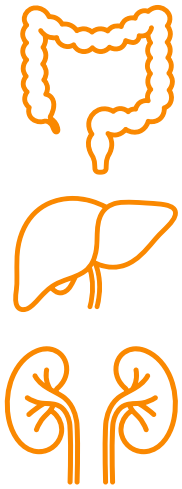

Case Study:

An Integrated Intestine-Liver-Kidney Microphysiological System (MPS) Model

Introduction

Toxicokinetics plays a pivotal role in chemical testing and drug development, yet traditional *in vivo* studies are costly in time and resources, often with poor translational relevance. Most microphysiological systems (MPS) contain a single organ or tissue type, limiting the opportunity for evaluating multi-organ responses, such as metabolism-mediated detoxification in one tissue that may affect another. Therefore, tissues in a multi-organ MPS should respond in a more human *in vivo*-like manner.

More relevant toxicokinetic and organ toxicity data for screening and risk assessment

LifeNet Health has developed a multi-organ MPS (Figure 1a) that uses low binding plastics, has isolated but connected organs, is connected with a simulated vascular flow, is adaptable to multiple configurations with different tissues and cells (i.e., single to multiple organs), and is accessible for sampling (e.g., media, cells) for bioanalysis (e.g., for parent/ metabolite and biomarkers) or to visualise (i.e., pathology).

REAL ADVANTAGE

The LifeNet Health MPS links combinations of two, three, or four organs generating toxicokinetic and organ toxicity data for screening and risk assessment. The multi-organ system differentiates LifeNet Health from its competitors with organ combinations including “Intestine + Liver + Kidney”, “Lung + Liver + Kidney”, and “Intestine + Liver + Thyroid”. LifeNet Health expert scientists design custom organ configurations and study designs with sample and data analysis and reporting based on client needs. Test systems are obtained

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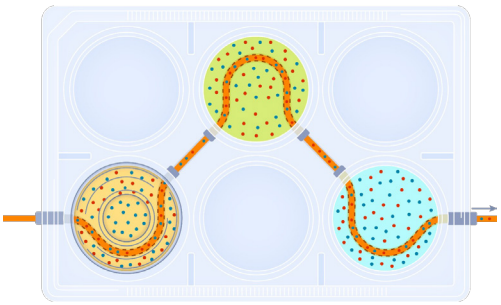
Figure 1.

Three-Organ MPS Model



A. *Wells with Continuous Flow Connection*

B. *3 Organs Connected by Simulated Blood*

Table 1.

Analyte(s)	Kit
GSH and GSSG[1]	GSH/ GSSG-Glo Assay
EpiIntestinal™ Viability	MTT Assay
Liver Cytotoxicity	CellTiter Glo® Luminescent Cell Viability Assay
Renal Cytotoxicity	b-N-acetylglucosaminidase (NAG) Lysosomal Assay
	KIM-12 ELISA
LDH[3] Release	CytoTox-ONE™ Homogeneous Membrane Integrity Assay

[1]Oxidized glutathione, [2]Kidney injury marker-1, [3]Lactate dehydrogenase

from LifeNet Health or commercially available vendors. Turnaround time depends on complexity of study design, availability of cells and tissues, availability of bioanalytical methods and level of validation and development of any new biomarker assays required. The data generated can be used in a Next Generation Risk Assessment (NGRA), defined as "an exposure-led, hypothesis driven risk assessment approach that integrates *in silico*, *in chemico* and *in vitro* approaches".

This application note highlights the "Intestine-Liver-Kidney" MPS model (Figure 1b) with acrylamide as a case study. The intestine is the primary route of exposure to food, drugs, chemicals, and environmental contaminants. The intestine is also a secondary route of exposure to crop protection products and cosmetics (after dermal and inhalational routes). The liver is the primary organ responsible for drug metabolism. The kidney is the primary organ responsible for chemical and metabolite excretion. These three organs also contribute to metabolism and excretion and generate cytokines which may impact other organs.

The details of the work were previously published (McKim et al., 2024).

Many reactive chemicals, including acrylamide, are formed during high temperature cooking. Reliable non-live animal test methods are needed for their rapid and cost-effective toxicological evaluation. Following ingestion, acrylamide can be metabolised and acrylamide and its primary reactive metabolite, glycidamide, can bind to glutathione (GSH) directly or via glutathione S-transferase activity. The key toxicological effects of acrylamide are genotoxicity, carcinogenicity, neurotoxicity, and effects on male reproductive parameters. Acrylamide-mediated

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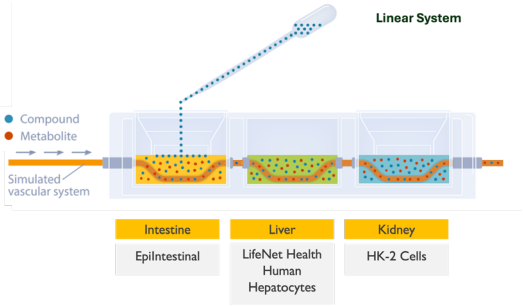
Figure 2.

Dosing of Acrylamide to EpiIntestinal™ in the MPS Connected by the Simulated Blood System



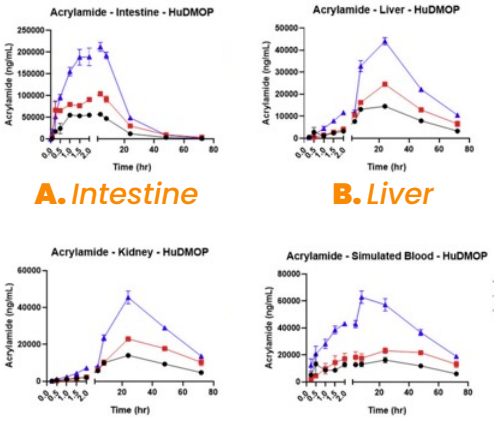
genotoxicity and carcinogenicity are primarily due to glycidamide. Therefore, acrylamide toxicity is of interest to human health.

Methods

A semipermeable dialysis membrane (effective 7-8 kDa molecular weight cut off) allowing small molecules to be exchanged by osmotic diffusion is used as simulated vasculature. Membrane tubing is custom fit into the plate with the membrane in contact with each organ compartment. Simulated blood (PBS and human serum albumin) was perfused through the tubing using a syringe pump controller and syringe pump. Non-specific binding was confirmed with acrylamide recovery of 87%.

Figure 3.

Concentration vs. Time Curves for Acrylamide Over a 72-hour Exposure Period



A. *Intestine* **B.** *Liver*

C. *Kidney* **D.** *Simulated Blood*

The intestinal compartment, EpiIntestinal™ (Mattek), was cultured on Transwell® inserts. Tight junctions were assessed by TEER (EVOM2 Epithelial Voltammeter and STX2 probe) alongside lucifer yellow exclusion. This was linked to the liver compartment containing fully characterized Primary Human Hepatocytes (LifeNet Health LifeSciences) cultured on collagen coated wells. This was linked to the kidney compartment, containing HK-2 normal human adult male kidney cell line (ATCC).

After equilibration, acrylamide (0.3, 0.5, and 1.0 mg) was applied to the apical side of the intestinal chamber to simulate an oral systemic exposure (Figure 2). The dose solution concentrations were chosen to result in a low oral dose exposure akin to that described following oral acrylamide ingestion by rats, but higher than that administered orally to humans, and on a par with the predicted daily consumer exposure. To monitor acrylamide movement, timed samples were collected from compartments.

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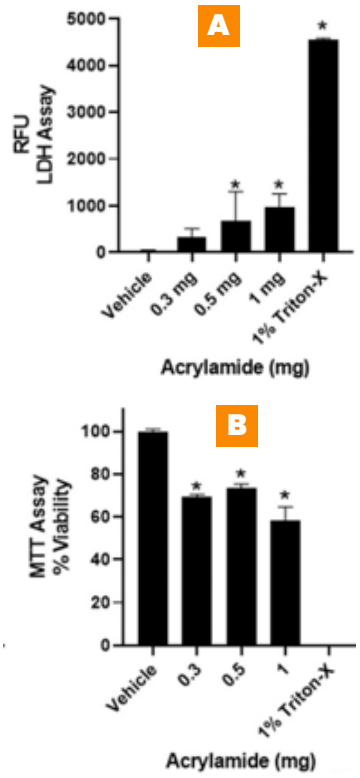
Figure 4.

Effect of Acrylamide on Intestinal Health



A. *Cytotoxicity assessed by LDH leakage*

B. *Cytotoxicity assessed by loss of mitochondrial function (MTT). Statistical significance, *p = <0.05*

Sample Analysis

An LC-MS/MS analytical method was developed for acrylamide. Standard curves and QC samples were prepared in PBS and compared to those in media. Samples were analyzed using this fit-for-purpose validated analytical method. A summary of sample analysis is provided in Table 1 and details in McKim et al., (2024).

Results

Acrylamide was rapidly absorbed through EpiIntestinal® within the first 30 minutes of exposure followed by a peak absorption and elimination phases (Figure 3A). This was also observed in the liver (Figure 3B), kidney (Figure 3C) and simulated blood (Figure 3D) with dose responses observed in all compartments. Kinetic parameters were Cmax (0.9 mg), time to Cmax (1.5-2 h), and T1/2 (15 h). Acrylamide dose recovery was ca 90%.

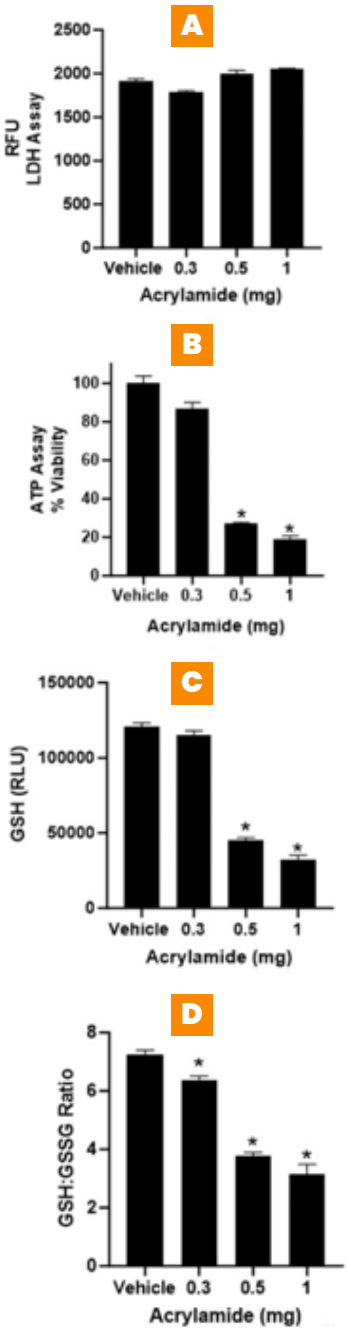
There was a small reduction in intestinal viability based on LDH release (Figure 4A). However, cell viability losses of >30% (MTT) were observed in mitochondrial function (Figure 4B) indicating a direct effect on mitochondria by acrylamide.

Acrylamide resulted in no effect on liver membrane integrity (LDH; Figure 5A) but caused a marked loss in liver cellular ATP (Figure 5B). GSH levels were reduced (Figure 5C) with accompanying increase in GSSG formation (Figure 5D), indicating direct conjugation of GSH by acrylamide and an increased oxidative environment in liver cells.

Acrylamide was toxic to HK-2 kidney cells as demonstrated by an increase in NAG (Figure 6A) and KIM-1 (Figure 6B) leakage.

Figure 5.

Effect of Acrylamide on Intestinal Health



A. *LDH leakage.*

B. *Intracellular ATP.*

C. *Intracellular GSH.*

D. *Intracellular GSSG. Statistical significance, *p = <0.05.*

Discussion

Initial uptake of Acrylate across EpiIntestinal® was dose-dependent and rapid with clear absorption and elimination phases. This was also observed for liver, kidney, and simulated blood. In animal and human studies, the fraction of the dose absorbed drives most pharmacokinetic work. However, when using *in vitro* systems, chamber dilution effects must be accounted for in the organ exposure and mass balance calculation in the final parameterization of the system. *In vitro* kinetic profiles represent Acrylamide uptake, dilution, distribution, metabolism, and elimination. This data could be used to develop models enabling extrapolation from the *in vitro* situation to *in vivo* animal or human exposure and hazard identification. *In vitro* distribution in organs was related to organ toxicity.

Acrylamide resulted in intestinal toxicity as evidenced by a reduction in mitochondrial function consistent with acrylamide-induced intestinal stress, inflammation, and oxidative stress observed in mice. Acrylamide induced hepatotoxicity was mediated through oxidative stress where the primary mechanism is disruption of the GSH homeostatic system. Acrylamide and glycidamide bind to GSH and damages mitochondria, which also contributes to oxidative stress via the release of ROS. Acrylamide and glycidamide can activate NF-kB, increasing inflammatory cytokines including IL-6, IL1β, and TNFβ. Acrylamide caused intracellular ATP and GSH depletion and increased GSSG, indicating direct effects on mitochondrial function, direct chelation of GSH, and increased oxidative stress. Loss of ATP and GSH without a significant loss in membrane integrity (LDH) may be observed when evaluating chemicals affecting mitochondrial function.

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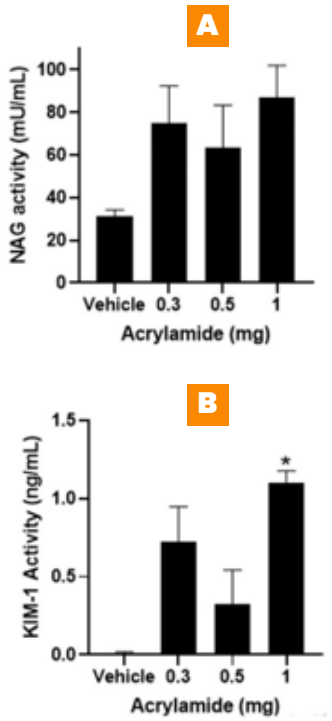
Figure 6.

The effect of Acrylamide on Kidney (HK-2) Health



***A.** NAG activity.*

***B.** KIM-1 levels. Statistical significance, *p = <0.05.*

Renal toxicity was confirmed by increased release of NAG and KIM-1. Acrylamide caused kidney damage as evidenced by increased blood urea nitrogen and creatinine in rats.

Conclusions

The novel *in vitro* human "Intestine-Liver-Kidney" MPS platform provided toxicokinetic, organ toxicity and mechanisms of toxicity consistent with *in vivo* published literature at doses consistent with rodent and predicted human exposures. There was a rapid uptake of acrylamide from the intestine with intestine, liver and kidney toxicity confirmed. Kinetic parameters and ADME endpoints data could be used to develop PBPK models to assist extrapolation from *in vitro* to *in vivo* exposure and hazard identification for use in NGRA. Therefore, this multi-organ MPS platform can be used in support of chemical testing and drug development.

Reference

McKim JM, Austin DJ, Sprando R, Hermansky S, Mattes W, and Fitzpatrick S (2024). Developing kinetic and organ toxicity data with a novel *in vitro* human-based multiple organ MPS: Acrylamide as a case study. Applied *In Vitro* Toxicology 10(2): 33-42. https://doi.org/10.1089/aivt.2024.0021.

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