# Microphysiological Systems as Predictive Tools for Nephrotoxicants: A Comparison Across Four Models

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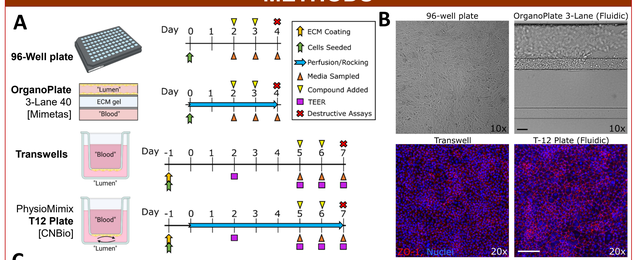
## BACKGROUND AND RATIONALE

The kidneys are crucial for eliminating drugs and chemicals from the body, with renal epithelial cells being particularly vulnerable to damage by xenobiotics and their metabolites. Complex in vitro models of the kidney, including Microphysiological Systems (MPS), can be used to faithfully predict clinical response to toxicants. However, their high cost and low throughput often limit their application in broad contexts of use, and constant technological development complicates the process of model selection. This study compared effects of 16 compounds in concentration response design using the TERT1-OAT1 renal proximal tubule epithelial cell (RPTEC) line cultured on four platforms: standard 96-well plates, static and fluidic Transwells (PhysioMimix T12), and OrganoPlate® 3-lane 40. Cytotoxicity was monitored via LDH leakage activity and transepithelial electrical resistance (TEER, in Transwell-based models). Samples were collected after 2, 24, and 48 hours of exposure, and drug transport was measured by LC-MS/MS. Additionally, transcriptomic analysis was performed using the TempO-seq® S1500+ assay suite.

## METHODS

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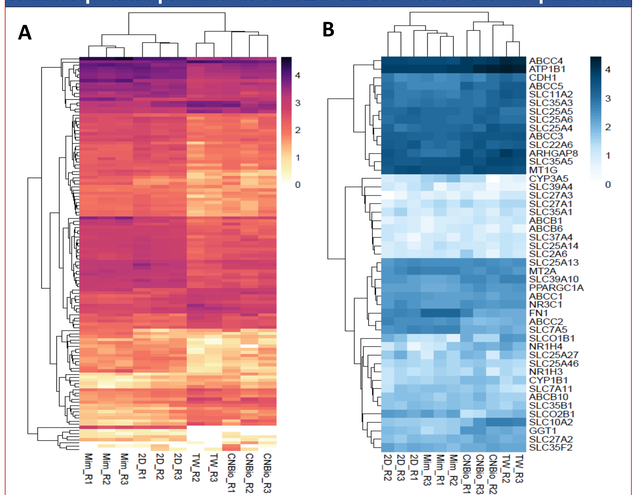
| Chemical | Cmax (uM) | Concentrations (uM) | Assignment |
|---|---|---|---|
| Benzophenone-4 | 2.08 | 1, 10, 100 | Negative |
| Buspirone | 0.007 | 1, 10, 100 | Negative |
| Streptomycin | 74.9 | 100, 1000, 10000 | Negative |
| Gentamicin | 33 | 100, 1000, 10000 | Positive |
| Tobramycin | 40.4 | 100, 1000, 10000 | Positive |
| Tenofovir (TFV) | 0.58 | 1, 10, 100 | Negative |
| Tenofovir (TDF) | 0.67 | 1, 10, 100 | Positive |
| Polymyxin B | 7.68 | 1, 10, 100 | Positive |
| Rifampin | 9.7 | 1, 10, 100 | Positive |
| Nefazodone | 1.6 | 1, 10, 100 | Negative |
| Chlorpromazine | 0.25 | 1, 10, 100 | Negative |
| Indomethacin | 8.4 | 1, 10, 100 | Positive |
| Ibuprofen | 97 | 1, 10, 100 | Negative |
| Sulforaphane | 2.2 | 1, 10, 100 | Negative |
| PFOS | 0.01 | 1, 10, 100 | Negative |
| PFHxA | 0.01 | 1, 10, 100 | Negative |

Table 1. Compounds tested in RPTEC/TERT1-OAT1 across platforms

**(A)** Overview of MPS platforms and experimental timelines for testing and treatment of RPTEC/TERT1-OAT1 cells. **(B)** Morphology of RPTECs cultured across different platforms. Cells are grown as a monolayer in 96-well plates and Transwells (both static and fluidic conditions enabled by the PhysioMimix T12 platform). In the OrganoPlate 3-Lane 40, cells are cultured against a gel matrix. **(C)** Tested chemicals and their respective concentrations are listed in the table. Human $C_{max}$ values and binary classifications ("positive" or "negative") are reported based on adverse event data reporting in PharmaPendium.

## Transcriptomic profiles of RPTEC/TERT1-OAT1 across platforms

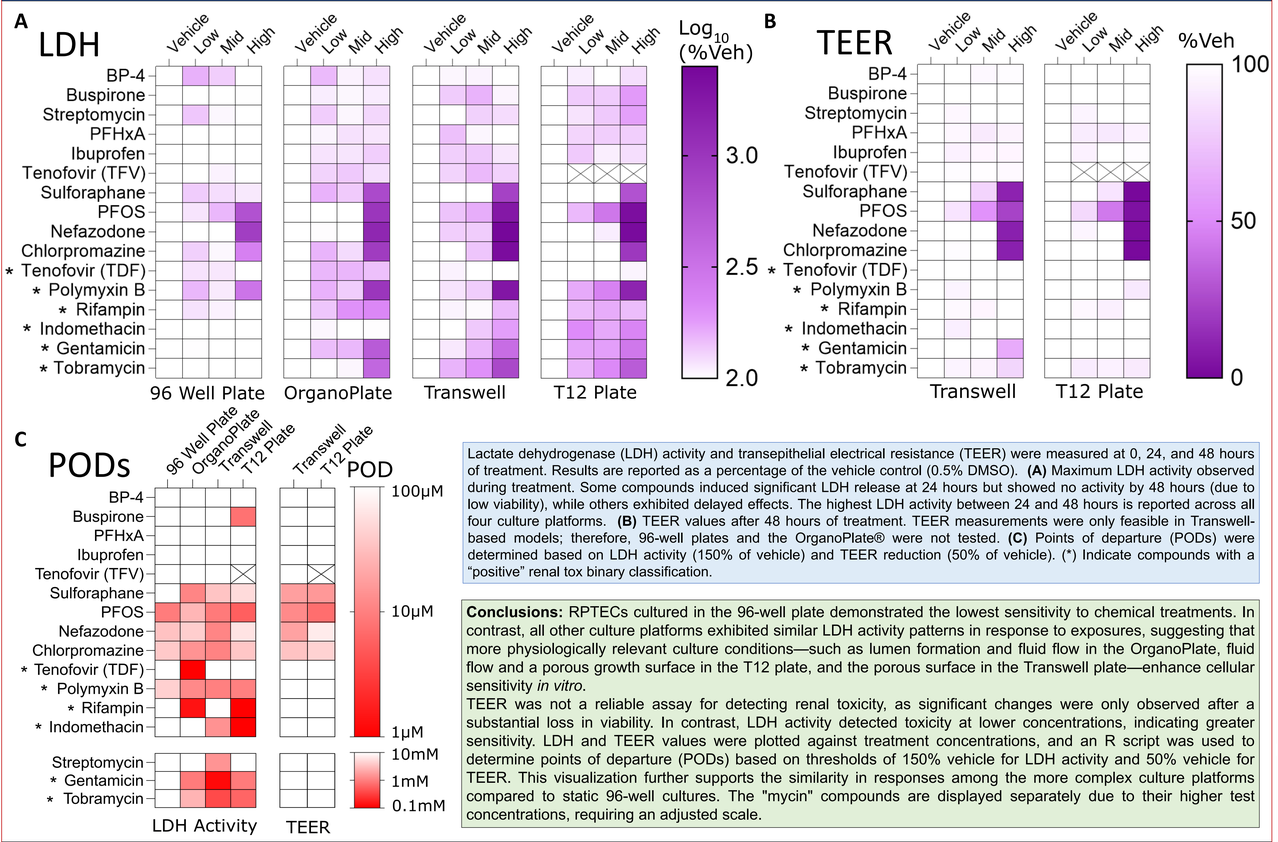



**(A)** An unsupervised clustering heatmap visualizing differentially expressed genes (DEGs) across platforms ($Log_{10}$ copy counts). **(B)** An unsupervised clustering heatmap visualizing baseline differences among platforms for a subset of ADME and "toxicology biomarker genes".

**Conclusions:** RPTECs cultured in the OrganoPlate platform and 96-well plates exhibit similar clustering patterns, while RPTECs grown on Transwells cluster together regardless of whether flow is present. When analyzing a subset of ADME and biomarker genes, baseline gene expression is largely consistent across cell culturing platforms.

## RESULTS

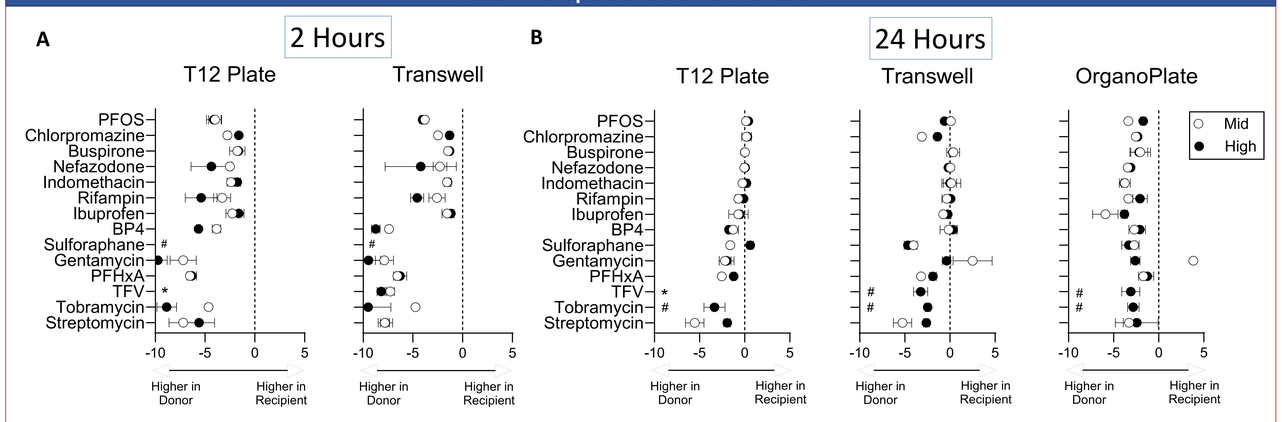
### Chemical Effects on Cell Viability and TEER Across Four Models




Lactate dehydrogenase (LDH) activity and transepithelial electrical resistance (TEER) were measured at 0, 24, and 48 hours of treatment. Results are reported as a percentage of the vehicle control (0.5% DMSO). **(A)** Maximum LDH activity observed during treatment. Some compounds induced significant LDH release at 24 hours but showed no activity by 48 hours (due to low viability), while others exhibited delayed effects. The highest LDH activity between 24 and 48 hours is reported across all four culture platforms. **(B)** TEER values after 48 hours of treatment. TEER measurements were only feasible in Transwell-based models; therefore, 96-well plates and the OrganoPlate® were not tested. **(C)** Points of departure (PODs) were determined based on LDH activity (150% of vehicle) and TEER reduction (50% of vehicle). (*) Indicate compounds with a "positive" renal tox binary classification.

**Conclusions:** RPTECs cultured in the 96-well plate demonstrated the lowest sensitivity to chemical treatments. In contrast, all other culture platforms exhibited similar LDH activity patterns in response to exposures, suggesting that more physiologically relevant culture conditions—such as lumen formation and fluid flow in the OrganoPlate, fluid flow and a porous growth surface in the T12 plate, and the porous surface in the Transwell plate—enhance cellular sensitivity *in vitro*.
TEER was not a reliable assay for detecting renal toxicity, as significant changes were only observed after a substantial loss in viability. In contrast, LDH activity detected toxicity at lower concentrations, indicating greater sensitivity. LDH and TEER values were plotted against treatment concentrations, and an R script was used to determine points of departure (PODs) based on thresholds of 150% vehicle for LDH activity and 50% vehicle for TEER. This visualization further supports the similarity in responses among the more complex culture platforms compared to static 96-well cultures. The "mycin" compounds are displayed separately due to their higher test concentrations, requiring an adjusted scale.

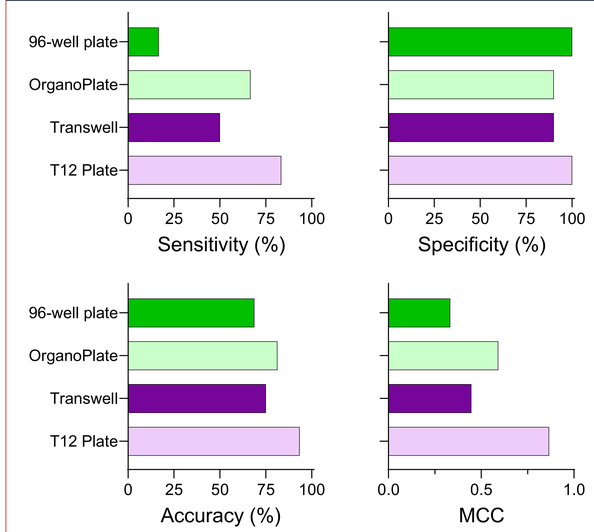
### Renal Transport and Barrier Function




**Renal Transport and Barrier Function Following Compound Exposure**. Chemicals were added to the apical side of Transwells (static or fluidic T12) or channels (OrganoPlate), with media sampled at (**A**) 2 hrs (T12 and Transwell only) and (**B**) 24 hrs (all platforms). Open circles represent transport/barrier data from "mid" exposures (1 mM for aminoglycosides and 10 µM for all other compounds), while filled circles indicate data from "high" exposures (10 mM for aminoglycosides and 100 µM for others). Media from 96-well plate cultures was not analyzed due to the absence of a barrier function in this model. Additionally, media was not analyzed from 1 µM exposure samples or from the Mimetas 2-hour time point due to detection limitations for some compounds on the recipient side. Compounds are ranked from highest transport to lowest transport in the CNBio platform at 24h hours. (*) Indicates the compound was not tested in that model, and (#) indicates the samples were below LOQ.

**Conclusions:** Chemical transport patterns were similar between the T12 fluidic Transwell and the standard static Transwell, indicating that flow in the T12 system does not impact the barrier function of TERT1/RPTEC-OAT1 cells. While some compounds reached equilibrium by 24 hours, approximately half remained in the donor (apical) compartment, suggesting either active renal secretion or a diffusion barrier. In contrast, the OrganoPlate platform did not reach equilibrium within 24 hours due to the gel barrier between compartments. Transport was comparable between "mid" and "high" treatment concentrations, indicating no saturation of compounds.

### Nephrotoxicity Predictions Across Four Models




Platforms were ranked for their ability to correctly predict nephrotoxicity. "Sensitivity" refers to the predictive rate of "true positives"; "Specificity" refers to the predictive rate of "true negatives"; "Accuracy" refers to the proportion of "true positive" and "true negative" in all evaluated cases; and the Matthew's Correlation Coefficient (MCC) is a single-value classification metric which helps to summarize true positives, true negatives, false positives, and false negatives.

**Conclusions:** The T12 platform demonstrated the highest overall performance across all classification metrics followed by the OrganoPlate, suggesting that increased physiological complexity and fluidic conditions may enhance predictive accuracy. In contrast, the 96-well plate showed the lowest sensitivity and MCC, indicating limitations in detecting positive outcomes using the simplest experimental model.

## SUMMARY

- The study demonstrated that RPTECs cultured in the OrganoPlate and 96-well plate showed similar gene expression patterns, while RPTECs cultured in static and fluidic (T12 plate) Transwells clustered together regardless of flow. However, when comparing ADMET and "biomarker" genes, expression patterns were similar across cells in all platforms.
- The 96-well plate model exhibited the lowest sensitivity with respect to detecting nephrotoxic effects in response to chemical treatments, while more physiologically relevant platforms (Transwells, OrganoPlate, and T12) showed greater prediction accuracy, suggesting the importance of microenvironment complexity in toxicity prediction.
- TEER was not a reliable assay for detecting nephrotoxicity toxicity, because significant changes were only observed after a substantial loss in cell viability. By contrast, LDH activity detected toxicity at lower concentrations, making it a more sensitive indicator of nephrotoxicity in vitro.
- Chemical transport patterns between the T12 fluidic Transwell and standard static Transwell were similar, suggesting that flow in the T12 system does not effectively impact the barrier function of TERT1/RPTEC-OAT1 cells. However, the OrganoPlate platform showed limited transport at 24 hours due to the presence of a gel barrier.
- These results support the use of MPS for ADME-Tox studies of nephrotoxicity; however, cost and throughput limitations must be considered when selecting platforms for use.

## FUTURE DIRECTIONS

- Testing Bi-Directional Transport – Since many of the tested chemicals are secreted rather than reabsorbed, future studies should assess both apical-to-basolateral and basolateral-to-apical transport to improve the model's utility for renal clearance predictions.
- Integration with PBPK Modeling – The transport data generated from this study can be incorporated into physiologically based pharmacokinetic (PBPK) models to refine in vitro-in vivo extrapolation (IVIVE) and enhance predictions of renal drug clearance.
- Expanding Cell Sources – Future studies should include additional RPTEC sources, primary renal cells, or transporter-overexpressing lines to assess inter-individual variability and enhance the model's translational relevance.

## ACKNOWLEDGEMENTS

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