Evaluating Acrylamide Toxicokinetics in an *In Vitro* Integrated Organ Platform

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Abstract
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ABSTRACT

The formation of Acrylamide (AA) in certain food types can occur when cooking at high temperatures. The formation of AA as well as other chemicals during high temperature food preparation and the subsequent formation of reactive metabolites have prompted regulatory agencies globally to investigate risk to human health. Given the large number of reactive chemicals formed during high temperature cooking and the need to develop human toxicokinetic and organ toxicity data for these compounds, alternative methods may provide a faster and less expensive means of performing initial assessments. Cell and tissue models today are more sophisticated in both their morphology and function. The use of these enhanced cell and tissue models in combination with new *in vitro* platforms is beginning to show promise for the eventual replacement of animal testing. The aim of this study was to test the ability of a novel Human Dynamic Multiple Organ Plate to provide data for key ADME parameters and organ toxicity using AA as the model compound. A three compartment (Intestine-Liver-Kidney) circuit was established. The intestine was EpiIntestinal from MatTek, Corp, human hepatocytes were from LifeNet Health Life Sciences, and Kidney cells (HK-2) were from ATCC. Each organ compartment was linked by a simulated blood system. The tubing of the blood system inside the organ compartment consisted of a semipermeable membrane. The movement of the test compound and its metabolites from the point of application to the other organ compartments was by osmotic diffusion into the simulated blood system. The simulated blood circulation was achieved with a micro syringe pump. Each organ compartment contained culture medium optimal for that organ's growth and there was no net change in compartment fluid volumes.

To begin the study, aliquots of an AA stock (300 mg/mL) were diluted with medium to provide final exposure concentrations of 3, 5, and 10 mg in a final volume of 100 µL. This was then applied to the apical surface of the MatTek EpiIntestinal model. Samples from each organ compartment and from simulated blood were collected at 0, 5, 15, 30, 60, 90, 120, 240, 480, 1440 (24 hr), 2880 (48hr), and 4320 min (72 hr). AA movement was measured using LC/MS/MS. At 72 hr samples were collected for determining cytotoxicity. AA was detectable in the intestinal compartment at 30 min, reaching a maximum at 1.5-2 hr. Values returned to baseline after 24-48 hr. In liver and kidney, AA was detected at 1-1.5 hr reaching a maximum after 20-24 hr. AA concentrations were dose and time dependent in each compartment. After 72 hr of exposure to 3 mg AA, intestinal viability was 70%, while the 10 mg dose resulted in a viability less than 60%. Liver ATP and GSH decreased in a dose-dependent manner after 72hr. In kidney, NAG and KIM-1 release also followed a dose-dependent pattern. Analysis of kinetic data enabled key ADME parameters (Cmax, AUC, and T1/2) to be estimated. In conclusion, the novel Human Dynamic Multiple Organ Plate system predicted AA liver and kidney toxicity and provided key ADME data points. These findings indicate the system can be used as a rapid response model for ADME and toxicity, as well as a model to explore metabolites and mechanisms of adverse effects.

INTRODUCTION

Microphysiological Systems (MPS) are *in vitro* models that comprise multiple organ models (cells, 3D tissues, spheroids) interconnected by simulated blood flow. In recent years several iterations of MPS systems have appeared each with unique advantages and also with limitations. The ability to interconnect physiologically relevant organ systems offers the possibility of using *in vitro* testing to predict *in vivo* responses. Pharmacokinetic data combined with metabolism and organ specific safety data would enable the development of physiologically based pharmacokinetic models (PBPK) which in turn would provide much stronger and more reliable *in vitro* to *in vivo* extrapolations. The aim of this study was to evaluate a novel human dynamic integrated organ platform (Intestine-Liver-Kidney) with chemicals of interest to the US FDA by studying kinetic movement, metabolism, and cytotoxicity.

MATERIALS & METHODS

Preparation of Plates. A three-organ plate system (Intestine-Liver-Kidney circuit) (Figure 1) was used and equipped with a simulated blood system that 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 dialysis membrane was in contact with each organ compartment. A perfusion rate of 5 µl/min was used in each experiment.

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 plate systems (Figure 1) and connected to the liver compartment via simulated blood system (Figure 2).

Liver Compartment. The liver compartment consisted of fully characterized human primary hepatocytes (LifeNet Health) cultured on a collagen coated wells. Cells were seeded in the system culture cups in culture media at a density of 500,000 cells/well and incubated at 37°C, 5% CO_2 for 48 hr prior to beginning the experiments.

Kidney Compartment: The kidney compartment consisted of a human renal cell line (HK-2) from ATCC. The cells were added to the culture cup in culture media at a density of 1.1 x 10^6 cells/well and incubated at 37°C, 5% CO_2 for 24 hr 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. Acrylamide was administered as an applied doses of (0.3, 0.5, and 1.0 mg total mass) in a volume of 100 µL from stock solutions of 3, 5 and 10 mg/mL.

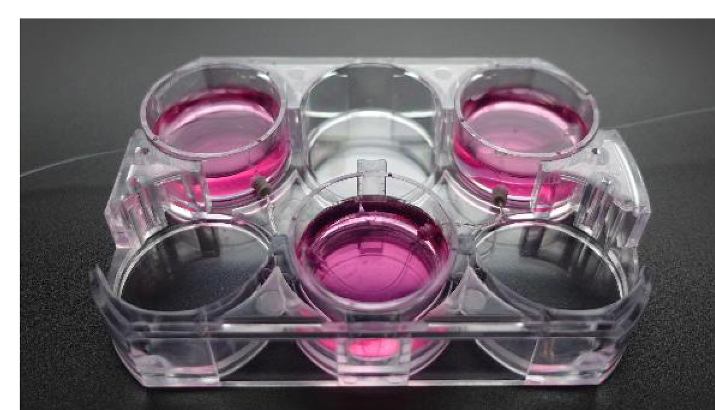
Analytical Procedures. Acrylamide was monitored by LC-MS/MS. Standard curves and QC samples were prepared in PBS and compared to standard curves and QC samples in media with and without serum.

RESULTS

Figure 1. LifeNet Health's Novel Human Dynamic Multiple Organ System

Human tissues representative of specific organs cultured in a custom designed mesoscale plate. Organs are connected by a simulated vascular system that allows small molecules to enter and leave via passive diffusion through a semi-permeable membrane.

Syringe pump controller and Syringe pump. This system controls the flow rate of the simulated blood. Syringe sizes of 1.0, 5.0, and 10 mL have been used successfully.



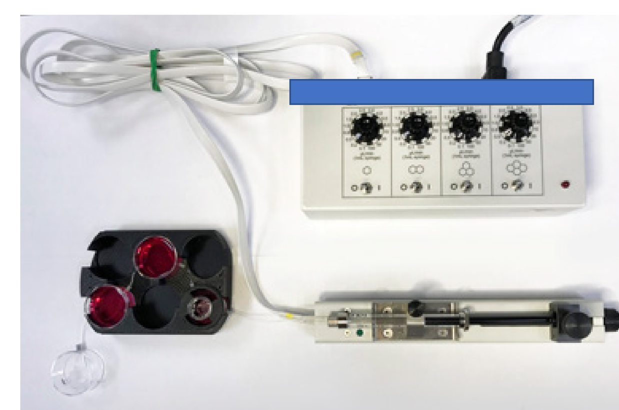


Figure 2. Human Dynamic Multiple Organ System with three organs connected by a simulated blood system.

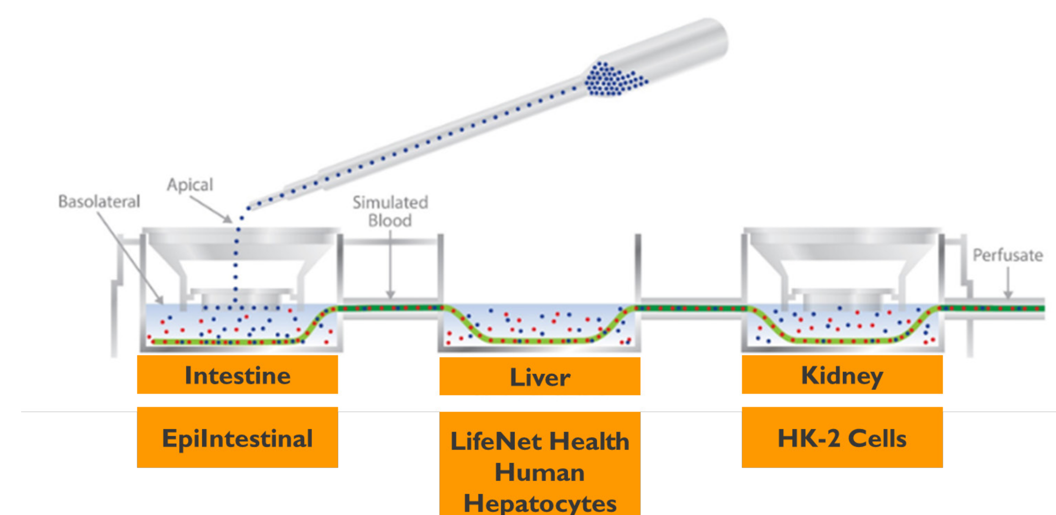
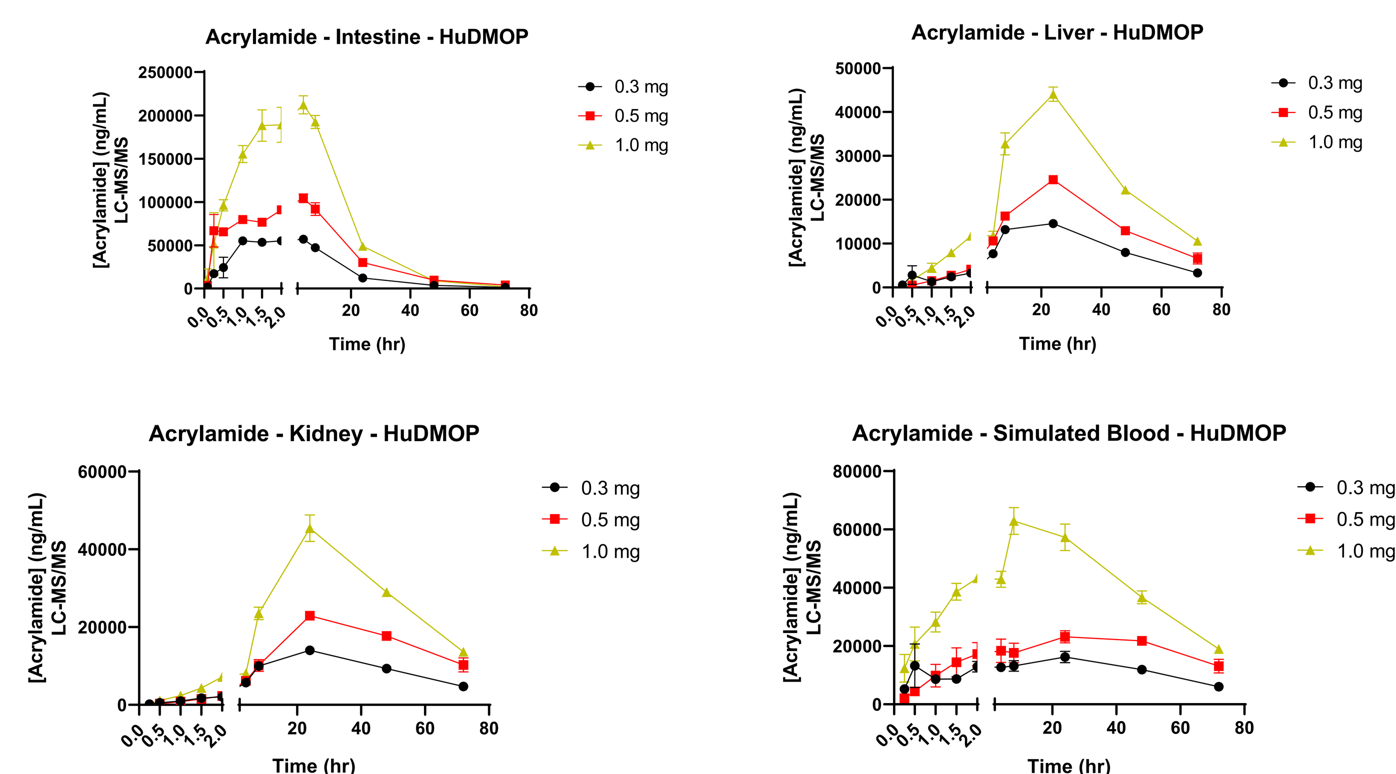
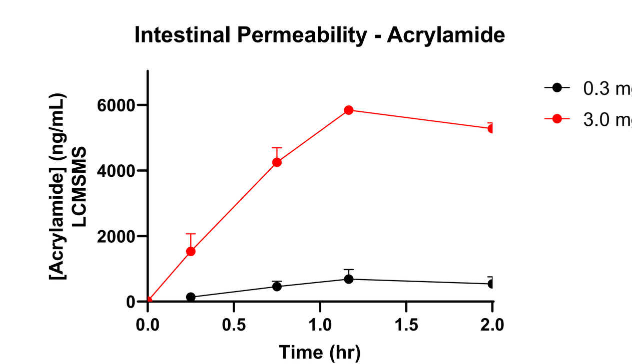


Figure 3. Acrylamide Kinetic Data for Intestine, Liver, and Kidney Chambers.



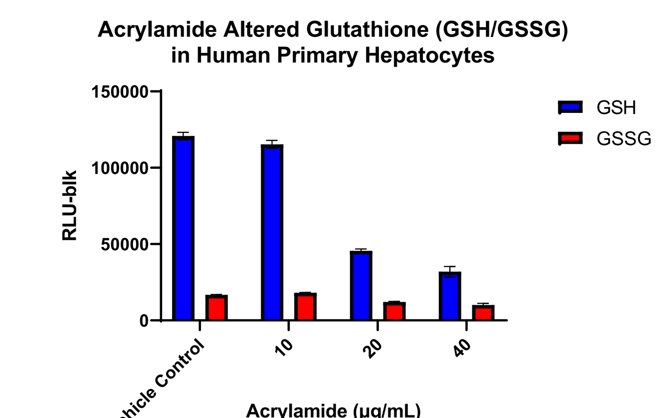
To monitor the movement of Acrylamide in the Integrated Organ System. Samples were collected from each organ compartment (intestine, liver, kidney, simulated blood) at the time points indicated over a 72 hr time period. The samples were analyzed by LC/MS/MS for Acrylamide. The intestinal basolateral compartment was used as systemic exposure. Bioavailability, Cmax (0.9 mg), time to maximum (1.5-2 hr), AUC values could be determined and a T1/2 of 15 hr estimated. The amount of the dose recovered was approximately 90%. Acrylamide was rapidly and efficiently taken up across the intestinal epithelium. Values represent the mean ±SEM of N=3 circuits.

Figure 4. Acrylamide Intestinal Permeability



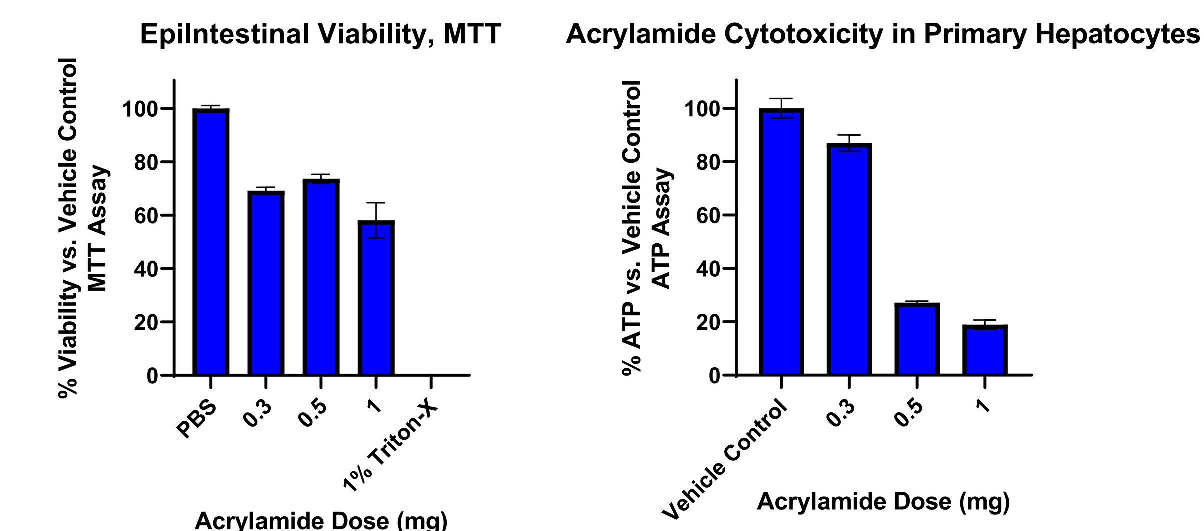
A human EpiIntestinal model (MatTek Corp) was used to determine permeability after administration of 0.3 and 3.0 mg of Acrylamide. This was done as a separate organ system. Values represent the mean± SEM of N=3 wells.

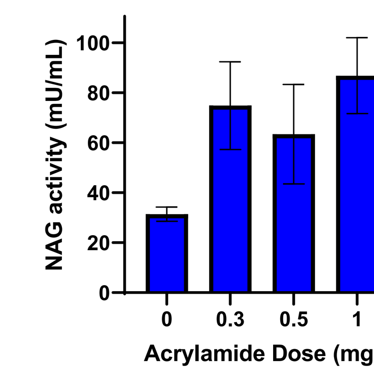
Figure 5. Effects of Acrylamide on Glutathione Levels

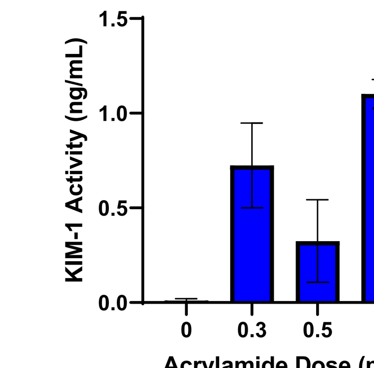


Low acrylamide exposure concentrations were tested in the liver chamber. There was a significant loss in reduced glutathione (GSH) while the oxidized form (GSSG) remained relatively stable. These data indicate direct reactivity.

Figure 6. Effects of Acrylamide in the Integrated Organ System on Intestine, Liver, and Kidney Cell Health







Organ specific toxicity was assessed in the integrated organ system described. After 72 hr of exposure to 0.3, 0.5, and 1.0 mg of Acrylamide, cytotoxicity was monitored in each organ. Intestinal viability ranged from 70%, to 60% as observed with an MTT reduction assay. In the liver chamber there was a pronounced dose-dependent reduction in ATP levels and in the kidney chamber there was an increase in NAG and kidney injury molecule (KIM-1) release. Values represent the mean ± SEM of N=3 for each organ.

CONCLUSIONS

- The novel *in vitro* human dynamic integrated organ platform (Intestine-Liver-Kidney) described provided a pharmacokinetic data and cytotoxicity data that is in close agreement with those reported in the published literature.
- There was rapid and efficient uptake of Acrylamide from the intestine.
- Organ specific toxicity (liver and kidney) was identified.
- The novel system demonstrated an ability to determine kinetic parameters, and some ADME endpoints. These findings indicate that the system described could be used to develop PBPK models to assist *in vitro* to *in vivo* extrapolation.

REFERENCES / ACKNOWLEDGEMENTS

1. Fuhr, U., et al. (2006) Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide. Cancer Epid Biomarkers 15, 266-271.
2. Wikswo,J. (2014) The relevance and potential roles of microphysiological systems in biology and medicine. Exp Biol Med 239, 1061-1072.