# A mechanistically-anchored and human stem cell-based in vitro test battery for assessing liver steatogenic potential of chemicals

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### Organisation

**Name of the organisation** Vrije Universiteit Brussel (VUB)

**Specific Research Group or Service**

In Vitro Toxicology and Dermato-Cosmetology (IVTD)

**Country** Belgium

**Geographical Area** Brussels Region

## SCOPE OF THE METHOD

| | |
|---|---|
| **The Method relates to** | Human health |
| **The Method is situated in** | Translational - Applied Research |
| **Type of method** | In vitro - Ex vivo |
| **Specify the type of cells/tissues/organs** | human skin-derived precursor cells differentiated into hepatic cells (hSKP-HPC) |

## DESCRIPTION

### Method keywords

adult stem cells

in vitro

test battery

Adverse outcome pathway

AOP

NAM

new approach methodologies

Stem cells

tiered approach

## Scientific area keywords

chemical toxicity

Steatosis

hepatic cells

safety assessment

fatty liver disease

## Method description

Fatty liver disease, which can result from various factors including chemical exposure, is an increasing clinical concern. A key event in its development is steatosis, referring to the accumulation of lipids within hepatocytes. To enable early detection of chemical-induced liver steatosis, we developed a mechanistically-anchored, human-relevant new approach methodology (NAM). This NAM consists of a 2-tiered *in vitro* test battery, aligned with the adverse outcome pathway (AOP) network for steatosis, and utilizes human skin-derived precursor cells differentiated into hepatic cells (hSKP-HPC), previously shown to be responsive to steatogenic triggers. In total, 6 well-known steatogenic compounds, including 3 pharmaceuticals, a pesticide, and 2 plasticizers alongside 2 non-steatogenic chemicals were tested over a 72-hour period. Tier 1 evaluated transcriptional changes in key lipid metabolism pathways, and modulations were observed in nuclear receptors (peroxisome proliferator-activated receptor), fatty acid uptake (fatty acid translocase), de novo lipogenesis (diacylglycerol acyl

transferase 2, fatty acid synthase and stearoyl-CoA desaturase 1), as well as in VLDL secretion (apolipoprotein B100). Tier 2 assays assessed and confirmed downstream functional disruptions in fatty acid uptake and lipid accumulation as ultimate specific key events for steatogenic chemicals. Overall, this human stem cell-based NAM offers a promising tool for supporting early hazard identification of steatogenic chemicals across diverse sectors, bridging mechanistic insights to outcomes relevant to the initiation of fatty liver disease.

### Lab equipment

- Cell culture facilities,
- CellTiter-Glo kit,
- Flow cytometer,
- Microscope.

### Method status

Published in peer reviewed journal

## PROS, CONS & FUTURE POTENTIAL

### Advantages

- Human stem cell-derived hepatic cell models offer an unlimited cell source, capture population diversity, and show the ability to detect idiosyncratic liver toxicities, making them valuable tools for both academic research and regulatory safety assessments.
- Among them, hSKP-HPC cells have previously demonstrated to be able to express typical fatty liver markers and accumulate intracellular lipid droplets upon exposure to steatogenic pharmaceuticals.
- hSKP-HPC cells possess the necessary cellular machinery required to recapitulate steatosis, supporting their application in NAMs for hazard identification of steatogenic chemicals.
- This strategy offers a targeted and mechanistically informed approach for supporting the early detection of a chemical’s steatogenic potential. Tier 1 serves as an initial screening to identify potential pathways that contribute to a chemical’s steatogenic

properties. Tier 2 quantifies functional changes in steatogenic-associated anabolic pathways, adding weight to the evidence and enhancing the predictive performance of the *in vitro* test battery.

### Challenges

While hSKP-HPC cells have previously been shown to be suitable for liver toxicity assessments, these may have certain limitations in fully reproducing the intricacies of mitochondrial function and regulation.

### Future & Other applications

The NAM holds promise for supporting early hazard screening in the safety assessment of pharmaceuticals, industrial chemicals and environmental contaminants, bridging mechanistic insights to outcomes relevant to the initiation of fatty liver disease.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Links

A mechanistically-anchored and human stem cell-based in vitro test battery for …

Prof. Mathieu Vinken - Team

IVTD Research Group

### Other remarks

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