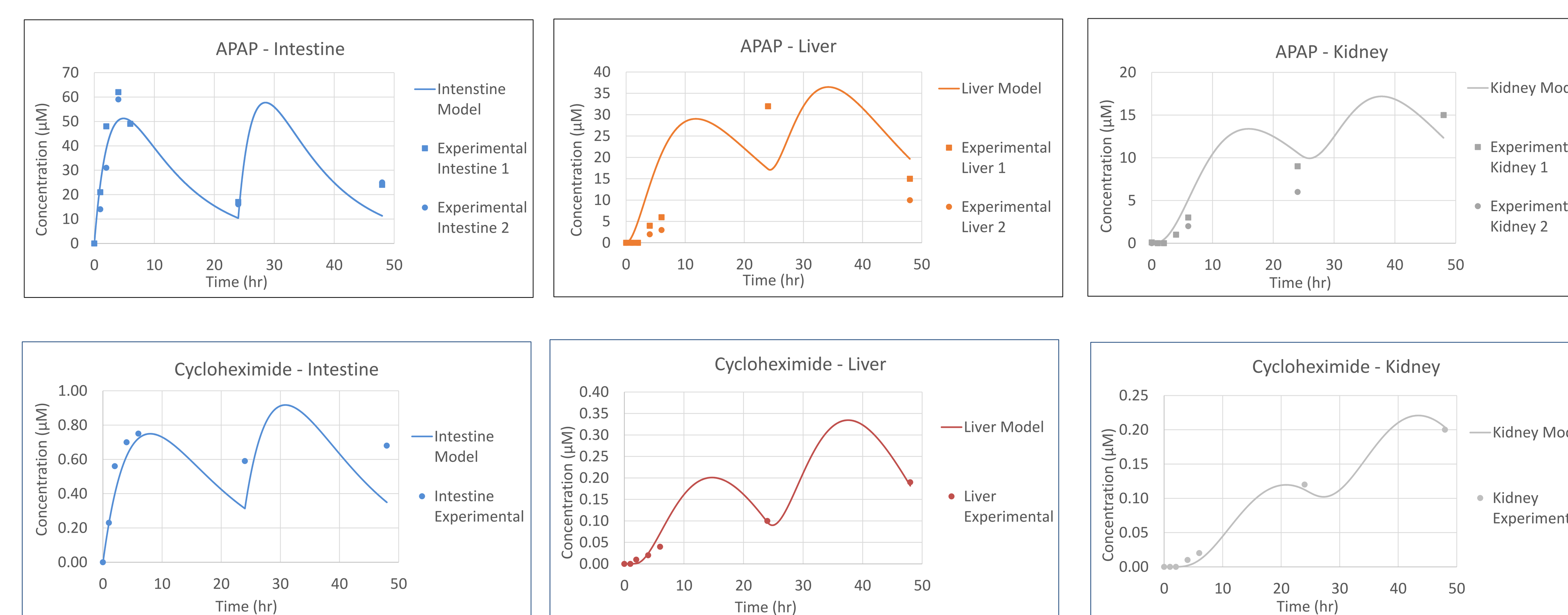


Figure 3: Concentration in the first three compartments fitted to the data for APAP (top) and Cycloheximide (bottom)

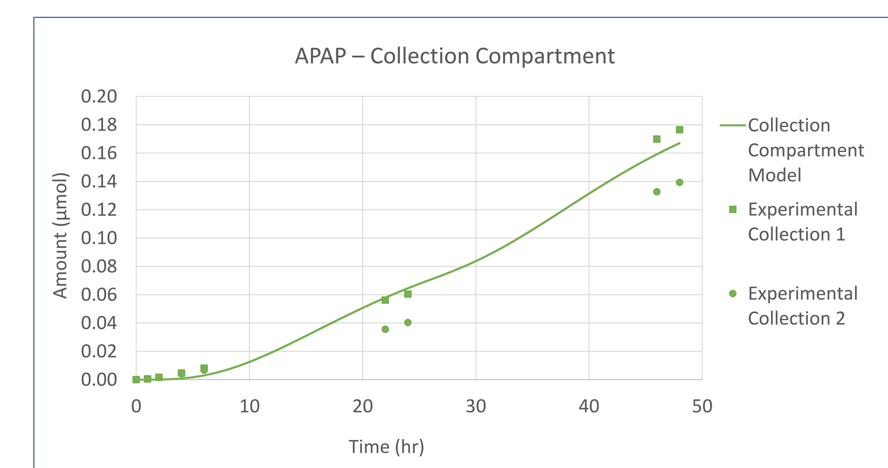


Figure 4: Amount collected the collection compartment for APAP.

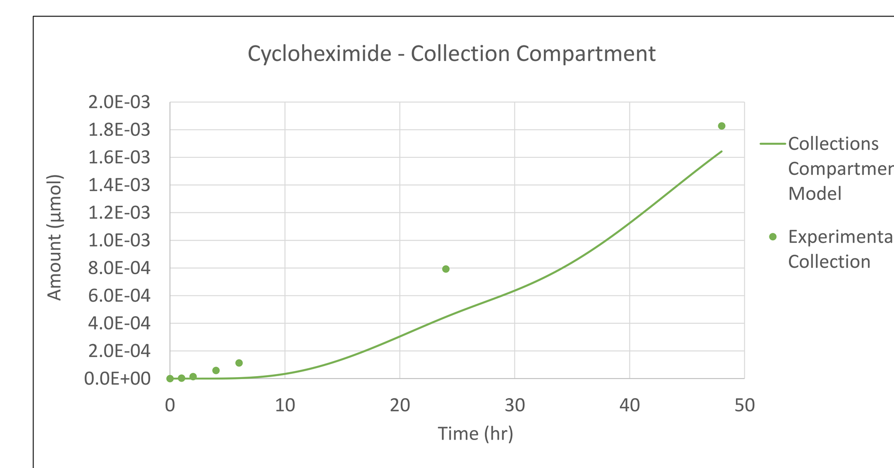


Figure 5: Amount collected the collection compartment for cycloheximide

CONCLUSIONS

In vitro methods capable of describing systemic effects of chemicals require use of multiple tissue types connected with a common perfusate. This arrangement allows integration of absorption, metabolism and toxicity data over extended times *in vitro* and provides a novel, animal-free tool for chemical, cosmetic, and pharmaceutical testing. Integration with computational modeling is key to transitioning these unique data to *in vivo* application, and the interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to expected in-life behavior of various chemicals.

The current data provide a basis for in silico modeling of the *in vitro* system. The computational model predictions represent the data well, though there appears to be more abrupt appearance in the final perfusate collection for both chemicals, and the cause is under investigation. Current thoughts for computational probing include the possibility for nonspecific binding to plastic in the system, and rate-limiting uptake into and out of the semipermeable membrane perfusing the compartments.

The computational modeling approach is increasingly used as a way of mathematically representing, interpreting, and extrapolating experimental data from in vitro (and in vivo) systems. The development and model-based interrogation of such novel in vitro systems to better inform chemical kinetics and toxicity in future testing of chemicals holds significant promise for reducing animal use, time, and money, with the ultimate goal of predicting human kinetics and toxicity without animal testing.

FUTURE DIRECTIONS

The ultimate goal of this partnership of a novel *in vitro* system and computational modeling is to predict the human health effects of chemical exposure. Through iterative computational and laboratory innovation, we can achieve this goal.

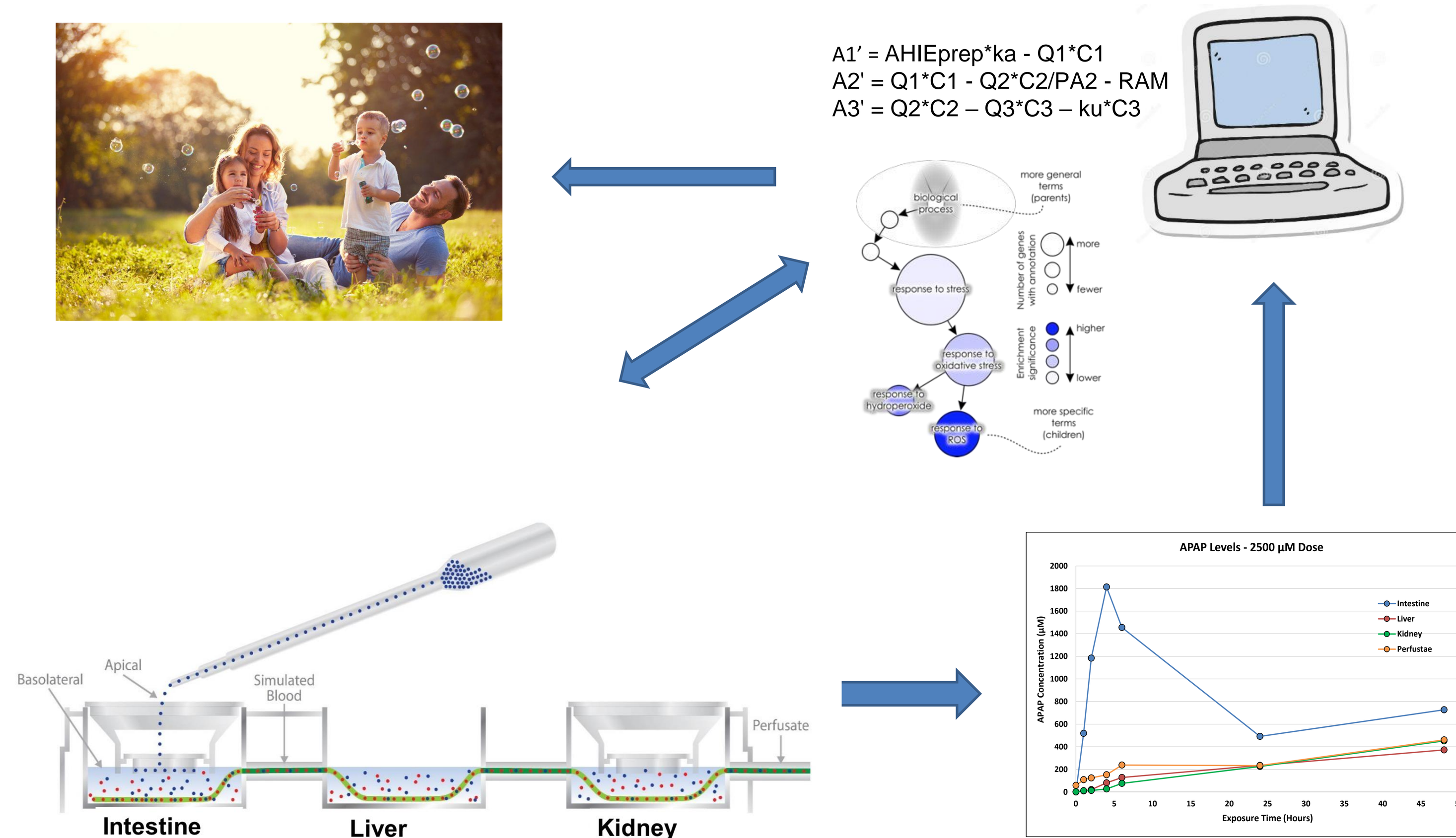


Figure 6: A flow chart representation of iterative model-experimentation process.

Computational and experimental improvements continue dynamically. The integration and flexibility of the *in vitro* model system and the computational model allows unique opportunities for discerning the systemic response to chemical exposure. Individual tissue compartments can be isolated for focused measurement of key parameters, such as absorption and hepatic clearance. Multiple tissue compartments can be integrated, including recirculation to simulate a closed in vivo system.

Toxicity metrics, such as transcriptomic response data, can also be collected from the *in vitro* system. Computational toxicology models may be employed to analyze these data, and possibly extrapolate to whole human responses.

The ability to dynamically integrate the computational modeling and experimental data gathering capability gives this partnership a unique advantage in regard to the development of new approach methodologies (NAMs) in support of human health safety assessment without animal testing.