# High Content Imaging of Cellular Energy Metabolism to Support Chemical Safety Testing for Thyroid Disruption

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This project aims to develop a human thyrocyte/hepatocyte screening model to elucidate the mechanism by which chemical exposures disrupt human thyroid kinetics and action and support risk-assessment in population level. Thyroid hormones (TH) regulate energy balance by controlling cellular energy expenditure. Levels of TH are tightly controlled whereby Hypothalamic-pituitary-thyroid (HPT) feedback regulates systemic TH levels, the liver regulates TH kinetics, and intracellular deiodination locally regulates TH action. Chemicals including polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and per- and polyfluoroalkyl substances (PFAS) can disrupt TH balance in humans and animals. The traditional mechanism is that chemicals (including PCDD/Fs and PFAS) act through receptor binding and/or induction of TH-glucuronidation and TH clearance. However, data from mutant rodent strains show similar chemically induced hypothyroidism despite loss of glucuronidation capacity. We tested metabolic endpoints using high-content confocal imaging. Treatment of HepG2 cells with 1,2,7,8-TCDD showed lipid uptake (bodipy), and mitochondrial membrane potential (TMRM) exhibited dose-response decreases with IC50s at 0.1 and 0.9 nanomolar. PCDD/F congeners, 1,2,3,7,8-PCDF, and 1,2,3,4,6,7,8-HCDF, showed 10-fold and 100fold lower IC50s compared to 1,2,7,8-TCDD. The PFOA and GenX treatments did not affect metabolic endpoints, and PFOS significantly induced TMRM and bodipy intensity. CYP1A1 luminescent assay showed dose-response relationships with 1,2,7,8-TCDD and congeners with IC50s at 0.44, 3.00 and 0.84 nanomolar. TMRM is the most sensitive endpoint in 2D culture. We further evaluated TMRM in 3D hepatocyte spheroids and found IC50 at 0.13 nanomolar. Combined toxicity effects were tested in 1,2,7,8-TCDD and PFOS mixture. TMRM and bodipy response to the mixture treatment.