Supplementary Material

Combining a microphysiological system of three organ equivalents and transcriptomics to assess toxicological endpoints for cosmetics ingredients

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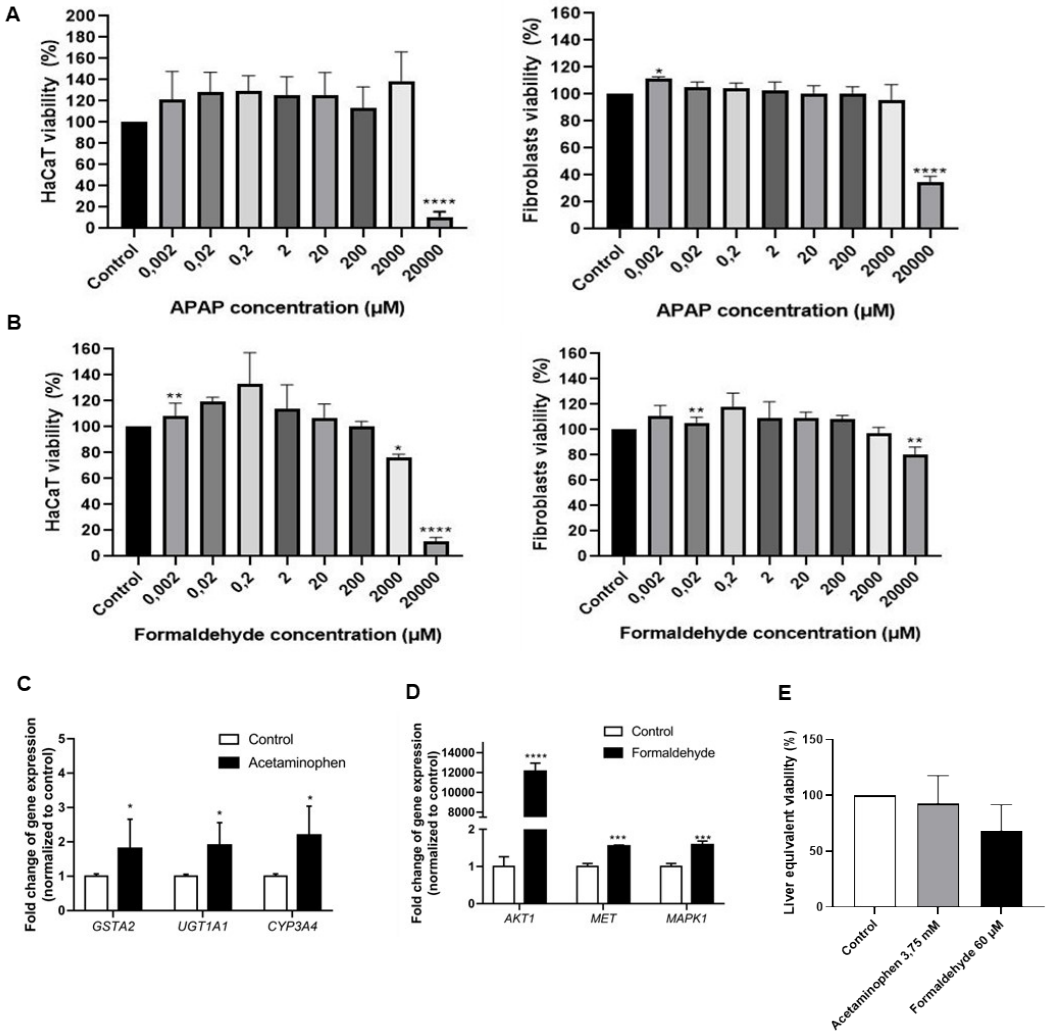
Supplementary Figure 1. Acetaminphen and formaldehyde dose selection. **A)** Cell viability of HaCaT and fibroblasts after treatment with different doses of acetaminophen**. B)** Cell viability of HaCaT and fibroblasts after treatment with different doses of formaldehyde**. C)** Normalized relative expression of systemic toxicity marker genes in liver spheroids after treatment with 3.75 mM acetaminophen. **D)** Normalized relative expression of carcinogenicity marker genes in liver spheroids after treatment with 60 µM formaldehyde. **E)** Liver equivalents viability after treatment with 3.75 mM acetaminophen and 60 µM formaldehyde assessed using MTT assay.



Supplementary Table 1. List of the main genes evaluated in our genic panels. **A)** List of genes for systemic toxicity panel. **B).** List of genes for carcinogenicity panel.

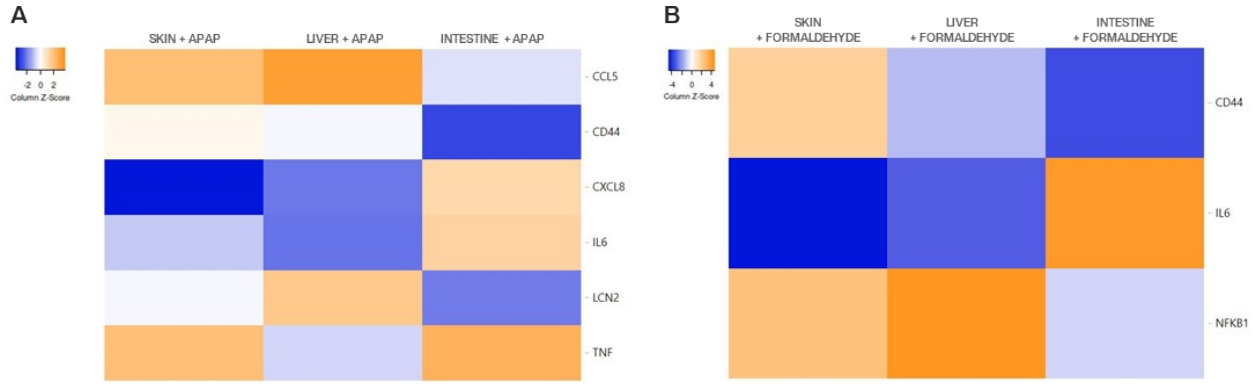
A

Systemic toxicity gene signature	
Gene	**Name**
AFP	Alpha-fetoprotein
ALB	Albumin
ALPI	Alkaline phosphatase, intestinal
ALPL	Alkaline phosphatase
APOE	Apolipoprotein E
CCL5	C-C Motif Chemokine Ligand 5
CFD	Complement factor D
CLDN1	Claudin 1
CXCL16	C-X-C motif chemokine ligand 16
CXCL8	Interleukin-8
CYP2E1	Cytochrome P450 family 2 subfamily E member 1
CYP3A4	Cytochrome P450 family 3 subfamily A member 4
FABP1	Liver fatty acid binding protein 1
GPT	Glutamic-pyruvic transaminase
GSTA1	Glutathione S-transferase alpha 1
LCN2	Lipocalin 2
MIR215	MicroRNA 215
MLXIPL	MLX Interacting Protein Like
NR1H3	Nuclear receptor subfamily 1 group H member 3
NR1H4	Nuclear receptor subfamily 1 group H member 4
NT5E	5'-nucleotidase ecto
SI	Sucrase-isomaltase
SLC22A1	Solute carrier family 22 member 1
SPP1	Secreted phosphoprotein 1
SREBF1	Sterol response element binding protein 1c
SULT2A1	Sulfotransferase Family 2A Member 1
TJP1	Tight Junction Protein 1
TJP3	Tight junction protein 3
TNF	Tumor necrosis factor
UGT2B4	UDP glucuronosyltransferase family 2 member B4

B

Carcinogenicity gene signature	
Gene	**Name**
AKT1	AKT Serine/Threonine Kinase 1
AKT2	AKT Serine/Threonine Kinase 2
BCL2	BCL2 Apoptosis Regulator
CCND1	Cyclin D1
CCNE1	Cyclin E1
ERBB2	Erb-b2 receptor tyrosine kinase 2
FOS	Fos proto-oncogene, AP-1 transcription factor subunit
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha
IL6	Interleukin 6
JAG1	Jagged canonical Notch ligand 1
JAK2	Janus kinase 2
JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
KIT	KIT Proto-Oncogene, Receptor Tyrosine Kinase
MDM2	MDM2 Proto-Oncogene
MTOR	Mechanistic target of rapamycin kinase
RICTOR	RPTOR Independent Companion Of MTOR Complex 2
RPTOR	Regulatory Associated Protein Of MTOR Complex 1
STAT5A	Signal transducers and activators of transcription 5A
TWIST1	Twist family bHLH transcription factor 1
VEGFA	Vascular endothelial growth factor-A
YAP1	Yes associated protein 1

Supplementary Figure 2. Modulation of inflammatory genes after treatment in the MPS. **A)** Expression of CCL5, CD44, CXCL8, IL6, LCN2 and TNFα in skin, liver, and intestinal barrier equivalents after treatment with acetaminophen**. B)** Expression of CD44, IL6 and NFKB1 in skin, liver, and intestinal barrier equivalents after formaldehyde treatment.



Supplementary Table 2. IPA analysis show the main biological processes modulated by gene expression evaluation. At left, the top canonical pathways, diseases and toxicological functions found after acetaminophen treatment (A). At right, the top canonical pathways, diseases, biological functions and toxicological functions found after formaldehyde treatment (B).

Considering intestinal barrier, the gastrointestinal disease was the main disorder in which the genes modulated in our panel were involved, while considering the liver spheroids, the main disorder was hepatic system disease. These findings indicate that the proposed model effectively assesses the potential for systemic toxicity, as topical treatment on the skin equivalent impacted the other organ equivalents within the MPS.

A

Systemic toxicity top canonical pathways
Xenobiotic Metabolism Signaling
PXR/RXR activation
FXF/RXR activation
Hepatic cholestasis
Systemic toxicity top diseases and disorders
Gastrointestinal disease
Hepatic system disease
Organismal injury and abnormalities
Inflammatory disease
Systemic toxicity top toxicological functions
Liver cholestasis
Liver inflammation
Liver cirrhosis
Liver steatosis

B

Carcinogenicity top canonical pathways
Molecular mechanisms of cancer
Carcinogenicity top diseases and disorders
Cancer
Dermatological diseases and conditions
Gastrointestinal disease
Hepatic system disease
Carcinogenicity top biological functions
Cell death and survival
Cellular growth and proliferation
Cellular movement
Cell cycle
Carcinogenicity top toxicological functions
Necrosis/cell death
Hepatocellular carcinoma
Liver hyperplasia/hyperproliferation
Liver inflammation/hepatitis