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**Abstract title:**
Assessment of the performance of a New Approach Method (NAM) testing DIO1 inhibition using a human microsome based, colorimetric assay


1. Introduction

The interference of chemicals with thyroid homeostasis can lead to adverse effects in humans. Exogenous chemicals that alter functions of the endocrine system and consequently causes adverse health effects are termed "endocrine disruptors". So far *in vitro* testing of the interference with thyroid homeostasis has been limited and without regulatory acceptance. EURL ECVAM in cooperation with a network of EU laboratories (EU-NETVAL), is coordinating the validation of multiple in vitro methods to address different key events potentially affecting thyroid homeostasis. One such method is the DIO1-SK assay. Deiodinases (DIO) are important, local regulators of TH action. DIO1, one of three isoforms and mainly expressed in thyroid, liver, and kidney, serves as one main source of the active hormone T3 via deiodination of T4 and plays a role in recycling of iodide via deiodination of inactive TH metabolites.

2. Objectives

The reproducibility of the method has previously been established (Weber et. al 2022; DOI: 10.1089/aivt.2022.0010). Here, we report on the predictivity assessment, where the predictivity of the method is investigated by testing a blinded set of substance and developing a prediction.

3. Materials & methods

A set of 22 test substances consisting of known DIO *in vitro* inhibitors as well as inactive substances and substances otherwise interfering with the thyroid axis were tested in the standardized DIO1-SK assay. The released iodide was quantified via the Sandell-Kolthoff (SK) reaction. Experiments were performed on blinded substances that were later unblinded; results were statistically evaluated and compared to literature data. Finally, an *in vitro* prediction model was generated.

4. Results

Seven test substances produced a maximum DIO1 inhibition greater than 90% and eleven test substances below 20%; they were regarded as inhibitors and non-inhibiting substances, respectively. Two test substances, Ketoconazole and Silicristin, were found to be not applicable based on assay interferences. Inhibition data were consistent with results of relevant *in vitro* and computational models.

5. Conclusion

Using the variation of control data, an *in vitro* prediction model was defined categorizing test substances (i) by efficacy using the maximum inhibition data into three different categories. The test substances that produced a maximum DIO1 inhibition greater 90% where further categorized based on potency using the IC50 data of the test substance.