Grouping of UVCB Substances with Dose-Response Transcriptomics Data from Human Cell-Based Assays

Supplementary Data

Table S1: Average number of reads per expressed transcript for each cell type and treatment combination
Please see Methods for explanation of the vehicle, media and DMSO concentrations for each cell type. Each test substance was examined in 3 concentrations ("Conc.") with "100" being the most concentrated and "1" being 10-fold subsequent dilutions.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Organ</th>
<th>Origin</th>
<th>Vehicle</th>
<th>Media</th>
<th>DMSO</th>
<th>Conc. 1</th>
<th>Conc. 10</th>
<th>Conc. 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCell Hepatocytes</td>
<td>Liver</td>
<td>iPSC-derived</td>
<td>639</td>
<td>589</td>
<td>612</td>
<td>557</td>
<td>531</td>
<td>546</td>
</tr>
<tr>
<td>iCell Cardiomyocytes</td>
<td>Heart</td>
<td>iPSC-derived</td>
<td>721</td>
<td>729</td>
<td>660</td>
<td>641</td>
<td>484</td>
<td>442</td>
</tr>
<tr>
<td>iCell Neurons</td>
<td>Brain</td>
<td>iPSC-derived</td>
<td>381</td>
<td>409</td>
<td>381</td>
<td>248</td>
<td>453</td>
<td>337</td>
</tr>
<tr>
<td>iCell Endothelial</td>
<td>Blood vessel</td>
<td>iPSC-derived</td>
<td>521</td>
<td>434</td>
<td>393</td>
<td>355</td>
<td>424</td>
<td>321</td>
</tr>
<tr>
<td>MCF7</td>
<td>Breast</td>
<td>Epithelial adenocarcinoma</td>
<td>562</td>
<td>599</td>
<td>550</td>
<td>586</td>
<td>423</td>
<td>485</td>
</tr>
<tr>
<td>A375</td>
<td>Skin</td>
<td>Malignant melanoma</td>
<td>733</td>
<td>760</td>
<td>689</td>
<td>704</td>
<td>569</td>
<td>685</td>
</tr>
</tbody>
</table>

Supplementary description of cell culture conditions

iCell hepatocytes 2.0
Vials of hepatocytes were thawed for 3 min at 37°C in a water bath and subsequently resuspended in RPMI medium containing 2% (v/v) iCell hepatocyte medium supplement, 0.1 μM dexamethasone, 2% (v/v) B27 supplement, 25 μg/mL gentamicin, and 20 ng/mL Oncostatin-M. Following microscopic evaluation of the cell density, the suspension was further diluted to a final concentration of 6.72 × 10^5 cells/mL. 25 μL of this suspension was then added to each well on collagen I coated 384-well plates (Corning, Product# 354664), yielding a final cell density of 16,800 cells per well. Plates were initially kept at room temperature (RT) for 30 min and then transferred to an incubator set at 37°C and 5% CO2. After 4 h of incubation, the plating medium was replaced with 25 μL fresh medium, a step that was repeated daily for 4 days. On day five, the plating medium was exchanged with 25 μL per well maintenance medium, consisting of RPMI containing 2% (v/v) iCell hepatocyte medium supplement, 0.1 μM dexamethasone, 2% (v/v) B27 supplement, 25 μg/mL gentamicin. Maintenance medium was exchanged daily for the duration of the experiment. See additional details in (Grimm et al., 2015).

iCell Neurons
Cryopreserved cells were thawed and plated according to the protocol provided by Cellular Dynamics International. Briefly, cells were plated on poly-d-lysine precoated 384-well plates (Greiner, Bio, Ref#: 781946) with iCell Neural Base Medium (Catalog#: M1010) added with iCell Neural Supplement A (Catalog#: M1032) and 3.3 mg/mL of laminin. Cells were plated at densities of 7,500 cells/well. Plates were initially kept at RT for 30 min before transferring to an incubator set at 37°C and 5% CO2 for 48 h until assay day. See additional details in

iCell Cardiomyocytes (also see Grimm et al., 2016)
384-well microplates were precoated with 25 μL 0.1% (w/v) gelatin solution per well for 2 h at 37°C and 5% CO2. Cryopreserved cells were thawed according to the manufacturer’s instruction using iCell cardiomyocyte plating medium with 1:500 (v/v) penicillin/streptomycin. Cell suspension was diluted in plate medium to provide a final cell concentration of 2 × 10^5 cells/mL. Subsequently, the gelatin solution was aspirated from the plates and 25 μL cell suspension was added to each well, making the final cell plating density 5000 viable cells/well. Plates were kept at room temperature for 30 min before they were incubated at 37°C and 5% CO2. 48 h following cell seeding, the plating medium was exchanged with 40 μL of maintenance medium containing 1:500 penicillin/streptomycin. Maintenance medium was subsequently changed every other day for another 12 days until assay day.

iCell Endothelial cells also see (Iwata et al., 2017)
Endothelial cells were plated and expanded on T-75 tissue culture flasks coated with human fibronectin solution at 3 μg/cm2. Cells were cultured with maintenance medium containing the VascuLife VEGF Medium Complete Kit (SKU: LL-0003), with FBS, and iCell Endothelial cells medium supplement. Cell density was determined using Trypan Blue exclusion test and a cell suspension was prepared that resulted in 1.0 × 10^4 cells/cm2. The fibronectin solution was aspirated and cells were seeded in a T-75 flask. Cells were incubated at 37°C and 5% CO2 with media changes every 2 days and passed every 3-4 days by TrypLE Express.

Experiments were conducted with cells between passages 1 and 5. Cells were transferred into 384-well plates with 50 μL maintenance medium at a density of 750 cells/well for cytotoxicity assay and 7,500 cells for angiogenesis assay. Cells were kept in microplates for 2-3 days until a monolayer formed before adding chemicals for cytotoxicity assays.
Cell lines were obtained from ECACC. A375 were maintained in DMEM HG (Gibco) without phenol red 10% HI FBS (1050064 South America Origin), 2mM L-Glutamine, pen/strep 100µg/mL/ 100U/mL split every 5-7 days 1:6 and MCF7 in EMEM (Gibco), 10% HI FBS (1050064 South America Origin), 2mM NEAA (5mL), 2mM L-Glutamine, pen/strep 100µg/mL/ 100U/mL split every 7-10 days 1:3. All cell lines were used through maximum 20 passages and then replaced from frozen stock. For use the harvest cell suspension was counted using a haemocytometer and diluted for seeding in 384 well plates for treatment at 12,000-14,000 cells/ well gave 90% confluence within 2-3 days of seeding in 50ul of the 10% FBS growth medium.

For assay the media for growth and maintenance, 50µL/ well, was removed on the morning of the day of treatment and replaced with to 20µL well fresh media without FBS. 200X stock plates for log10 dilutions of the UVCB substances where pure extracted substance is 1X and then dilutions from there of 10X, 100X and 1000X were set up. Positive controls at 200X final concentration were also included in this plate. The UVCB substance 200X with positive control plate was first diluted 40 fold by diluting 4 µL stock chemical with 156uL fresh media without FBS. After mixing by trituration and microplate spinning, 5µL of treatment solution from these diluted plates was added to the20 µl medial on the cells to give a final 200-fold dilution from the 200X stock. Thus, final concentrations of the UVCB substance extract were 200X, 2000X, 20,000X and 200,000X. Treated plates were incubated for total 24 hr and processed according to the individual assay requirements according to the manufacturer's protocols.

References
Fig. S1: Top 50 differentially expressed genes by cell type across 141 petroleum substances when comparing the maximum dose to method blank controls. The bar width represents the number of times a given gene was differentially expressed (FDR = 10%) when assessed across each of 141 substances. Gold represents down-regulated gene expression while green represents up-regulation.
Fig. S2: Top 50 concentration responsive genes by cell type across 141 petroleum substances
The bar width represents the number of times a given gene elicited a concentration response (FDR = 10%) when assessed across each of 141 substances. Gold represents decreasing expression with concentration, while green represents increasing gene expression with concentration.
Cardiomyocyte

CR Genes @ FDR = 10%

Number of Petroleum Substances (Out of 141) Eliciting a Concentration Response

Neuron

CR Genes @ FDR = 10%

Number of Petroleum Substances (Out of 141) Eliciting a Concentration Response
Fig. S3: Class-specific effects of petroleum substances on gene expression in the multi-cell in vitro transcriptomic analysis by cell type.
Box and whiskers plots show the range in the number of genes significantly (FDR≤10%) differentially expressed by the substances in each class when comparing maximum dose to method blank controls.
Fig. S4: Class-specific effects of petroleum substances on gene expression throughout a concentration-response in the multi-cell *in vitro* transcriptomic analysis by cell type.

Box and whiskers plots show the range in the number of genes significantly (FDR≤10%) exhibiting a concentration response by the substances in each class when comparing maximum dose to method blank controls.
Fig. S5: Class-specific effects of petroleum substances on pathway enrichment in the multi-cell in vitro transcriptomic analysis and differential gene expression at max dose

An FDR of 5% was used to select any genes differentially expressed for any substance within a category. Pathway analysis using C2Reactome ontologies was conducted with an FDR of 5% applied for pathway analysis. Bar plots show enriched C2Reactome ontologies by cell type. The background was set to all genes assessed after QC by each cell type.
Fig. S6: Class-specific effects of petroleum substances on pathway enrichment in the multi-cell in vitro transcriptomic analysis of concentration response

An FDR of 5% was used to select any concentration-responsive genes for any substance within a category. Pathway analysis using C2Reactome ontologies was conducted with an FDR of 5% applied for pathway analysis. Bar plots show enriched C2Reactome ontologies by cell type. The background was set to all genes assessed after QC by each cell type.