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Screening of Emerging Contaminants Using Cell Bioassays (OEHHA)
– Study Design –

Objective

To generate toxicity data for data-poor chemicals using in vitro bioassays.

Chemical Selection and Stock Preparation

A total of 18 chemicals will be tested (Table 1). These chemicals were identified based on OEHHA's interests and priorities, chemical solubility, purity, availability and pricing (up to $250 per chemical).

Stock solutions of the selected chemicals will be made in DMSO at concentrations of 0.2 to 0.5 M. This concentration range was determined based on an evaluation of the range of chemical concentrations tested in the Tox21 program (Shi et al. 2022). Ethanol may be used to create stock solutions if solubility is limited in DMSO. In this case, stocks would be further diluted in DMSO to a final concentration of less than 0.05% ethanol.

In Vitro Bioassay Analysis

Cell-based assays will be performed using commercially available fluorescence-based glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor gamma (PPARγ) GeneBLAzer™ assays purchased from ThermoFisher Scientific. Human dividing cells (HEK 293H) will be plated in 96-well plates and exposed to various concentrations of each chemical. The final amount of DMSO per well will be no greater than 0.5%, consistent with the manufacturer's recommendations. After incubation for 16 h at 37°C and 5% CO_2, LiveBLAzer and PrestoBlue reagents will be added to each well to measure receptor activity (460:530 nm) and cell viability (590 nm), respectively, using a microplate reader.

The selected chemicals will be tested in two phases. In the first phase, a range finding exercise will be conducted by testing 12 concentrations using a 1:3 or 1:4 serial dilution. Each chemical concentration will be tested in duplicate wells. Data will be used to narrow down the concentration range where a sigmoidal dose-response is produced. In the second phase, each chemical will be serial diluted to produce 8 concentrations that capture the complete dose-response. The chemicals will then be tested as described above. In this phase, each assay plate will also include 8 concentrations of a known reference compound (see Table 2), a solvent control, and a negative (cells only) control. All selected chemicals will be run in duplicate wells per plate and on two separate plates. Thus, four replicate data points will be generated per chemical of interest.

Table 1. List of chemicals selected for in vitro bioassay testing

Chemical Name	Chemical Group	CAS No.	Assay Endpoint
Methyl linoleate	Fatty acid esters	112-63-0	GR inhibition
Phenol, 4,4'-[1-[4-[1-(4-hydroxyphenyl)-1-methylethyl]phenyl]ethylidene]bis-	Bisphenol	110726-28-8	GR inhibition
Butyl cis-9-octadecenoate	Fatty acid esters	142-77-8	GR inhibition
Dodecyl(2-hydroxy-3-sulphonatopropyl) dimethylammonium	QACs	13197-76-7	GR inhibition
4-(2-Methylbutan-2-yl)phenol	Benzene substituted derivatives	80-46-6	GR inhibition
2-Octen-1-ylsuccinic anhydride	Carboxylic acids and derivatives	42482-06-4	GR inhibition
1,3-Butylene diacrylate	Carboxylic acids and derivatives	19485-03-1	GR inhibition
Perfluorooctanesulfonamide	PFAS	754-91-6	PPARγ activation
2-(N-Ethylperfluorooctanesulfonamido) acetic acid	PFAS	2991-50-6	PPARγ activation
2-(N-Methylperfluorooctanesulfonamido) acetic acid	PFAS	2355-31-9	PPARγ activation
Levocarnitine	QACs	541-15-1	PPARγ activation
Perfluoroheptanesulfonic acid	PFAS	375-92-8	PPARγ activation
Perfluoro-2-methyl-3-oxahexanoic acid	PFAS	13252-13-6	PPARγ activation
Perfluoro-3,6,9-trioxadecanoic acid	PFAS	151772-59-7	PPARγ activation
(Perfluorobutyryl)-2-thenoylmethane	PFAS	559-94-4	PPARγ activation
Perfluorooctanesulfonic acid	PFAS	1763-23-1	PPARγ inhibition
Perfluorooctanoic acid	PFAS	335-67-1	PPARγ inhibition
Perfluoroheptanoic acid	PFAS	375-85-9	PPARγ inhibition

Table 2. Assay-specific reference chemical to be used

Assay Endpoint	Reference compound	Chemical Group
GR inhibition assay	Mifepristone	Pharmaceutical; steroidal antiglucocorticoid and antiprogestogen
PPARγ activation assay	Rosiglitazone	Pharmaceutical; insulin regulation
PARRγ inhibition assay	GW9662	Synthetic irreversible PPARγ antagonist

Data Analysis

Range finding bioassay data will be plotted to identify the range of concentrations that will produce full dose–response curves.

Data generated in the second phase will be plotted and analyzed using a non-linear curve fitting model with GraphPad Prism or R (e.g., drda or drm packages) and derive the curve R^2, and 10 and 50% effect concentrations (EC10 and EC50).

Effect concentration (ECx %) for each chemical will be calculated as:

ECx % = $\left(\frac{X}{100-X}\right)^{\frac{1}{Hillslope}} * EC_{50}$, where hillslope and EC50 are determined by fitting the non-linear model.

Relative chemical potency defined as the potency of the chemical of interest relative to the reference compound will be calculated as follows.

$REP_{chemical}$ = (EC_x reference compound) / (EC_y chemical of interest)

Cell viability will be evaluated across the plate by comparing the raw data for the test chemicals against that of the mean response measured in the negative control wells. Percent cell viability will be calculated as:

Cell viability (%) = (mean viability (test sample) / mean viability (negative control))*100

Quality Assurance Measures

For each chemical, data quality will be validated against a set of performance-based quality assurance/quality control (QA/QC) parameters recommended in Mehinto et al. 2024 (see Table 3).

Table 3. Performance-based QA/QC measures

Criterion	**Acceptable limits**
R^2	≥ 0.95
Induction ratio	≥ 4
Cell viability	≥ 80% cell viability in chemical-treated wells at test termination compared to the viability of the negative control wells.
Reproducibility	≤ 20% relative standard deviation for raw data values for replicate wells. Up to 30% RSD is acceptable for responses below LOD.
Vehicle control (VC)	Mean response in VC wells ±15% of the mean response in negative control wells.

References

Mehinto, A.C., McGruer, V., Schlenk, D., 2024. Development and standardization of bioanalytical screening tools, Part II – protocols for laboratory and data analysis. Technical Report 1381.B. Southern California Coastal Water Research Project. Costa Mesa, CA.

Shi, Z., Xia, M., Xiao, S., Zhang, Q., 2022. Identification of nonmonotonic concentration-responses in Tox21 high-throughput screening estrogen receptor assays. Toxicology and Applied Pharmacology 452, 116206. https://doi.org/10.1016/j.taap.2022.116206