CATEGORIZATION OF PETROLEUM SUBSTANCES THROUGH HIGH-CONTENT SCREENING OF INDUCED PLURIPOTENT STEM CELL (iPSC) DERIVED CARDIOMYOCYTES AND HEPATOCYTES

Grimm FA1, Iwata Y1, Sirenko O2, Crittenden C2, Roy T3, Boogaard P4, Ketelslegers H5, Rohde A5, and Rusyn I1

1Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA; 2Molecular Devices LLC, Sunnyvale, CA, USA; 3University of South Carolina, Beaufort, SC, USA; 4SHELL International BV, The Hague, NL; 5E Saxion MOBIL Petroleum and Chemicals, Machelen, BE; and 6Concawe, Brussels, BE

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INTRODUCTION

While hazard assessment of data-limited chemicals by chemical structure-based category read-across is sensitive for chemically-characterized compounds, it cannot be used to assess complex chemicals, such as petroleum substances. Therefore, we hypothesized that a biological data-based read-across, i.e., safety evaluation category (SERC)-like categorizing substances according to similarities in their biological response, may represent a feasible alternative. To test this, we applied high-content, multi-parametric toxicity screening of induced pluripotent stem cell-derived (iPSC) cardiomyocytes1,2 and hepatocytes3 that were exposed to petroleum substances from six distinct product categories in a concentration- and time-response design. Cell-specific effects were observed and used as high-dimensional ‘biological’ data inputs for evaluation of the similarities and differences within and across different categories in ToxPi Graphical User Interface.

GOALS OF THE STUDY

1. To address the challenge of safety assessment of UVCB (Unknown or Variable composition, Complex reaction products and biological materials) materials by using a "biological" category read-across and a case study of petroleum substances;
2. To collect toxicological data on in vitro effects of petroleum substances using high-content screening of iPSC-derived cardiomyocytes and hepatocytes;
3. To use toxicological data as an integrative "biological" high-dimensional matrix for category read-across that can be visualized using Toxicological Priority Index (ToxPi) approach.

MATERIALS & METHODS

POTENTIAL SUBSTANCE EXTRACT PREPARATION

Petroleum substances were sorted into specific categories (SRGO - Straight Run Gas Oils, ADD - Other Oils, SRGO - Vacuum or Hydrocrack Gas Oils, Volume & Viscosity) in concurrence with Concawe, a European intergovernmental association that acts in the field of petroleum products. For each sample, 2 µl were aspirated into 28 µl DMSO and the DMSO solution was subsequently analyzed by liquid chromatography.

CELL CULTURE

High-content imaging and CYTOTOXICITY SCREENING

High-content imaging of iPSC cardiomyocytes and hepatocytes was performed on a custom-built high-content imaging system (Molecular Devices, LLC). Cells were grown on iCell Cultureware (Molecular Devices, LLC) for 4 days and stained with calcein AM (Molecular Devices, LLC) and Hoechst 33342 (Molecular Devices, LLC) for visualization and quantifying applications included in MacProbe (Molecular Devices).

IN VITRO CARDIOTOXICITY ASSAY

The effects on cardiotoxicity were assessed by monitoring the intrinsic and extrinsic signaling activities of cardiomyocytes using a high-content imaging system (Molecular Devices). Briefly, cells were seeded onto 96-well plates with 60% confluence 3 days before testing. After the wells were seeded, viability was monitored using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). The concentration of the test substance was confirmed by cell counting using a hemocytometer. The plates were washed twice with 100 µl of fresh growth media. Subsequently, 100 µl of 1:50 diluted Calcein-AM (Molecular Devices) was added to each well and incubated at 37°C for 4 hours. The fluorescence was quantified using an iFluor plate reader with excitation at 485 nm and emission at 510 nm.

SAINIS-FI-ICHI MASS SPECTROMETRY

Chemical characterization of petroleum substances was performed by isolated controls and low endogenous compounds. Isolated compounds were identified by matched models. The total ion current encoding was performed on a Thermo Scientific Q Exactive Hybrid Quadrupole Orbitrap Mass Spectrometer using the positive ion mode. Spectra were processed using massLynx software (version 4.1; Waltham, MA).

RESULTS

High-content imaging of iPSC cardiomyocytes exposed to varying concentrations of SRGO extracts of petroleum substances. Cell viability was assessed by live cell staining with Hoechst 33342 (nuclei, blue) and Calcein AM (intracellular, red) and Calcein AM viability marker, green. Cells were also exposed to the vehicle control (DMSO) or a positive control (SRGO) and images were acquired at three time points (4, 6, and 8 h). The white box (1% DMSO) did not result in any changes of the beating pattern.

POTENTIAL SUBSTANCE CATEGORIZATION BASED ON THE BIOLOGICAL PROFILES USING ToxPi SOFTWARE

Substance categorization was performed using the ToxPi approach. ToxPi data were integrated to provide a ToxPi score, i.e., a relative toxicity score equivalent to the relative toxicity for each substance. The higher the ToxPi score, the higher the relative toxicity of the substance. Figures for both iPSC cardiomyocytes and hepatocytes indicate strong correlations between ToxPi scores and substances categories. In most cases, the two are considerably similar between individual phenotypic responses of substances within a certain category. Moreover, there were also similarities between different substance groups, particularly Straight Run Gas Oils (SRGO) and Vacuum or Hydrocrack Gas Oils (VHGO).

CONCLUSIONS

1. In vitro toxicity testing of petroleum substances, a prototypical example of UVCB, demonstrates appreciable similarities in potential hazard properties of the individual products both within the same category and between related categories;
2. Quantitative high-content imaging using diverse cell-based models provides “biological” means for exercising the similarity principle through category read-across;
3. Effective communication of the complex multi-dimensional datasets comprising of various information streams (e.g., physicochemical properties, manufacturing process details, toxicity profiling) can be achieved using ToxPi-enabled data integration;
4. Extensions of this approach to additional cell-based model systems representing various tissues, coupled with high-throughput gene expression profiling, will further increase confidence in the “biological” based read-across of UVCB.

REFERENCES

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