Table S1. Paracetamol cytotoxicity, metabolism and mechanistic endpoints evaluation in different cell types and cell culture systems.

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| --- | --- | --- | --- | --- | --- | --- |
| **Cell Type** | **Cell Culture System** | **Time of Exposure** | **IC50 /EC50 / LC50 / TC50 (mM)** | **Cytotoxicity Endpoints** | **Biotransformation | Mechanistic Endpoints** | **References** |
| HepG2 | 2D | 24h | 29.7 | WST-1 assay | NA | (Wang et al., 2002) |
| 24.0 | ATP quantification | CYP activity, glucuronidation and sulfation activity, hepatobiliary transport | NA | (Ramaiahgari et al., 2014) |
| 23.8 | Calcein-AM (live) / ethidium homodimer (dead) fluorescence | CYP1A1/2 and glucuronidation activity, drug-drug interaction | NA | (Deng et al., 2019) |
| 72h | 15.8 | CYP1A1/2 induction | NA | (Jang et al., 2015) |
| 5 days (compound addition at D0 and 2) | 2.251 | Live cell protease activity | CYP activity, glucuronidation and sulfation activity | NA | (Atienzar et al., 2014) |
| 3D Spheroids with Matrigel | 6 days (repeated exposure) | 9.4 | ATP quantification | CYP activity, glucuronidation and sulfation activity, hepatobiliary transport | NA | (Ramaiahgari et al., 2014) |
| 72h | 11.8 | Calcein-AM (live) / ethidium homodimer (dead) fluorescence | CYP1A1/2 induction | NA | (Jang et al., 2015) |
| 3D Spheroids in a microfluidic chip – OrganoPlateTM (MIMETAS) | 7.1 |
| 3D liver-sinusoid-on-a-chip (LSOC) | 24h | 9.8 | CYP1A1/2 and glucuronidation activity, drug-drug interaction | NA | (Deng et al., 2019) |
| HepG2/C3A | 2D | 24h | 33.8 | ATP Quantification | CYP2E1 expression and transporter function | NA | (Gaskell et al., 2016) |
| 3D Spheroids | 24h after 21 days in culture | 40.0 | ATP Quantification | NA | (Fey and Wrzesinski, 2012) |
| 3D Spheroids on Agarose overlay | 24h | 7.2 | CYP2E1 expression and transporter function | NA | (Gaskell et al., 2016) |
| 3D Spheroids on a perfused microbioreactor | 12h | 12.3 | TUNEL assay | NA | Mitochondrial function and metabolic activity (oxygen uptake), phospholipidosis and microvesicular steatosis induction | (Prill et al., 2016) |
| Hep3B | 2D | 72h | 0.3 | MTT Assay | NA | (Yu et al., 2018) |
| 3D Miniaturized cell-culture array (DataChip + Metachip) | 24h | 1.200 | Calcein-AM (live) / ethidium homodimer- (dead) fluorescence |
| 3D Miniaturized cell-culture array (DataChip) + CYP450 enzymes (MetaChip) | 0.068 |
| 3D Miniaturized cell-culture array (DataChip) + CYP450 + phase II enzymes (MetaChip) | > 1.200 |
| 3D Miniaturized cell-culture array (DataChip) + human liver microsomes (MetaChip) | >1.200 |
| HepaRG | 2D | 24h | Toxicity starting on 4.0 mM\* | Alamar Blue | Metabolic competence and metabolite quantification | Mitochondrial ROS levels and MMP measurement | (Zhang et al., 2020) |
| 24h after 5 days in culture 24h after 22 days in culture | 26.334.6 | ATP Quantification | CYP2E1 and MRP-2 activity | NA | (Gunness et al., 2013) |
| 48h7 days14 days | 5.9161.5871.311 | *CYP, GSTT1, UGT1A1, ABCB11, ABCC1* and *SLCO1B1* gene expression | NA | (Bell et al., 2017) |
| 3D Spheroids | 24h after 5 days in culture24h after 22 days in culture | 2.710.1 | ATP Quantification | CYP2E1 and MRP-2 activity | NA | (Gunness et al., 2013) |
| 24h after 6/7 days in culture24h after 21/22 days in culture | 11.6 | CYP induction | mitochondrial function (OCR) and glycolytic activity (ECAR), fibrosis (HSC activation, collagen secretion and deposition) | (Leite et al., 2016) |
| 3D Spheroids with pulverized liver biomatrix scaffolds | 24h | 20.0 (toxicity starting on 0.8 mM\*) | Alamar Blue | Metabolic competence and metabolite quantification | mitochondrial ROS levels and MMP measurement | (Zhang et al., 2020) |
| HLCs(hESC) | 2D | 24h | 46.0 | MTT Assay | CYP activity | NA | (Tasnim et al., 2015) |
| HLCs(hiPSC) | 2D | 48h7 days14 days | >10.0>10.09.439 | ATP Quantification | *CYP, GSTT1, UGT1A1, ABCB11, ABCC1* and *SLCO1B1* gene expression | NA | (Bell et al., 2017) |
| rpHep | 2D | 12h | 30.0 | MTT assay | NA | Oxidative stress, lipid peroxidation (MDA release), DNA strand breaks | (Lewerenz et al., 2003) |
| 24h | 7.6 | CYP2E1 activity | NA | (Jemnitz et al., 2008) |
| 14.0 | WST-1 assay | NA | (Wang et al., 2002) |
| ~3.75 | LDH Leakage | NA | Intracellular GSH,ROS and MDA production, MMP visualization | (Kučera et al., 2017) |
| 30.0 | Calcein AM (live) / PI (dead) fluorescent stain | CYP activity | NA | (Zhang et al., 2011) |
| 3D Scaffold perfused bioreactor (RoboTox) | 24h  | 7.0 | Calcein AM (live) / PI (dead) fluorescent stain |
| mpHep | 2D | 24h | ~1.25 | LDH Leakage | NA | Intracellular GSH,ROS and MDA production, MMP visualization | (Kučera et al., 2017) |
| 3D Collagen sandwich | 24h | 3.8 | MTT assay | CYP2E1 activity | NA | (Jemnitz et al., 2008) |
| hpHep | 3D Collagen sandwich | 24h | 28.2 | MTT assay | CYP2E1 activity | NA | (Jemnitz et al., 2008) |
| Cryo hpHep | 2D | 24h | 5-10 | Live cell protease /caspase-3/7 | NA | Mitochondrial dysfunction (OCR) | (Goda et al., 2016) |
| 45.2 | MTT Assay | CYP activity | NA | (Tasnim et al., 2015) |
| ~20.0 | ATP Quantification | Sulfation andglucuronidation assessment | NA | (Riches et al., 2009) |
| >20.0  | NA | inflammatory response | (Li et al., 2020) |
| 48h | 4.596 | NA | miR-122, HMGB1 and α-GST | (Proctor et al., 2017) |
| 5 days (compound addition at D0 and 2) | 2.987 | Live cell protease activity | CYP activity, glucuronidation and sulfation activity | NA | (Atienzar et al., 2014) |
| 3D Spheroids | 48h7 days14 days | >10.02.7030.644 | ATP Quantification | *CYP, GSTT1, UGT1A1, ABCB11, ABCC1* and *OATP-C* gene expression | NA | (Bell et al., 2017) |
| 24h after repeated dosing at D8, 12 and 15 | 3.280 | NA | inflammatory response | (Li et al., 2020) |
| Co-culture of HepaRG andHSC | 3D Spheroids | 24h after 6/7 days in culture24h after 21/22 days in culture | 10.08.0 | ATP Quantification | CYP induction | mitochondrial function (OCR) and glycolytic activity (ECAR), fibrosis (HSC activation, collagen secretion and deposition) | (Leite et al., 2016) |
| Co-culture of dog hepatocytes and NPC | 2D | 5 days (compound addition at D0 and 2) | 8.489 | Live cell protease activity | CYP activity, glucuronidation and sulfation activity | NA | (Atienzar et al., 2014) |
| Co-culture of cryo hpHep and NPC | 3D Spheroids | 24h72h7 days10 days | >102.91.91.7 | ATP Quantification | Metabolite quantification | miR-122, α-GST | (Foster et al., 2019) |
| 3D Spheroid human liver microtissues (3D hLiMT) | 72h | 1.348 | Biotransformation phase I, II and III enzymes induction | Mitochondrialoxidative stress, NAPQI-protein adducts quantification | (Bruderer et al., 2015) |
| 5-6 days14 days | 0.91100.5728 | NA | miR-122, HMGB1 and α-GST | (Proctor et al., 2017) |
| 14 days | 0.7542 | NA | (Messner et al., 2013) |
| Co-culture of cryo hpHep and KC | 3D Spheroids | 5 days | 2.247 | ATP Quantification | NA | inflammatory response | (Li et al., 2020) |
| Co-culture of cryo hpHep and LSEC | 3D Human Liver-Chip model | 10 days | 2.4 | ATP Quantification | Metabolite quantification | miR-122, α-GST | (Foster et al., 2019) |

ABC, ATP Binding Cassette; Ca2+, calcium; cryo, cryopreserved; CYP, cytochrome P450; ECAR, extracellular acidification rates; GSH, glutathione; GST, glutathione S-transferase; HepG2, HepG2/C3A, FaO, Huh7, HCCT-T, hepatic cell lines; hESC, human embryonic stem cells; HLC, hepatocyte-like cells (stem cell derived); HMGB, high mobility group box; hnMSC, human neonatal mesenchymal stem cells; hpHep, human primary hepatocytes; KC, Kupffer cells; MDA, malondialdehyde; miR, microRNA; MMP, mitochondrial membrane potential; MRP, multidrug resistance-associated protein; NA, not applicable; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NPC, primary human non-parenchymal cells; OATP, organic-anion-transporting polypeptides; OCR, oxygen consumption rate; ROS, reactive oxygen species; rpHep, rat primary hepatocytes; SCSIT, cell spreading inhibition test; UGT, UDP-glucuronosyltransferase.

\* in these reports, no IC50 was calculated and the values presented correspond to the concentration levels (mM) in which toxicity was observed.