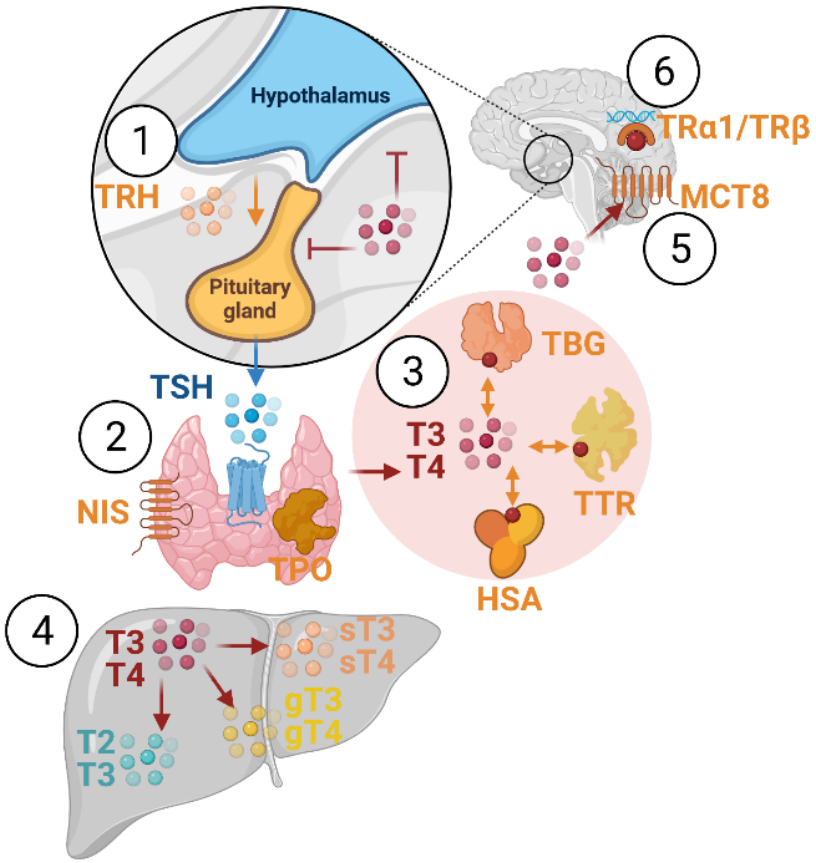
STUDY REPORT

for the TPO-catalyzed tyrosine iodination assay using liquid chromatography – Part 2

EURL ECVAM validation study of a battery of mechanistic methods relevant for the detection of chemicals that can disrupt the thyroid hormone system



2022

This study report has been prepared within the context of a collaboration agreement signed in 2019 with the Joint Research Centre (JRC) Directorate for Health, Consumers and Reference Materials (Chemicals Safety and Alternative Methods Unit F3 / EURL ECVAM), for the validation of mechanistic methods to identify potential modulators of thyroid hormone signalling. For information on the methodology and quality underlying the data presented in this report, users should contact the referenced source.

This study report describes the experimental design and includes data generated in Part 2 of the validation study. The method was developed and experimentally assessed by EU-NETVAL laboratory Charles River Laboratories, Den Bosch.

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FINAL REPORT

Test Facility Study No. 20382705

In Vitro Determination of the Potential of Thirty Test Items to Suppress the Thyroid Peroxidase (TPO)-Catalyzed Iodination using FTC 238 hrTPO Cell Homogenates

Non-GLP

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REPORT APPROVAL

All electronic signatures appear at the end of this Report upon finalization.

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Role/Phase	Quality Assurance Unit	Name	Contact Information
Study Director	Charles River	Jelle Reinen, PhD, ERT	Address as cited for Test Facility
Test Facility Management	Charles River	Beppy van de Waart, MSc, ERT	Address as cited for Test Facility

2. SUMMARY

This study was performed for PART 2 of the EURL ECVAM coordinated Thyroid Validation Study. After the full description of method 2C (Tyrosine iodination using liquid chromatography) in standard operating procedures (SOPs) and successful assessment of the robustness and reliability of the in vitro method to determine the suppression of human thyroid peroxidase (TPO)-catalyzed iodination during PART 1 of the Thyroid Validation Study (Charles River Test Facility Study No. 20309164), in PART 2 the effect of in total 30 randomized test items were assessed in a blinded manner.

TPO is an enzyme which is present on the apical membrane of thyroid follicular cells where it reduces hydrogen peroxide (H_2O_2), thereby elevating the oxidation state of iodide to an iodinating species (often considered to be hypoiodous acid) and iodinates tyrosyl residues in the thyroglobulin (Tg) glycoprotein. Initial iodination of Tg produces monoiodotyrosine (MIT) and diiodotyrosine (DIT) while subsequent oxidation of MIT and DIT by TPO to radical species couples two residues of DIT, both still linked to the Tg, to produce thyroxine (T4) and couples one residue of MIT and one residue of DIT to produce triiodothyronine (T3). When thyroid hormones are needed, hormone-rich Tg is taken up into thyroid epithelial cells by endocytosis and digested by proteases which results in the release of T4 and T3 into the blood circulation through the action of their transporters. Chemicals potentially can suppress TPO-catalyzed iodination and/or coupling and in that way alter thyroid hormone homeostasis in vivo.

For each test item, at least two valid and independent TPO-catalyzed iodination experiments were performed. During each experiment, the test materials were tested at eight concentrations (in triplicate) together with vehicle controls, no-vehicle controls, no-peroxide controls, non-enzymatic iodination controls, the negative control suppressor bis-(2-ethylhexyl)-phthalate (DEHP) at a single concentration and a complete dose-response curve for the reference material 6-propyl-2-thiouracil (PTU). Dimethyl sulfoxide (DMSO) was used as the vehicle and the concentration of vehicle in the incubations was kept constant at 1% (v/v).

For each of the experiments, the percentage of TPO-catalyzed iodination compared to the vehicle control was ≥ 93% for the negative control DEHP which was well above the acceptance criterion of > 80%. For each of the experiments, the mean MIT concentrations of the no-peroxide controls were always below the LLOQ (0.0761 µM) and were therefore considered background. These results confirmed that MIT formation in the incubations was dependent on the presence of H_2O_2. For each of the experiments, the mean percentage of TPO-catalyzed iodination of the no-vehicle controls when compared to the average TPO-catalyzed iodination in the vehicle control samples varied between 98% and 109% and thus was within the range of 80%-120%. As all acceptance criteria for the controls were met, all TPO-catalyzed iodination experiments were considered valid.

For each of the experiments, the curves for the reference material PTU were sigmoidal and were composed of a minimum of six concentrations. The reference material PTU suppressed the TPO-catalyzed iodination in a dose-dependent manner with IC_{50} values which varied between 1.27 µM and 2.45 µM and thus were within the acceptance range of 0.5 – 5 µM. As all acceptance criteria for the reference material PTU were met, all TPO-catalyzed iodination experiments were considered valid.

The test materials IVA_012, IVA_071, IVA_135, IVA_140, IVA_151, IVA_226, IVA_254, IVA_301, IVA_303, IVA_310, IVA_341, IVA_387, IVA_401, IVA_431, IVA_482, IVA_515, IVA_532, , IVA_563, IVA_597, IVA_696, IVA_779, IVA_805, IVA_820,

IVA_992 were found to be positive and suppressed the FTC-238-hrTPO-catalyzed iodination in a dose-dependent manner.

The test materials IVA_136, IVA_246, IVA_550, IVA_661, IVA_834, IVA_920 were found to be negative and did not suppress the FTC-238-hrTPO-catalyzed iodination.

3. INTRODUCTION

This study was performed for PART 2 of the EURL ECVAM coordinated Thyroid Validation Study. After the full description of method 2C (Tyrosine iodination using liquid chromatography) in standard operating procedures (SOPs) and successful assessment of the robustness and reliability of the in vitro method to determine the suppression of human thyroid peroxidase (TPO)-catalyzed iodination during PART 1 of the Thyroid Validation Study (Charles River Test Facility Study No. 20309164), in PART 2 the effect of in total 30 randomized test items were assessed in a blinded manner.

TPO is an enzyme which is present on the apical membrane of thyroid follicular cells where it reduces hydrogen peroxide (H_2O_2), thereby elevating the oxidation state of iodide to an iodinating species (often considered to be hypoiodous acid) and iodinates tyrosyl residues in the thyroglobulin (Tg) glycoprotein. Initial iodination of Tg produces monoiodotyrosine (MIT) and diiodotyrosine (DIT) while subsequent oxidation of MIT and DIT by TPO to radical species couples two residues of DIT, both still linked to the Tg, to produce thyroxine (T4) and couples one residue of MIT and one residue of DIT to produce triiodothyronine (T3). When thyroid hormones are needed, hormone-rich Tg is taken up into thyroid epithelial cells by endocytosis and digested by proteases which results in the release of T4 and T3 into the blood circulation through the action of their transporters. Chemicals potentially can suppress TPO-catalyzed iodination and/or coupling and in that way alter thyroid hormone homeostasis in vivo.

FTC-238-hrTPO cells are human thyroid carcinoma cells stably transfected with an expression clone coding for human recombinant (hr) TPO and can be used to prepare cell lysates containing the hrTPO enzyme. To evaluate potential interference with TPO-catalyzed iodination, FTC-238-hrTPO cell lysates containing TPO enzymes will be incubated together with L-tyrosine, potassium iodide and H_2O_2 in the presence or absence of the individual test items, the negative control bis-(2-ethylhexyl)-phthalate (DEHP) or the reference material 6-propyl-2-thiouracil (PTU). During incubation, TPO enzymatically converts L-tyrosine into MIT and formation of this metabolite can be monitored by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as a direct measurement of TPO-catalyzed iodination. A small amount of MIT may also be formed non-enzymatically, its formation will be assessed in separate incubations.

This study is not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

The Study Plan is presented in Appendix 1.

Study Initiation Date: 30 May 2022

Experimental Start Date: 31 May 2022

Experimental Completion Date: 13 Jul 2022

4. MATERIALS AND METHODS

4.1. Test Materials

The terms “item” and “material” (i.e., test item, control item) are both used in this study report and do have the same meaning. Therefore, both terms can be used interchangeably.

4.1.1. Test Materials

The test material information as provided by EURL ECVAM is summarized in the table below.

Test Item No.	Appearance	Storage Conditions	Approx. Molecular Weight (g/mol)
IVA_012	Solid	-20°C	475
IVA_071	Solid	-20°C	750
IVA_135	Solid	4°C	325
IVA_136	Solid	RT	200
IVA_140	Solid	RT	350
IVA_151	Solid	4°C	550
IVA_226	Solid	RT	175
IVA_246	Solid	RT, inert gas	200
IVA_254	Solid	RT	550
IVA_301	Solid	4°C	500
IVA_303	Solid	4°C	275
IVA_310	Solid	4°C	350
IVA_341	Solid	RT	175
IVA_387	Solid	RT	275
IVA_401	Solid	RT	300
IVA_431	Liquid	RT	1 M solution
IVA_482	Solid	4°C	150
IVA_515	Solid	RT	250
IVA_532	Solid	RT	275
IVA_550	Solid	RT	125
IVA_563	Solid	RT	250
IVA_597	Solid	-20°C	350
IVA_661	Liquid	RT	300
IVA_696	Solid	RT	125
IVA_779	Solid	RT	325
IVA_805	Solid	RT	125
IVA_820	Solid	RT, inert gas	300
IVA_834	Solid	4°C	700
IVA_920	Solid	RT	200
IVA_992	Solid	RT, inert gas	375

-20°C: Freezer set to maintain -20°C; 4°C: Refrigerator set to maintain 4°C; RT: Room temperature (15 to 25°C).

4.1.2. Control Materials

4.1.2.1. Vehicle

Dimethyl sulfoxide (DMSO) and milli-Q water (MQ) were used as vehicle.

4.1.2.2. Substrate

L-tyrosine (CAS# 60-18-4) was used as substrate for the TPO-catalyzed iodination assay.

4.1.2.3. Metabolite

Monoiodotyrosine (MIT) (CAS# 70-78-0) was the metabolite that was evaluated in the TPO-catalyzed iodination assay.

4.1.2.4. Internal Standard

Monoiodotyrosine-$^{13}C_6$ (MIT-$^{13}C_6$) was used as internal standard (IS) in the TPO-catalyzed iodination assay.

4.1.2.5. Negative Control

Bis-(2-ethylhexyl)-phthalate (DEHP, CAS# 117-81-7) was used as a negative control.

4.1.2.6. Reference Material

6-Propyl-2-thiouracil (PTU, CAS# 51-52-5) was used as a reference material.

4.2. Reserve Samples

Reserve samples of the test materials were not collected and maintained.

4.3. Test and Control Material and Internal Standard Inventory and Disposition

The test and control materials and IS were received by the Test Facility for distribution as needed. Records of the receipt, distribution, and storage of test and control materials were maintained. With the exception of the reserve sample, all unused Sponsor-supplied test material will be discarded or returned to the Sponsor after completion of the scheduled program of work. Records of the decisions made will be kept at the Test Facility.

4.4. Dose Formulation and Analysis

4.4.1. Preparation of Test Materials

No correction for the purity/composition of the test materials was performed.

Before performing the first TPO-catalyzed iodination experiment, a solubility test was performed for the test materials to determine the appropriate concentration range to be tested (see Section 4.7.1.1).

4.4.1.1. Stock Solutions of the Test Materials

For IVA_301, a 50 mM stock solution was prepared in DMSO by dissolving the entire vial containing 10 mg of the test material into 400 µL of DMSO. The resulting solution was transferred to another glass vial in steps of 100 µL and by rinsing the vial during transfer. IVA_301 stock solutions were stored in the freezer set to maintain -20°C for a maximum of one month.

For IVA_597, a 100 mM stock solution was prepared in DMSO by weighing up to 10 mg of the test material and dissolving this in the appropriate volume of DMSO. IVA_597 stock solutions were stored in the freezer set to maintain -20°C for a maximum of one month.

For all other test materials, based on the solubility assessments, appropriate stock solutions of the test material were prepared freshly in DMSO or MQ for each experiment on the day of use.

4.4.1.2. Spiking Solutions of the Test Materials

For each experiment, the test material stock solution was further diluted in DMSO or MQ to obtain eight 100× spiking solutions. The spiking solutions were further diluted in the incubation mixtures. The final concentrations of the test material in the different TPO-catalyzed iodination experiments are presented in Text Table 1. Any residual volumes were discarded.

Text Table 1
Final Test Material Concentrations

Test Material	Final Concentrations used for First Experiment	Final Concentrations used for Second Experiment *
IVA_012	100 pM, 1 nM, 10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM and 10 µM	1 nM, 10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM and 10 µM
IVA_071	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM
IVA_135	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 316 µM	10 nM, 100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM and 316 µM
IVA_136	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM
IVA_140	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	10 nM, 100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM and 1 mM
IVA_151	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM
IVA_226	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 100 µM
IVA_246	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM
IVA_254	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 31.6 µM
IVA_301	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 500 µM	10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 100 µM
IVA_303	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 31.6 µM
IVA_310	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_341	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 100 µM
IVA_387	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_401	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 316 µM	100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM and 316 µM
IVA_431	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 316 µM	10 nM, 100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM and 316 µM
IVA_482	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 100 µM
IVA_515	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	1 nM, 3.16 nM, 10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM and 10 µM
IVA_532	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 316 µM	100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM and 316 µM
IVA_550	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_563	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 316 µM	100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM and 316 µM
IVA_597	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_661	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 31.6 µM and 100 µM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM
IVA_696	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM
IVA_779	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_805	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_820	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_834	100 pM, 1 nM, 10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM and 10 µM	1 nM, 10 nM, 31.6 nM, 100 nM, 316 nM, 1 µM, 3.16 µM and 10 µM
IVA_920	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM	100 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM, 100 µM, 316 µM and 1 mM
IVA_992	100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 316 µM	10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM, 31.6 µM and 100 µM

* For IVA_135 the same concentration range as in experiment 2 was used in experiment 3.

4.4.2. Preparation of Control Material Stock and Spiking Solutions

100 mM DEHP stock solutions were prepared in DMSO and stored in the freezer set to maintain -20°C for a maximum of one month.

The negative control suppressor DEHP was tested at one concentration. For this purpose, the DEHP stock solution was further diluted in the incubation mixture. The final DEHP concentration in the TPO suppression assay incubation mixture was 1 mM.

For each experiment, a 3.16 mM PTU stock solution was prepared freshly in DMSO on the day of use.

The PTU stock solution was further diluted in DMSO to obtain eight 100× spiking solutions. The spiking solutions were further diluted in the incubation mixtures. The final concentrations in the TPO-catalyzed iodination assay incubation mixtures were 1 nM, 10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 31.6 µM.

Any residual volumes were discarded.

4.4.3. Preparation of Internal Standard Solutions

No correction was made for the purity/composition of the IS.

A stock solution of the IS was prepared in 0.1 M HCl at a target concentration of 1 mg/mL. The stock solution was aliquoted in glass vials and stored in the freezer set to maintain -20°C.

The IS stock solution was diluted in methanol containing 300 µM sodium thiosulfate to obtain a solution of 3000 ng IS/mL (IS working solution). The IS working solution was prepared freshly on the day of use.

Any residual volumes were discarded.

4.5. Test System

Test system	FTC-238-hrTPO cell lysates.
Rationale	The FTC-238 human follicular thyroid carcinoma cell line was established from a lung metastasis of a follicular thyroid carcinoma from a 42-year-old male. The cells are polymorphic showing flat polygonal to spindle-like morphologies. The FTC-238 cells are genetically modified to incorporate human recombinant TPO and a neomycin resistance gene. Prepared cell lysates of hematin-stimulated FTC-238-hrTPO cells contain active human thyroid peroxidase.
Source	The FTC-238-hrTPO cell line was provided by the study Sponsor EURL ECVAM, who obtained it from Charité.
Storage	FTC-238-hrTPO cell lysates were stored in an ultra-low freezer set to maintain -80°C.

4.6. Reagents

2-Propanol	LiChrosolv, Merck KGaA, Darmstadt, Germany
6-Propyl-2-thiouracil (PTU)	Batch BCBX0879, Sigma Aldrich Chemie GmbH, Schnelldorf, Germany

Acetonitrile (ACN)	ULC/MS, Biosolve B.V., Valkenswaard, The Netherlands
Bis-(2-ethylhexyl)-phthalate (DEHP)	Batch BCBX5578, Sigma Aldrich Chemie GmbH, Steinheim, Germany
Dimethyl sulfoxide (DMSO)	SeccoSolv, Merck KGaA
Dipotassium hydrogen phosphate (K_2HPO_4)	p.a. Merck KGaA
Formic acid (FA)	99%, ULC/MS, Biosolve B.V.
Hydrochloric acid (HCl) 1N	TitriPUR, Merck KGaA
Hydrogen peroxide (H_2O_2) 30%	EMPROVE, Merck KGaA
L-tyrosine	Batch L0121, BioConnect BV, Huissen, The Netherlands
Methanol (MeOH)	Absolute, ULC/MS, Biosolve B.V.
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion exchange cartridges; Millipore, Bedford, MA, USA
Monoiodotyrosine (MIT)	Batch BCBZ6000, Sigma-Aldrich, Saint Louis, USA
Monoiodotyrosine-$^{13}C_6$ (MIT-$^{13}C_6$)	8-NAV-34-2, Toronto Research Chemicals, Toronto, Canada
Potassium dihydrogen phosphate (KH_2PO_4)	p.a. Merck KGaA
Potassium iodide (KI)	p.a. Merck KGaA
Sodium thiosulfate ($Na_2S_2O_3$)	Honeywell, Seelze, Germany

4.7. Experimental Design

Incubations were performed with FTC-238-hrTPO cell lysates and the test materials to evaluate the possible suppressive effect of the test material on the TPO-catalyzed iodination by measuring the formation of MIT.

For IVA-135, three independent and valid runs were performed. For all other test items, two independent and valid runs were performed.

4.7.1. Main Study

4.7.1.1. Solubility Test

For each test material, a preliminary test was performed to determine whether the test material had any solubility problems, i.e., the presence of cloudiness or precipitate was evaluated. For IVA_301, a stock solution was prepared in DMSO at an initial concentration of 50 mM. All other test materials were dissolved in DMSO or MQ to prepare a stock solution at an initial concentration of 100 mM. The presence of cloudiness or precipitate was evaluated by visual inspection and under the microscope. If necessary, the test material stock solution was further diluted (steps of ½ log lower) to define the highest soluble concentration of test material in vehicle.

Subsequently, a 100-fold dilution of the stock solution was prepared in the incubation buffer and it was determined whether the test material had any solubility problems in the incubation buffer, i.e., the presence of cloudiness or precipitate was evaluated by visual inspection and under the microscope. If necessary, the test material stock solution in vehicle was further diluted (e.g., ½ log lower) to define the highest soluble concentration of test material in the incubation buffer.

4.7.1.2. TPO-Catalyzed Iodination Assay

In the TPO-catalyzed iodination assay, incubations were performed with FTC-238-hrTPO cell lysates to determine the possible suppressory effect of the test materials on the TPO-catalyzed iodination by measuring the formation of the metabolite MIT. Two or three (IVA_135 only) independent and valid runs were performed for each test material. If applicable, concentrations of the test material used in the second experiment were adjusted. For IVA_135, the same concentrations were used for the and third experiments.

Incubation mixtures were prepared on ice by mixing phosphate buffer (0.1 M, pH 7.4), potassium iodide (final concentration 150 µM), L-tyrosine (final concentration 500 µM) FTC-238-hrTPO cell lysate (final concentration 1000 µg/mL) and vehicle, test material or control material. After shaking, the samples were pre-incubated for 5 minutes at 37±1°C in a water bath and the reaction was started by the addition of 20 µL H_2O_2 (final concentration 250 µM). The total volume was 300 µL and incubations were also performed in the water bath at 37±1°C. Incubations were stopped after 15 minutes by transferring the reaction tubes to ice and addition of a half reaction volume of the 3000 ng/mL IS working solution. After vortex-mixing of the containers, the samples were kept on ice until centrifugation (2000 *g* for at least 5 minutes at 4°C) and prepared for MIT analysis by UPLC-MS/MS by diluting them 100-fold in potassium phosphate buffer (0.1 M, pH 7.4).

Vehicle controls, assay buffer controls (no vehicle controls), no peroxide controls (water instead of H_2O_2), non-enzymatic iodination controls and the negative control DEHP at a single concentration (1 mM) were included.

An overview of the incubations included for each independent experiment is presented in the table below.

Text Table 2
Composition of Incubation Samples

Constituent (final concentration)	**No peroxide control**	**Vehicle control**	**No vehicle control**	**Non-enzymatic iodination control**	**Test material, reference material or negative control material incubations**
Phosphate buffer (0.1 M, pH 7.4)	X	X	X	X	X
Tyrosine (500 µM)	X	X	X	X	X
Potassium Iodide (150 µM)	X	X	X	X	X
H_2O_2 (250 µM)	---	X	X	X	X
FTC-238-hrTPO cell lysate	X	X	X	---	X
Inactivated FTC-238-hrTPO cell lysate	---	---	---	X	---
Vehicle (1%)	X	X	---	X	---
Test item or PTU (in triplicate) and DEHP (in triplicate)	---	---	---	---	X

4.7.2. Analysis of MIT

4.7.2.1. MIT Stock and Spiking Solutions

Duplicate MIT stock solutions (stocks A and B) were prepared at a 10 mM concentration in 0.1 M HCl in glass vials. Stock solutions were prepared freshly on the day of use.

MIT stock solutions were diluted in 0.1 M potassium phosphate buffer pH 7.4 to obtain spiking solutions for the preparation of calibration standards and quality control (QC) samples. Spiking solutions were prepared freshly each experimental day as presented in the tables below.

Text Table 3
Preparation of MIT Spiking Solutions used for the Preparation of Calibration Standards

Code	Applied solution	Volume applied (µL)	Volume buffer added (µL) [1)]	Target concentration (µM)
Spike 0.0761 µM	Spike 0.352 µM	108	392	0.0761
Spike 0.163 µM	Spike 0.756 µM	108	392	0.163
Spike 0.352 µM	Spike 1.63 µM	108	392	0.352
Spike 0.756 µM	Spike 3.50 µM	108	392	0.756
Spike 1.63 µM	Spike 7.55 µM	108	392	1.63
Spike 3.50 µM	Spike 16.2 µM	108	392	3.50
Spike 7.55 µM	Spike 35.0 µM	108	392	7.55
Spike 16.2 µM	Spike 75.0 µM	108	392	16.2
Spike 35.0 µM	150 µM – B	105	345	35.0
Spike 75.0 µM	150 µM – A	300	300	75.0
150 µM – B	Stock B	15	985	150
150 µM – A	Stock A	15	985	150

[1)] The buffer consisted of 0.1 M potassium phosphate buffer pH 7.4.

Text Table 4
Preparation of MIT Spiking Solutions used for the Preparation of QC Samples

Code	Applied solution	Volume applied (µL)	Volume buffer added (µL) [1)]	Target concentration (µM)
Spike 15 µM	Spike 75 µM	200	800	0.150
Spike 75 µM	Spike 600 µM	125	875	0.750
Spike 600 µM	Spike 6 mM	100	900	NA
Spike 6 mM	Stock A	600	400	60.0

[1)] The buffer consisted of 0.1 M potassium phosphate buffer pH 7.4.
NA: Not applicable, the spiking solution was used as an intermediate solution only.

4.7.2.2. Calibration Standards

Ten calibration standards were prepared from MIT spiking solutions which were prepared from two MIT stock solutions (A and B as described in Section 4.7.2.1). An aliquot of the appropriate spiking solution was added to the IS working solution as presented in the table below. Samples were vortex-mixed and prepared for UPLC-MS/MS analysis by diluting them 100-fold in phosphate buffer (0.1 M, pH 7.4). Calibration standards were prepared freshly on the first day of use.

Text Table 5
Preparation of MIT Calibration Standards

Code	Applied solution	Volume Applied		Target concentration (µM) [2)]
		Spiking solution (µL)	IS Working Solution (µL)	
MIT 0.0 µM	Buffer [1)]	300	150	0
MIT 0.0761 µM	Spike 0.0761 µM	300	150	0.0761
MIT 0.163 µM	Spike 0.163 µM	300	150	0.163
MIT 0.352 µM	Spike 0.352 µM	300	150	0.352
MIT 0.756 µM	Spike 0.756 µM	300	150	0.756
MIT 1.63 µM	Spike 1.63 µM	300	150	1.63
MIT 3.50 µM	Spike 3.50 µM	300	150	3.50
MIT 7.55 µM	Spike 7.55 µM	300	150	7.55
MIT 16.2 µM	Spike 16.2 µM	300	150	16.2
MIT 35.0 µM	Spike 35.0 µM	300	150	35.0
MIT 75.0 µM	Spike 75.0 µM	300	150	75.0

[1)] The buffer consisted of 0.1 M potassium phosphate buffer pH 7.4.
[2)] Concentration in the sample before addition of the IS working solution (see Section 4.4.3).

The LLOQ (lower limit of quantification) was defined as 0.0761 µM while the ULOQ (upper limit of quantification) was defined as 75.0 µM when the acceptance criteria were met. The values below LLOQ were reported but it was specified that they were LLOQ and the LLOQ was used for calculations.

4.7.2.3. Preparation of Quality Control (QC) Samples

MIT spiking solutions were applied to prepare quality control (QC)-0, -low (L), -middle (M), -high (H) samples. First, a blank matrix working solution was prepared by mixing the following components:

(1) 4631 µL 0.1 M potassium phosphate buffer pH 7.4
(2) 300 µL 10 mM L-Tyrosine dissolved in 0.1 M HCl
(3) 9 µL 100 mM potassium iodide prepared in buffer (1)
(4) 600 µL 1000 µg/mL of heat-inactivated FTC-238-hrTPO cell lysate solution in buffer

An aliquot of the appropriate spiking solution was added to the blank matrix working solution as presented in the table below. The QC samples were prepared freshly on the first day of use.

Text Table 6
Preparation of MIT QC-0, QC-L, QC-M and QC-H Samples

Code	Applied solution	Volume Applied			Target concentration (µM) [2)]
		Spiking solution (µL)	Blank Matrix Working Solution (µL)	IS Working Solution (µL)	
QC-0	Buffer [1)]	3	277	150	0
QC-L	Spike 15 µM	3	277	150	0.150
QC-M	Spike 75 µM	3	277	150	0.750
QC-H	Spike 6 mM	3	277	150	60.0

[1)] The buffer consisted of 0.1 M phosphate buffer pH 7.4.
[2)] Concentration in the sample before addition of the IS working solution (see Section 4.4.3).

QC-0, QC-L, QC-M and QC-H samples were prepared in polypropylene tubes in quadruplicate as presented in the table above and were vortex-mixed after which 20 µL 3.75 mM H_2O_2 was added to each tube. After vortex-mixing of the containers one by one, the samples were kept on ice until centrifugation (2000 *g* for at least 5 minutes at 4°C) and prepared for UPLC-MS/MS analysis by diluting them 100-fold in potassium phosphate buffer (0.1 M, pH 7.4).

4.7.2.4. UPLC-MS/MS Analysis

MIT and IS peak areas in the samples were determined by UPLC-MS/MS using the method validated in Charles River Test Facility Study No. 20278296 (see Table 91 in Appendix 2) and the following system:

- Acquity UPLC I-Class system (Waters, Milford, MA, USA)
- Xevo TQ-S mass spectrometer (Waters)

Data were acquired and interpreted with MassLynx software (Waters).

5. ACCEPTABILITY CRITERIA

5.1. Sample Analysis

An UPLC-MS/MS analytical run was considered acceptable if the criteria for the calibration curve and the QC samples were met.

5.1.1. Calibration Curve

The response of the calibration standards was correlated with the nominal MIT concentration of the calibration solutions using regression analysis with a $1/x^2$ weighting factor. Calibration curves with back calculated accuracies within the criterion range of 80-120% of the nominal concentration for the lowest calibration standard and 85-115% of the nominal concentration for the remaining calibration standards were accepted.

When a back calculated accuracy (once established) did not comply with the criterion range, the response of the calibration standard with the highest deviation was rejected and the calibration curve was re-evaluated. Calibration curves were accepted when ≥ 75% of the calibration standards fulfilled the acceptance criteria.

Zero was not part of the calibrated range. Blank samples were not taken into account in the fitting procedure.

5.1.2. QC Samples

The analytical method was considered applicable for the quantitative analysis of MIT in the samples when the accuracy of the QC samples was in the criterion range of 85-115%. Results outside the criterion range were discarded as long as per run 2/3 of the QC samples were accepted with ≥ 50% of each level.

5.2. TPO-Catalyzed Iodination Assay

An independent TPO-catalyzed iodination experiment was considered acceptable if the following criteria were met:

- The final curve for the positive control PTU was composed of a minimum of six concentrations obtained from the average of three replicates after excluding samples on the basis of insolubility, operator errors or other information.
- The final curve of the test material was composed of a minimum of six concentrations obtained from the average of three replicates after excluding samples on the basis of insolubility, operator errors or other information.
- The percentage of TPO-catalyzed iodination of the lowest test material concentration was within the range of 80%-120% when compared to the average activity in the vehicle control samples.
- A complete sigmoidal curve for the reference material PTU was obtained.
- The calculated IC_{50} for PTU was within the range of 0.5 – 5 µM.
- The percentage of TPO-catalyzed iodination compared to the vehicle control for the negative control DEHP was > 80%.
- The average percentage of TPO-catalyzed iodination in no-peroxide control samples was < 1% when compared to the average activity in the vehicle control samples.
- The mean percentage of TPO-catalyzed iodination of the no-vehicle controls was within the range of 80%-120% when compared to the average TPO-catalyzed iodination in the vehicle control samples.

5.3. Data Interpretation Criteria

For each run, a test material was considered negative when the percentage of TPO-catalyzed iodination compared to the average activity in the vehicle control samples remained above 80% for all concentrations tested.

For each run, a test material was considered positive when the percentage of TPO-catalyzed iodination compared to the average activity in the vehicle control samples was less or equal to 80% for at least one concentration and was showing a dose-dependent effect.

A run was considered inconclusive in all other cases.

6. ANALYSIS

6.1. MIT Analysis

Response (R) [units]	Peak area of the analyte × (IS Conc./ IS peak area)
Calibration curve	$R = a \times C_N + b$ where: a = linear regression factor

b = intercept

C_N = nominal concentration

Regression analysis was performed using the least squares method.

Analyzed concentration (C_A) $C_A = \frac{(R - b)}{a}$ [µM]

Accuracy of analytical QC samples $\frac{C_A - C_B}{C_N} \times 100$ [%]

where:

C_B = analyzed concentration in QC-0 sample

6.2. TPO-Catalyzed Iodination

The percentage of TPO-catalyzed iodination compared to the average TPO-catalyzed iodination in the vehicle control samples (= full activity) was calculated for each individual sample (vehicle control, no vehicle control, no peroxide control, DEHP, PTU and test material samples) using the following equation:

$$\%\text{TPO catalyzed iodination} = \frac{C_A \text{ in sample}}{\text{Average } C_A \text{ of vehicle control samples}} \times 100\%$$

If applicable, the IC_{50} value was calculated by plotting the percentage of control activity versus the logarithm of the concentration fitted by the Hill curve model (variable slope, 4 parameters) using GraphPad Prism (GraphPad Software 8.4, San Diego, USA) and the following equation:

$$y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{\left(1 + 10^{((\text{Log}IC_{50} - x) * \text{HillSlope})}\right)}$$

In which the variables were defined as follows:

Y = Percent of the control activity
X = Logarithm (base 10) of the concentration
Top = Top of the curve in same units as Y
Bottom = Bottom of the curve in same units as Y
Log IC_{50} = Logarithm of concentration at which 50% of maximum response is observed
HillSlope = Slope factor of the Hill curve

7. COMPUTERIZED SYSTEMS

Computerized systems used in the study are listed below. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 7
Computerized Systems

System name	Description of Data Collected and/or Analyzed
M-Files®	Reporting and collection of 21 CFR Part 11 compliant signature
MassLynx	System control, data acquisition and integration
REES Centron	Temperature monitoring

8. RETENTION AND DISPOSITION OF RECORDS AND SAMPLES

All study-specific raw data, documentation, Study Plan, and Final Report from this study were archived at the Test Facility at finalization of the report.

Electronic data generated by the Test Facility were archived as noted above, except that files stored on M-Files® (Study Plan and reporting files) were archived at the Charles River Laboratories facility location in Wilmington, Massachusetts, USA.

Disposition of residual/retained analytical samples was as described in the table below.

Text Table 8
Disposition of Residual/Retained Samples

Sample Type	Disposition
Analytical (and Test Material used in analysis)	Discarded

9. RESULTS

9.1. Solubility Assessments

9.1.1. IVA_012

The test material was not soluble in DMSO at a concentration of 100 mM and 31.6 mM but was soluble in DMSO at a concentration of 10 mM. A 100-fold dilution of this 10 mM stock solution in incubation buffer resulted in precipitation of the test material. A similar effect was observed for a test material stock concentration of 3.16 mM. A 100-fold dilution of a 1 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 1 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 10 µM).

9.1.2. IVA_071

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in in precipitation of the test material. A 100-fold dilution of a 10 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 100 µM).

9.1.3. IVA_135

The test material was soluble in DMSO at a concentration of 100 mM, however, a 100-fold dilution of this 100 mM stock solution in incubation buffer resulted in precipitation of the test material. A 100-fold dilution of a 31.6 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 31.6 mM stock solution was used for the test material as highest spiking solution in all three TPO suppression assay experiments (final concentration in incubation mixture: 316 µM).

9.1.4. IVA_136

The test material was not soluble DMSO at any concentration and also not soluble in MQ at a concentration of 100 mM and 31.6 mM but was soluble in MQ at a concentration of 10 mM. A 100-fold dilution of this 10 mM stock solution in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 100 µM).

9.1.5. IVA_140

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.6. IVA_151

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in in precipitation of the test material. A 100-fold dilution of a 10 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 100 µM).

9.1.7. IVA_226

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 1 mM). For the second TPO suppression assay experiment, a 10 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 100 µM).

9.1.8. IVA_246

The test material was soluble in DMSO at a concentration of 100 mM, however, a 100-fold dilution of this 100 mM stock solution in incubation buffer resulted in precipitation of the test material. A similar effect was observed for a test material stock concentration of 31.6 mM. A 100-fold dilution of a 10 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 100 µM).

9.1.9. IVA_254

The test material was soluble in DMSO at a concentration of 100 mM, however, a 100-fold dilution of this 100 mM stock solution in incubation buffer resulted in precipitation of the test material. A similar effect was observed for a test material stock concentration of 31.6 mM. A 100-fold dilution of a 10 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 100 µM). For the second TPO suppression assay experiment, a 3.16 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 31.6 µM).

9.1.10. IVA_301

The test material was soluble in DMSO at a concentration of 50 mM and a 100-fold dilution of the 50 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 50 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 500 µM). For the second TPO suppression assay experiment, the 50 mM stock solution was diluted 5-fold in DMSO to prepare a 10 mM solution which was used as highest test material spiking concentration (final concentration in incubation mixture: 100 µM).

9.1.11. IVA_303

The test material was soluble in DMSO at a concentration of 100 mM, however, a 100-fold dilution of this 100 mM stock solution in incubation buffer resulted in precipitation of the test material. A similar effect was observed for a test material stock concentration of 31.6 mM. A 100-fold dilution of a 10 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 100 µM). For the second TPO suppression assay experiment, a 3.16 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 31.6 µM).

9.1.12. IVA_310

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.13. IVA_341

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 1 mM). For the second TPO suppression assay experiment, a 10 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 100 µM).

9.1.14. IVA_387

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.15. IVA_401

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 31.6 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 316 µM).

9.1.16. IVA_431

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 31.6 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 316 µM).

9.1.17. IVA_482

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 1 mM). For the second TPO suppression assay experiment, a 10 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 100 µM).

9.1.18. IVA_515

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 1 mM). For the second TPO suppression assay experiment, a 1 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 10 µM).

9.1.19. IVA_532

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 31.6 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 316 µM).

9.1.20. IVA_550

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.21. IVA_563

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 31.6 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 316 µM).

9.1.22. IVA_597

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.23. IVA_661

The test material was soluble in DMSO at a concentration of 100 mM, however, a 100-fold dilution of this 100 mM stock solution in incubation buffer resulted in precipitation of the test material. A similar effect was observed for a test material stock concentration of 31.6 mM. A 100-fold dilution of a 10 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 10 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 100 µM).

9.1.24. IVA_696

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 1 mM). For the second TPO suppression assay experiment, a 10 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 100 µM).

9.1.25. IVA_779

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.26. IVA_805

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.27. IVA_820

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.28. IVA_834

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in precipitation of the test material. A similar effect was observed for a test material stock concentration of 10 mM and 3.16 mM. A 100-fold dilution of a 1 mM stock solution of the test material in incubation buffer resulted in formation of a clear solution.

Based on the solubility assessment, a 1 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 10 µM).

9.1.29. IVA_920

The test material was soluble in DMSO at a concentration of 100 mM and a 100-fold dilution of the 100 mM stock solutions in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 100 mM stock solution was used for the test material as highest spiking solution in both TPO suppression assay experiments (final concentration in incubation mixture: 1 mM).

9.1.30. IVA_992

The test material was not soluble in DMSO at a concentration of 100 mM but was soluble in DMSO at a concentration of 31.6 mM. A 100-fold dilution of this 31.6 mM stock solution in incubation buffer resulted in the formation of a clear solution.

Based on the solubility assessment, a 31.6 mM stock solution was used for the test material as highest spiking solution in the first TPO suppression assay experiment (final concentration in incubation mixture: 316 µM). For the second TPO suppression assay experiment, a 10 mM stock solution was used for the test material as highest spiking solution (final concentration in incubation mixture: 100 µM).

9.2. TPO-Catalyzed Iodination Assay

In total thirteen individual experiments were performed to evaluate the potential of the test materials to suppress TPO-catalyzed iodination. The experimental dates are given in the table below.

Text Table 9
Experimental Dates for the Thirteen Experiments

Experiment	Experimental Date [dd/Mmm/yyyy]
1-1	14 Jun 2022
1-2	15 Jun 2022
1-3	15 Jun 2022
1-4	17 Jun 2022
1-5	17 Jun 2022
1-6	20 Jun 2022
2-1	28 Jun 2022
2-2	28 Jun 2022
2-3	29 Jun 2022
2-4	29 Jun 2022
2-5	30 Jun 2022
2-6	30 Jun 2022
3	13 Jul 2022

The results of the experiments performed were considered valid and have been reported below.

9.2.1. Results for the Calibration Curves and QC Samples

The analytical run history is presented in Table 1. The results for the calibration curves and QC samples obtained for the different experiments are presented in Table 2 and Table 3, respectively.

A linear relationship was observed between response and concentration for MIT in the range of 0.0761 – 75.0 µM for each of the curves. As the back calculated accuracies of all data points were in the range of 85-115% for all calibration standards for each of the curves, all curves were accepted.

The mean accuracies of all QC samples at each QC level were within 85-115% and therefore, all analytical runs were accepted.

9.2.2. Results for Controls

The results obtained for the vehicle, no vehicle, non-enzymatic iodination, no peroxide and negative (DEHP) controls are presented for all thirteen valid experiments in Table 4 up to Table 16.

The percentage of TPO-catalyzed iodination compared to the vehicle control varied between 93% and 104% in all experiments for the negative control DEHP which was well above the acceptance criterion of > 80%.

For each of the experiments, the mean MIT concentrations of the no-peroxide controls were always below the LLOQ (0.0761 µM) and were therefore considered background. These results confirmed that MIT formation in the incubations is dependent on the presence of H_2O_2.

The mean percentage of TPO-catalyzed iodination of the no-vehicle controls when compared to the average TPO-catalyzed iodination in the vehicle control samples varied between 98% and 109% in all experiments and was thus within the range of 80%-120%.

As all acceptance criteria for the controls were met, all TPO-catalysed iodination experiments were considered valid.

9.2.3. Results for the Reference Material PTU

In each TPO-catalyzed iodination experiment, the reference material PTU was included at eight concentrations in triplicate. Detailed results obtained for PTU are presented in Table 17 up to Table 29. The individual dose response curves for the different experiments are presented in Figure 1 up to Figure 3. The curve fit parameters, log IC_{50} and IC_{50} obtained for PTU are presented in the tables below.

Text Table 10
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for PTU in Experiment 1

Parameter	Exp 1-1	Exp 1-2	Exp 1-3	Exp 1-4	Exp 1-5	Exp 1-6
Bottom (%)	-0.3425	-0.3979	0.6999	-0.4538	0.2609	0.8206
Top (%)	94.93	102.3	97.00	98.65	98.22	94.02
Log IC_{50} (M)	-5.746	-5.700	-5.741	-5.705	-5.739	-5.818
HillSlope	-2.147	-2.209	-3.038	-2.199	-2.495	-3.171
IC_{50} (µM)	1.794	1.997	1.815	1.972	1.824	1.521
Values	24	24	24	24	24	24

Text Table 11
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for PTU in Experiment 2

Parameter	Exp 2-1	Exp 2-2	Exp 2-3	Exp 2-4	Exp 2-5	Exp 2-6
Bottom (%)	1.543	-1.030	0.6724	-0.2821	1.715	-0.8139
Top (%)	96.28	99.20	99.33	101.3	95.38	97.00
Log IC_{50} (M)	-5.889	-5.610	-5.807	-5.726	-5.896	-5.651
HillSlope	-3.673	-2.246	-2.749	-2.242	-3.933	-2.162
IC_{50} (µM)	1.292	2.453	1.561	1.880	1.272	2.231
Values	24	24	24	24	24	24

Text Table 12
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for PTU in Experiment 3

Parameter	Exp 3
Bottom (%)	-0.2349
Top (%)	98.69
Log IC_{50} (M)	-5.725
HillSlope	-2.237
IC_{50} (µM)	1.884
Values	24

For each of the experiments, the curve for the reference material PTU was sigmoidal and composed of a minimum of six concentrations. The reference material PTU suppressed the TPO-catalyzed iodination in a dose-dependent manner in all experiments with IC_{50} values that varied between 1.27 µM and 2.45 µM and which thus were all within the acceptance range of 0.5 – 5 µM.

As all acceptance criteria for the reference material PTU were met, all TPO-catalyzed iodination experiments were considered valid.

9.2.4. Results for the Test Materials

9.2.4.1. IVA_012

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 30 and Table 31. The dose response curves for both experiments are presented in Figure 4. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 13
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%) *	0.000	0.000
Top (%)	92.47	99.07
Log IC_{50} (M)	-4.609	-4.517
HillSlope	-0.9431	-0.8999
IC_{50} (µM)	24.60	30.40
Values	24	24

* For the construction of the individual and the averaged dose response curves, the bottom of the curves was restrained at zero.

For each of the experiments, the curves for the test material were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 94% in experiment 1 and 101% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 24.6 µM and 30.4 µM, respectively. Please do note that in both experiments the percentage of TPO-catalyzed iodination remained above 50% (65% and 72% for experiment 1 and 2, respectively) which resulted in a non-sigmoidal curve as a result of which IC_{50} values were determined using curve fits for which the bottom was restrained at zero.

9.2.4.2. IVA_071

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 32 and Table 33. The dose response curves for both experiments are presented in Figure 5. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 14
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	-4.831	-2.174
Top (%)	94.19	100.6
Log IC_{50} (M)	-5.636	-5.689
HillSlope	-0.7477	-0.8516
IC_{50} (µM)	2.313	2.048
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 93% in experiment 1 and 100% in

experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 2.31 µM and 2.05 µM, respectively.

9.2.4.3. IVA_135

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 34 up to Table 36. The dose response curves for all three experiments are presented in Figure 6. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 15
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2	Exp 3
Bottom (%) *	0.000	0.000	0.000
Top (%)	92.70	102.1	98.07
Log IC_{50} (M)	-2.859	-2.672	-2.789
HillSlope	-0.9276	-0.7104	-0.8895
IC_{50} (µM)	1385	2126	1624
Values	24	24	24

* For the construction of the individual and the averaged dose response curves, the bottom of the curves was restrained at zero.

For each of the experiments, the curves for the test material were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 95% in experiment 1, 104% in experiment 2 and 98% in experiment 3 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

For each of the three experiments, it was found that the test material suppressed the TPO-catalyzed iodination in a dose-dependent manner. The percentage of TPO-catalyzed iodination for the highest test material concentration evaluated compared to the average activity in the vehicle control samples was 74%, 81% and 80% for experiment 1, 2 and 3, respectively. As the test material showed a dose-dependent suppression effect for all three runs while for two out of three runs the percentage of TPO-catalyzed iodination was less or equal to 80% for at least one concentration, the test material was considered to be positive.

Based on the obtained curves, IC_{50} values of 1385 µM, 2126 µM and 1624 µM were calculated for experiments 1, 2 and 3, respectively. Please do note that in all three experiments the percentage of TPO-catalyzed iodination remained above 50% which resulted in a non-sigmoidal curve as a result of which IC_{50} values were determined using curve fits for which the bottom was restrained at zero.

9.2.4.4. IVA_136

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 37 and Table 38. The dose response curves for both experiments are presented in Figure 7. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 16
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	No suppression	No suppression
Top (%)		
Log IC_{50} (M)		
HillSlope		
IC_{50} (µM)		
Values	24	22

For each of the experiments, the mean percentage of TPO-catalyzed iodination compared to the average activity in the corresponding vehicle control samples was not less than 80% for any of the concentrations evaluated.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was negative and did not suppress the TPO-catalyzed iodination. At the two highest concentrations tested (31.6 µM and 100 µM) it was observed that the MIT concentration in the incubation samples had increased when compared to the corresponding MIT concentration in the vehicle control samples. Most likely, the test material evaluated was L-tyrosine or a similar compound.

9.2.4.5. IVA_140

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 39 and Table 40. The dose response curves for both experiments are presented in Figure 8. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 17
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	-0.9928	0.03893
Top (%)	94.08	101.9
Log IC_{50} (M)	-5.013	-5.059
HillSlope	-1.095	-1.120
IC_{50} (µM)	9.711	8.735
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 96% in experiment 1 and 104% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 9.71 µM and 8.74 µM, respectively.

9.2.4.6. IVA_151

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 41 and Table 42. The dose response curves for both experiments are presented in Figure 9. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 18
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	1.507	14.11
Top (%)	98.79	100.1
Log IC_{50} (M)	-4.089	-4.219
HillSlope	-0.7213	-0.8581
IC_{50} (µM)	81.38	60.37
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 98% in experiment 1 and 99% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 81.4 µM and 60.4 µM, respectively.

9.2.4.7. IVA_226

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 43 and Table 44. The individual dose response curves for both experiments are presented in Figure 10. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 19
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.9871	-1.573
Top (%)	98.42	100.0
Log IC_{50} (M)	-5.849	-5.541
HillSlope	-4.006	-2.234
IC_{50} (µM)	1.415	2.876
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 100% in experiment 1 and 100% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 1.42 µM and 2.88 µM, respectively.

9.2.4.8. IVA_246

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 45 and Table 46. The dose response curves for both experiments are presented in Figure 11. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 20
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	No suppression	No suppression
Top (%)		
Log IC_{50} (M)		
HillSlope		
IC_{50} (µM)		
Values	23	24

For each of the experiments, the mean percentage of TPO-catalyzed iodination compared to the average activity in the corresponding vehicle control samples was not less than 80% for any of the concentrations evaluated.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was negative and did not suppress the TPO-catalyzed iodination.

9.2.4.9. IVA_254

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 47 and Table 48. The dose response curves for both experiments are presented in Figure 12. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 21
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.5232	-1.678
Top (%)	101.3	82.15
Log IC_{50} (M)	-6.253	-6.046
HillSlope	-1.523	-1.411
IC_{50} (µM)	0.5586	0.8997
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 102% in experiment 1 and 80% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 0.56 µM and 0.90 µM, respectively.

9.2.4.10. IVA_301

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 49 and Table 50. The dose response curves for both experiments are presented in Figure 13. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 22
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.8260	1.582
Top (%)	98.28	93.61
Log IC_{50} (M)	-6.076	-5.992
HillSlope	-2.104	-2.764
IC_{50} (µM)	0.8389	1.019
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 97% in experiment 1 and 88% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 0.84 µM and 1.02 µM, respectively.

9.2.4.11. IVA_303

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 51 and Table 52. The dose response curves for both experiments are presented in Figure 14. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 23
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
2Bottom (%)	0.4809	-0.4948
Top (%)	99.48	96.31
Log IC_{50} (M)	-6.403	-6.322
HillSlope	-1.342	-1.404
IC_{50} (µM)	0.3953	0.4763
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 101% in experiment 1 and 98% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 0.40 µM and 0.48 µM, respectively.

9.2.4.12. IVA_310

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 53 and Table 54. The dose response curves for both experiments are presented in Figure 15. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 24
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	-6.285	-10.66
Top (%)	100.4	100.4
Log IC_{50} (M)	-3.763	-3.588
HillSlope	-1.032	-0.9701
IC_{50} (µM)	172.5	258.1
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 102% in experiment 1 and 102% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 173 µM and 258 µM, respectively.

9.2.4.13. IVA_341

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 55 and Table 56. The dose response curves for both experiments are presented in Figure 16. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 25
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.9067	0.9087
Top (%)	100.8	94.89
Log IC_{50} (M)	-5.819	-5.850
HillSlope	-3.112	-4.258
IC_{50} (µM)	1.515	1.411
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 101% in experiment 1 and 97% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 1.52 µM and 1.41 µM, respectively.

9.2.4.14. IVA_387

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 57 and Table 58. The dose response curves for both experiments are presented in Figure 17. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 26
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%) *	0.000	0.000
Top (%)	99.67	96.48
Log IC_{50} (M)	-2.867	-2.751
HillSlope	-1.396	-0.9640
IC_{50} (µM)	1358	1774
Values	24	24

* For the construction of the individual and the averaged dose response curves, the bottom of the curves was restrained at zero.

For each of the experiments, the curves for the test material were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 100% in experiment 1 and 97% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 1358 µM and 1774 µM, respectively. Please do note that in both experiments the percentage of TPO-catalyzed iodination remained above 50% (60% and 61% for experiment 1 and 2, respectively) which resulted in a non-sigmoidal curve as a result of which IC_{50} values were determined using curve fits for which the bottom was restrained at zero.

9.2.4.15. IVA_401

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 59 and Table 60. The dose response curves for both experiments are presented in Figure 18. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 27
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.7790	-0.9481
Top (%)	99.98	95.24
Log IC_{50} (M)	-5.446	-5.444
HillSlope	-1.662	-1.289
IC_{50} (µM)	3.577	3.595
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 102% in experiment 1 and 98% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 3.58 µM and 3.60 µM, respectively.

9.2.4.16. IVA_431

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 61 and Table 62. The dose response curves for both experiments are presented in Figure 19. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 28
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%) *	0.000	0.000
Top (%)	98.82	102.2
Log IC_{50} (M)	-4.248	-4.281
HillSlope	-1.342	-1.336
IC_{50} (µM)	56.54	52.41
Values	24	24

* For the construction of the individual and the averaged dose response curves, the bottom of the curves was restrained at zero.

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 95% in experiment 1 and 101% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 56.5 µM and 52.4 µM, respectively.

9.2.4.17. IVA_482

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 63 and Table 64. The dose response curves for both experiments are presented in Figure 20. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 29
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.9527	-1.113
Top (%)	101.6	106.2
Log IC_{50} (M)	-5.853	-5.740
HillSlope	-3.340	-1.846
IC_{50} (µM)	1.402	1.819
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 102% in experiment 1 and 110% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in

a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 1.40 µM and 1.82 µM, respectively.

9.2.4.18. IVA_515

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 65 and Table 66. The dose response curves for both experiments are presented in Figure 21. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 30
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.9161	0.2012
Top (%)	103.0	92.47
Log IC_{50} (M)	-7.592	-7.487
HillSlope	-1.569	-1.664
IC_{50} (µM)	0.02558	0.03260
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 103% in experiment 1 and 94% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 25.6 nM and 32.6 nM, respectively.

9.2.4.19. IVA_532

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 67 and Table 68. The dose response curves for both experiments are presented in Figure 22. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 31
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%) *	0.000	0.000
Top (%)	100.9	94.95
Log IC_{50} (M)	-4.221	-4.211
HillSlope	-1.274	-1.213
IC_{50} (µM)	60.09	61.53
Values	23	24

* For the construction of the individual and the averaged dose response curves, the bottom of the curves was restrained at zero.

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 102% in experiment 1 and 97% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 60.1 µM and 61.5 µM, respectively.

9.2.4.20. IVA_550

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 69 and Table 70. The dose response curves for both experiments are presented in Figure 23. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 32
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	No suppression	No suppression
Top (%)		
Log IC_{50} (M)		
HillSlope		
IC_{50} (µM)		
Values	24	24

For each of the experiments, the mean percentage of TPO-catalyzed iodination compared to the average activity in the corresponding vehicle control samples was not less than 80% for any of the concentrations evaluated.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was negative and did not suppress the TPO-catalyzed iodination.

9.2.4.21. IVA_563

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 71 and Table 72. The dose response curves for both experiments are presented inFigure 24. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 33
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	1.372	-0.4367
Top (%)	98.91	102.0
Log IC_{50} (M)	-4.764	-4.762
HillSlope	-1.169	-0.9537
IC_{50} (µM)	17.23	17.32
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 99% in experiment 1 and 99% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in

a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 17.2 µM and 17.3 µM, respectively.

9.2.4.22. IVA_597

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 73 and Table 74. The dose response curves for both experiments are presented in Figure 25. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 34
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.1973	-1.856
Top (%)	98.27	98.08
Log IC_{50} (M)	-4.782	-4.703
HillSlope	-1.221	-0.9792
IC_{50} (µM)	16.54	19.84
Values	23	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 97% in experiment 1 and 100% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 16.5 µM and 19.8 µM, respectively.

9.2.4.23. IVA_661

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 75 and Table 76. The dose response curves for both experiments are presented in Figure 26. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 35
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	No suppression	No suppression
Top (%)		
Log IC_{50} (M)		
HillSlope		
IC_{50} (µM)		
Values	24	24

For each of the experiments, the mean percentage of TPO-catalyzed iodination compared to the average activity in the corresponding vehicle control samples was not less than 80% for any of the concentrations evaluated.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was negative and did not suppress the TPO-catalyzed iodination.

9.2.4.24. IVA_696

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 77 and Table 78. The dose response curves for both experiments are presented in Figure 27. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 36
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.9236	1.358
Top (%)	100.3	96.95
Log IC_{50} (M)	-5.811	-5.918
HillSlope	-2.346	-2.250
IC_{50} (µM)	1.545	1.208
Values	23	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 101% in experiment 1 and 99% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 1.55 µM and 1.21 µM, respectively.

9.2.4.25. IVA_779

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 79 and Table 80. The dose response curves for both experiments are presented in Figure 28. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 37
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	-3.609	-10.98
Top (%)	101.4	99.08
Log IC_{50} (M)	-3.760	-3.693
HillSlope	-1.173	-0.9759
IC_{50} (µM)	173.6	202.8
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 102% in experiment 1 and 99% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 174 µM and 203 µM, respectively.

9.2.4.26. IVA_805

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 81 and Table 82. The dose response curves for both experiments are presented in Figure 29. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 38
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	0.8649	0.7671
Top (%)	97.76	90.56
Log IC_{50} (M)	-7.886	-7.776
HillSlope	-1.746	-2.017
IC_{50} (µM)	0.01300	0.01674
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 99% in experiment 1 and 81% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 130 nM and 167 nM, respectively.

9.2.4.27. IVA_820

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 83 and Table 84. The dose response curves for both experiments are presented in Figure 30. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 39
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%) *	0.000	0.000
Top (%)	99.05	101.6
Log IC_{50} (M)	-2.956	-2.603
HillSlope	-5.703	-0.8107
IC_{50} (µM)	1106	2494
Values	24	24

* For the construction of the individual and the averaged dose response curves, the bottom of the curves was restrained at zero.

For each of the experiments, the curves for the test material were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 100% in experiment 1 and 102% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in

a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 1106 µM and 2494 µM, respectively. Please do note that in both experiments the percentage of TPO-catalyzed iodination remained above 50% (63% and 69% for experiment 1 and 2, respectively) which resulted in a non-sigmoidal curve as a result of which IC_{50} values were determined using curve fits for which the bottom was restrained at zero.

9.2.4.28. IVA_834

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 85 and Table 86. The dose response curves for both experiments are presented in Figure 31. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 40
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	No suppression	No suppression
Top (%)		
Log IC_{50} (M)		
HillSlope		
IC_{50} (µM)		
Values	24	24

For each of the experiments, the mean percentage of TPO-catalyzed iodination compared to the average activity in the corresponding vehicle control samples was not less than 80% for any of the concentrations evaluated.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was negative and did not suppress the TPO-catalyzed iodination.

9.2.4.29. IVA_920

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 87 and Table 88. The dose response curves for both experiments are presented in Figure 32. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 41
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	No suppression	No suppression
Top (%)		
Log IC_{50} (M)		
HillSlope		
IC_{50} (µM)		
Values	23	24

For each of the experiments, the mean percentage of TPO-catalyzed iodination compared to the average activity in the corresponding vehicle control samples was not less than 80% for any of the concentrations evaluated.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was negative and did not suppress the TPO-catalyzed iodination.

9.2.4.30. IVA_992

The test material was included at eight concentrations in triplicate in each TPO-catalyzed iodination experiment. Detailed results obtained for the test material are presented in Table 89 and Table 90. The dose response curves for both experiments are presented in Figure 33. The curve fit parameters, log IC_{50} and IC_{50} obtained for the test material are presented in the table below.

Text Table 42
Curve Fit Parameters, Log IC_{50} and IC_{50} obtained for the Test Material

Parameter	Exp 1	Exp 2
Bottom (%)	2.564	4.194
Top (%)	101.5	103.0
Log IC_{50} (M)	-6.453	-6.395
HillSlope	-1.270	-1.306
IC_{50} (µM)	0.3521	0.4031
Values	24	24

For each of the experiments, the curves for the test material were sigmoidal and were composed of a minimum of six concentrations. The mean percentage of TPO-catalyzed iodination of the lowest test material concentration was 104% in experiment 1 and 101% in experiment 2 and thus within the acceptance range of 80%-120% when compared to the average activity in the vehicle control samples.

Based on the outcome of the two valid TPO-catalyzed iodination experiments, it was concluded that the test material was positive and suppressed the TPO-catalyzed iodination in a dose-dependent manner in experiments 1 and 2 with IC_{50} values of 352 nM and 403 nM, respectively.

10. CONCLUSION

The test materials IVA_012, IVA_071, IVA_135, IVA_140, IVA_151, IVA_226, IVA_254, IVA_301, IVA_303, IVA_310, IVA_341, IVA_387, IVA_401, IVA_431, IVA_482, IVA_515, IVA_532, , IVA_563, IVA_597, IVA_696, IVA_779, IVA_805, IVA_820, IVA_992 were found to be positive and suppressed the FTC-238-hrTPO-catalyzed iodination in a dose-dependent manner.

The test materials IVA_136, IVA_246, IVA_550, IVA_661, IVA_834, IVA_920 were found to be negative and did not suppress the FTC-238-hrTPO-catalyzed iodination.

11. REFERENCES

1. Doerge, D.L., Chang, H.C., Divi, R.L., Churchwell, M.I., Mechanism for suppression of thyroid peroxidase by Leucomalachite Green. Chemical Research in Toxicology 11, p. 1098-1104 (1998).

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12. LIST OF ABBREVIATIONS

ACN	Acetonitrile
DEHP	Di-(2-ethylhexyl)phthalate
DMSO	Dimethyl sulfoxide
FA	Formic acid
GLP	Good laboratory practice
HCl	Hydrogen chloride
IC_{50}	Inhibition at 50% of vehicle control
IS	Internal standard
KI	Potassium iodide
LLOQ	Lower limit of quantification
MeOH	Methanol
MIT	Mono-iodotyrosine
MIT-$^{13}C_6$	Mono-iodotyrosine-$^{13}C_6$
MQ	Milli-Q water
NA	Not applicable
PTU	6-propyl-2-thiouracil
QAU	Quality assurance unit
r^2	Determination coefficient
SD	Standard deviation
TPO	Thyroid peroxidase
ULOQ	Upper limit of quantification
UPLC-MS/MS	Ultraperformance liquid chromatography tandem mass spectrometry
VC	Vehicle control

Figure 1
Dose Response Curves for the Reference Material PTU obtained in Experiment 1

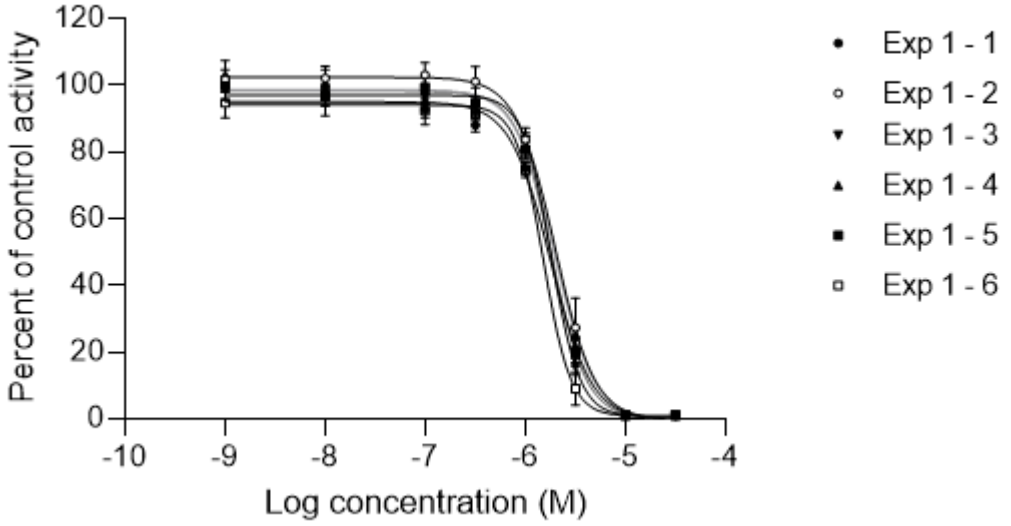

Figure 2
Dose Response Curves for the Reference Material PTU obtained in Experiment 2

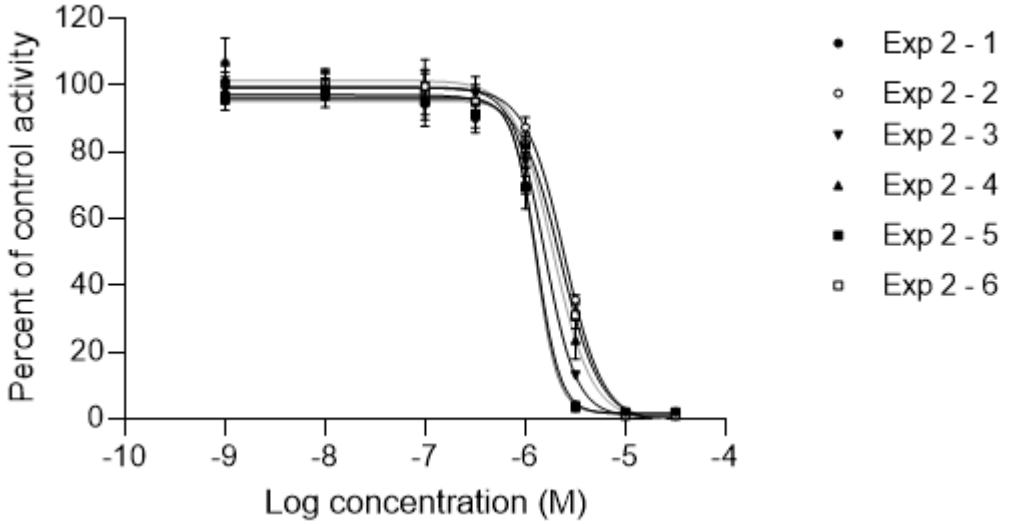

Figure 3
Dose Response Curves for the Reference Material PTU obtained in Experiment 3

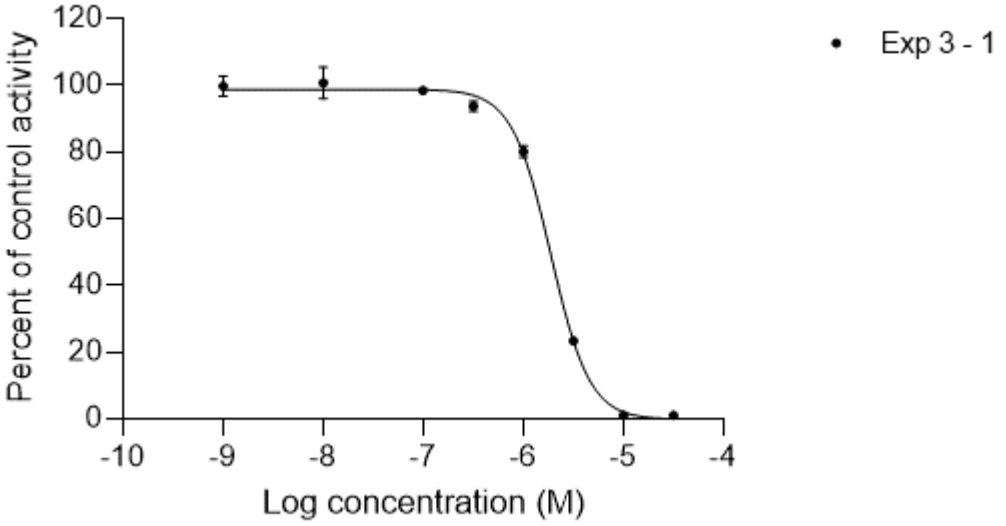

Figure 4
Dose Response Curves for IVA_012

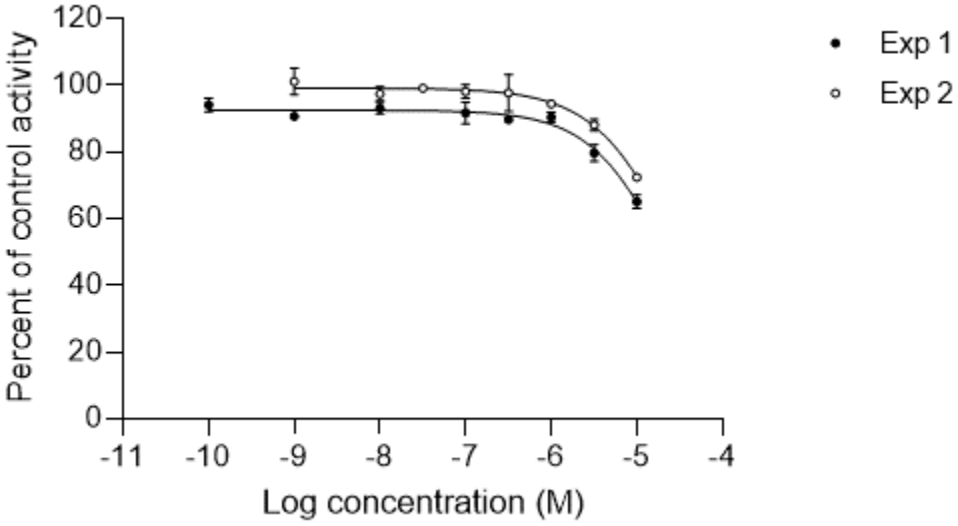

Figure 5
Dose Response Curves for IVA_071

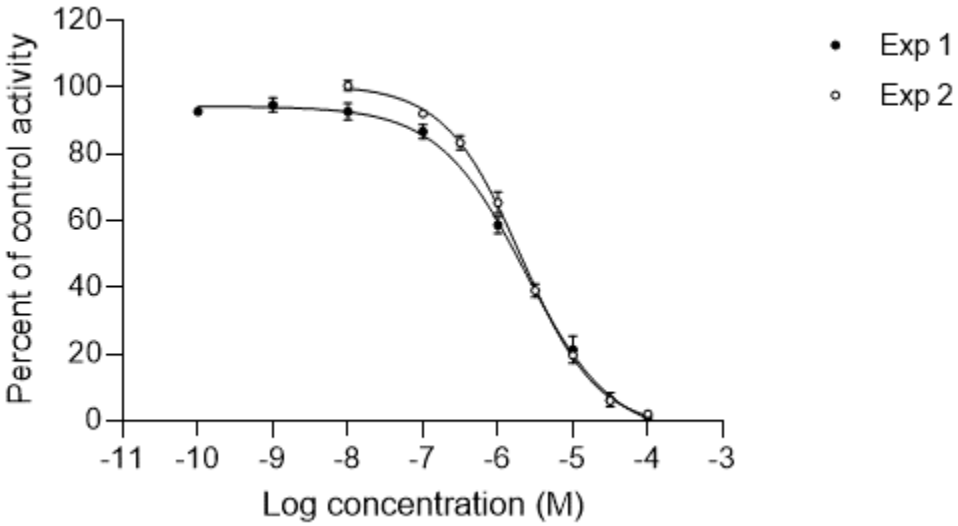

Figure 6
Dose Response Curves for IVA_135

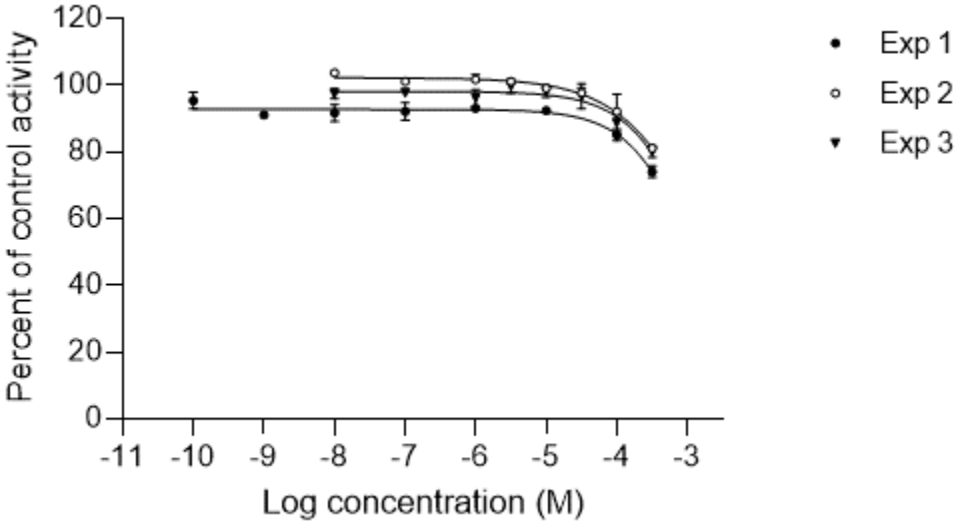

Figure 7
Dose Response Curves for IVA_136

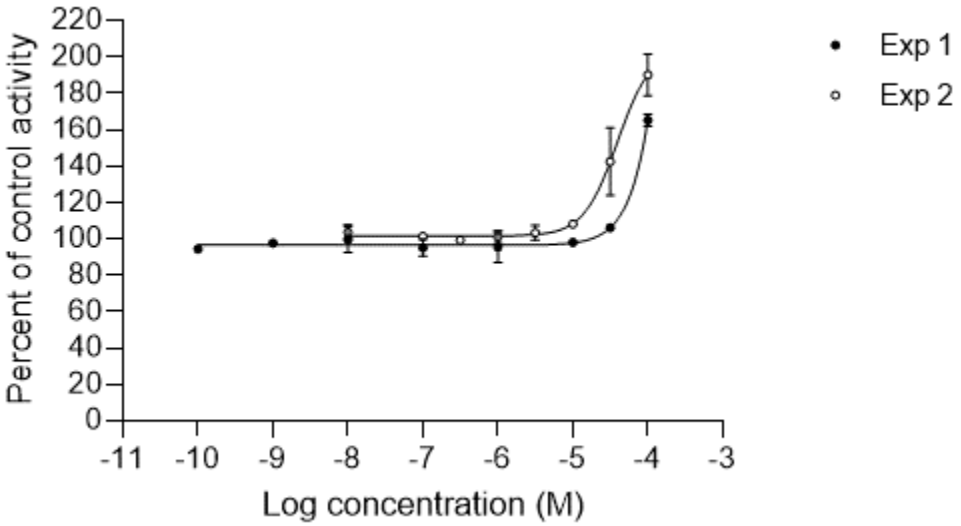

Figure 8
Dose Response Curves for IVA_140

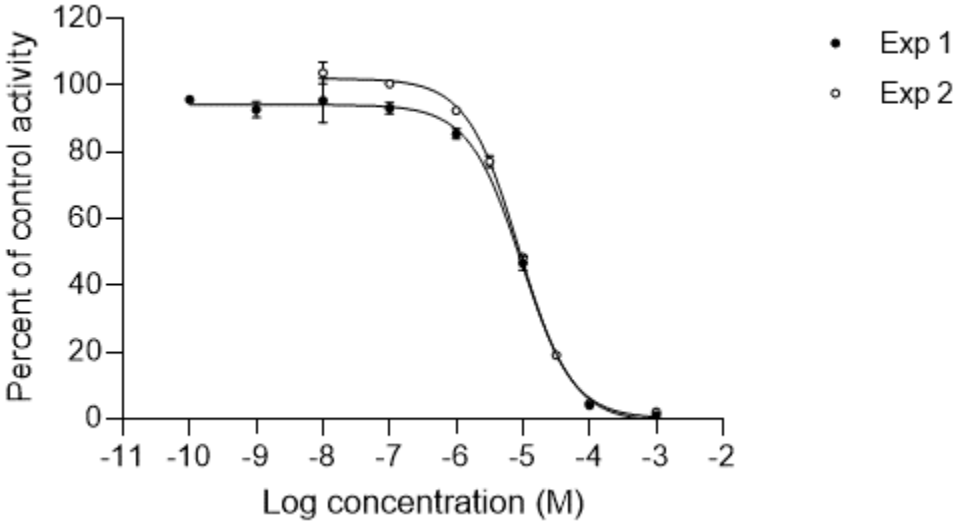

Figure 9
Dose Response Curves for IVA_151

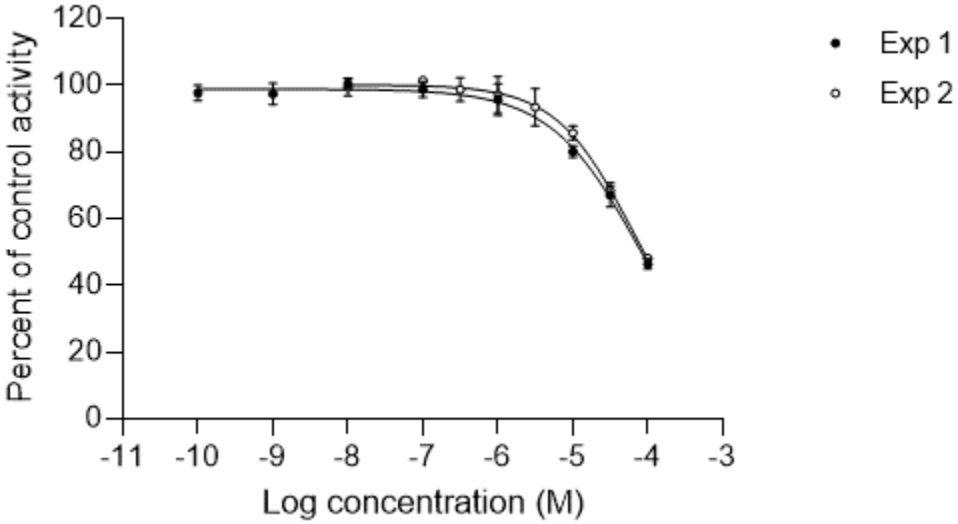

Figure 10
Dose Response Curves for IVA_226

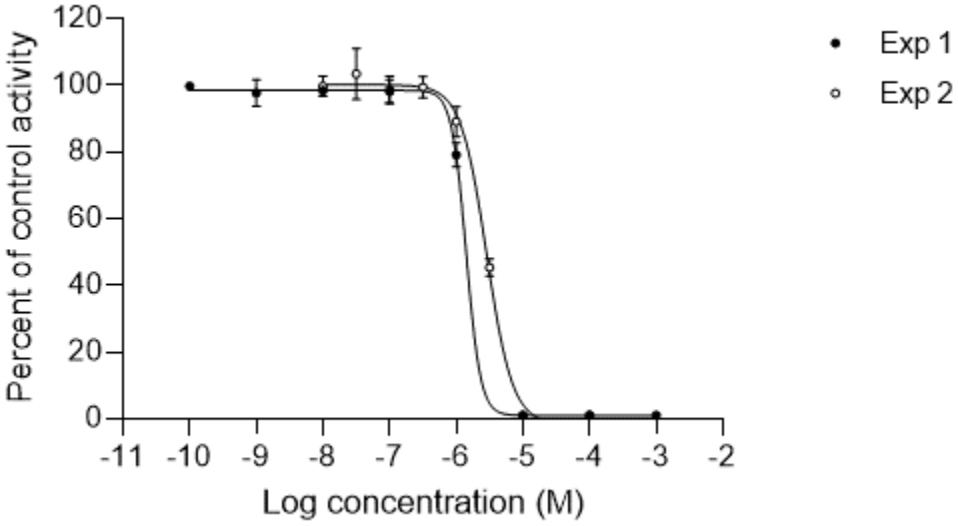

Figure 11
Dose Response Curves for IVA_246

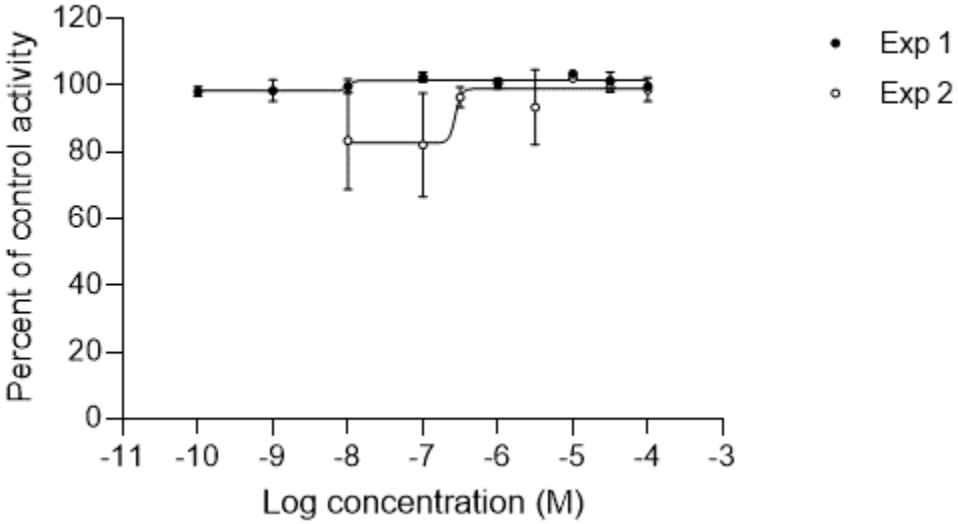

Figure 12
Dose Response Curves for IVA_254

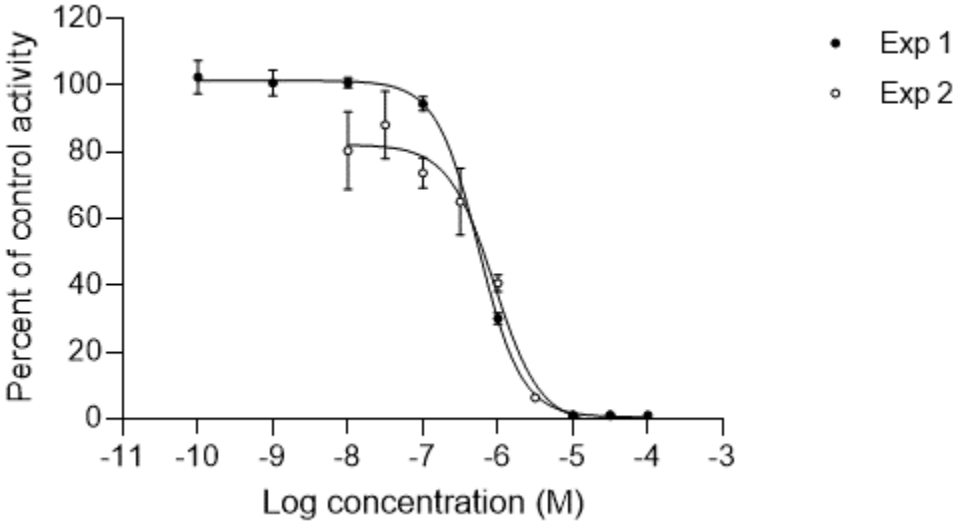

Figure 13
Dose Response Curves for IVA_301

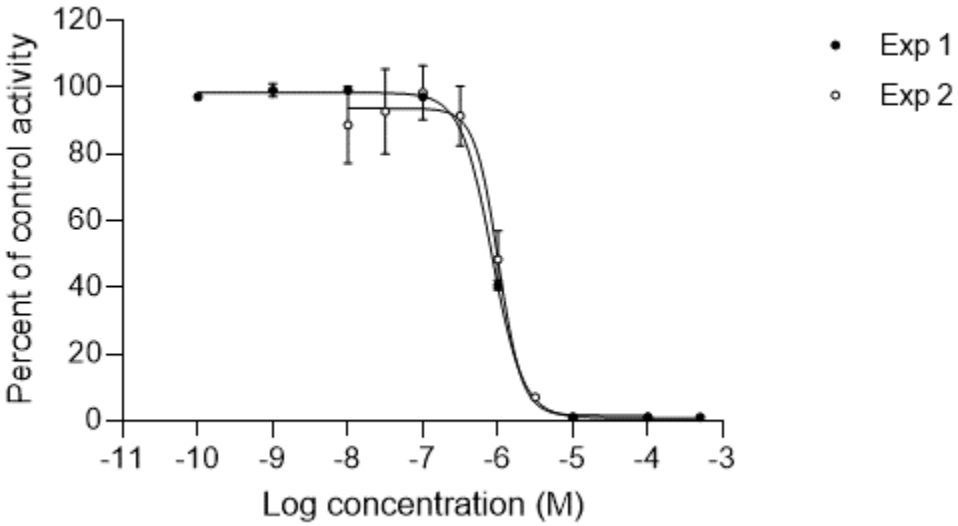

Figure 14
Dose Response Curves for IVA_303

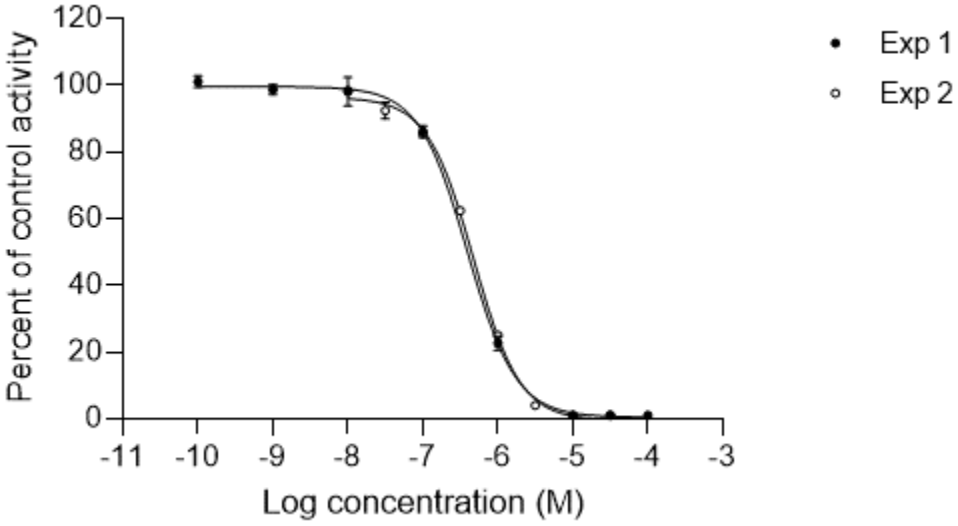

Figure 15
Dose Response Curves for IVA_310

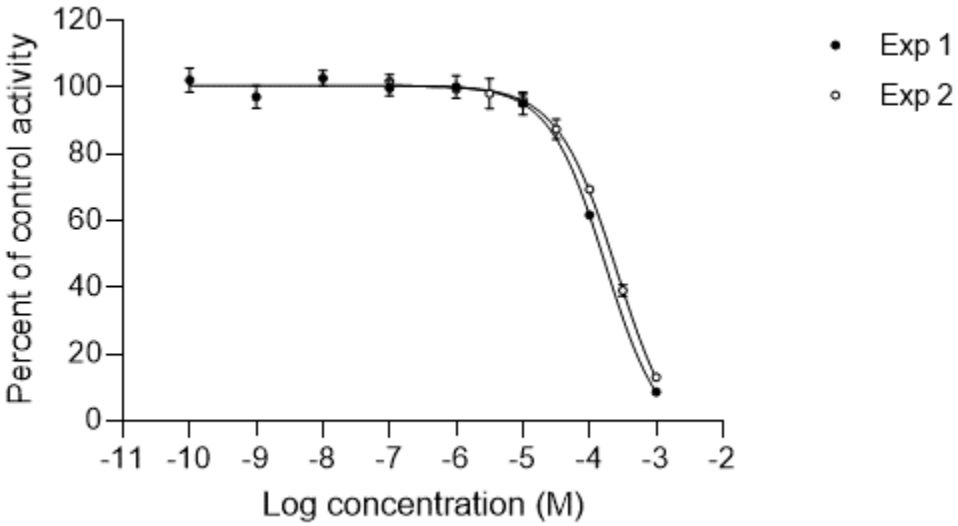

Figure 16
Dose Response Curves for IVA_341

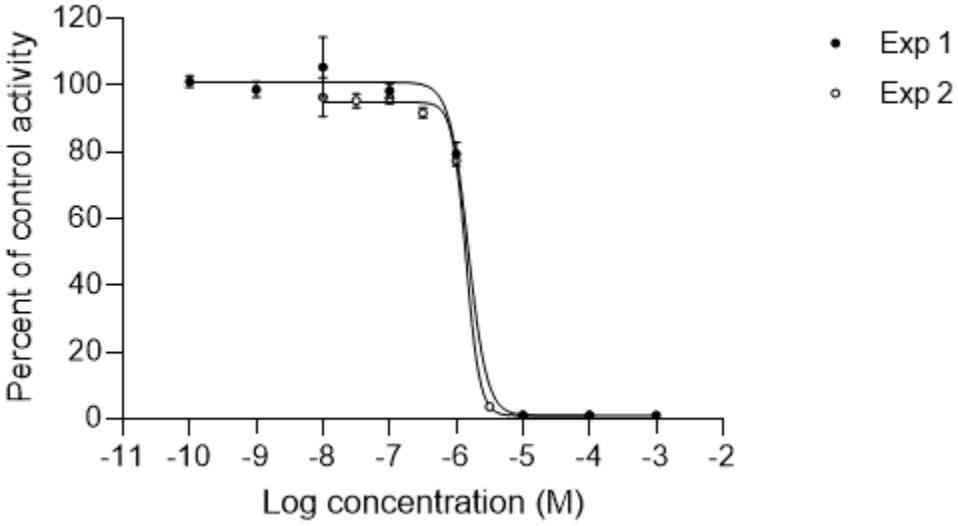

Figure 17
Dose Response Curves for IVA_387

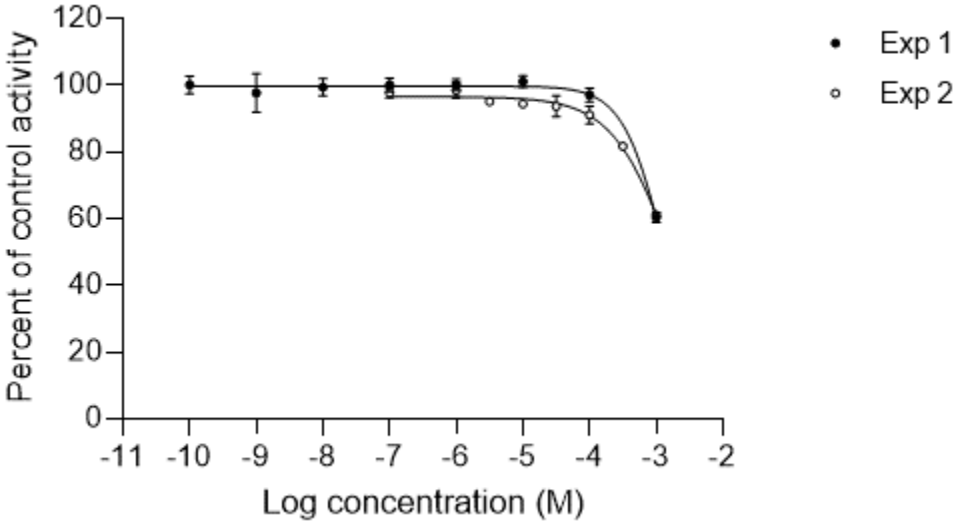

Figure 18
Dose Response Curves for IVA_401

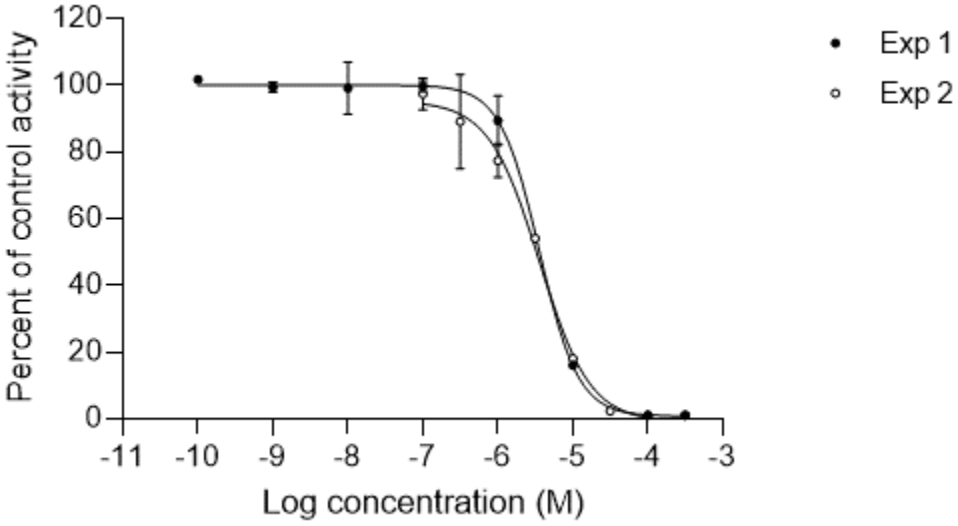

Figure 19
Dose Response Curves for IVA_431

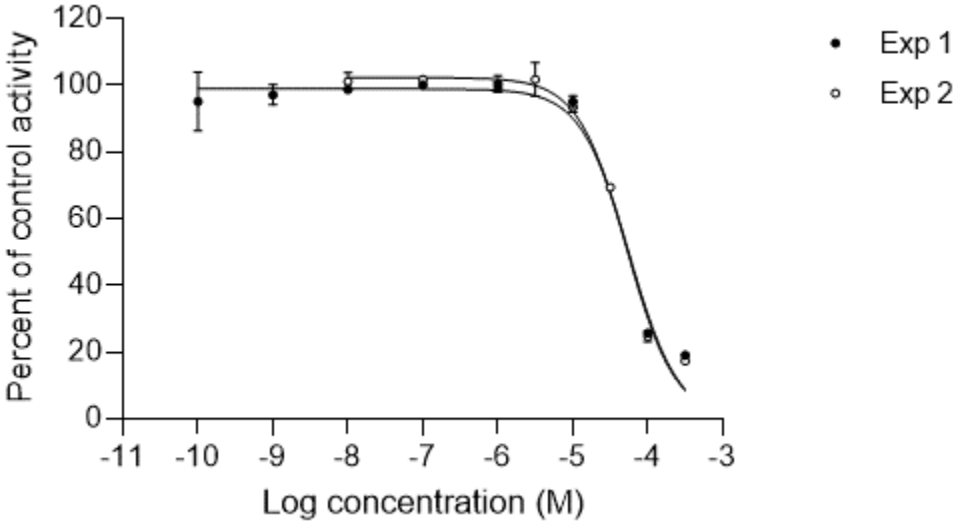

Figure 20
Dose Response Curves for IVA_482

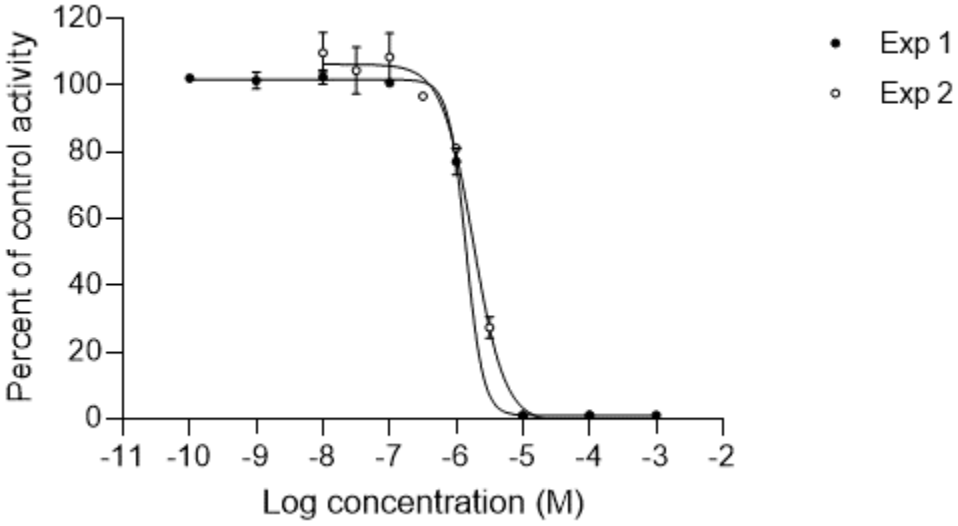

Figure 21
Dose Response Curves for IVA_515

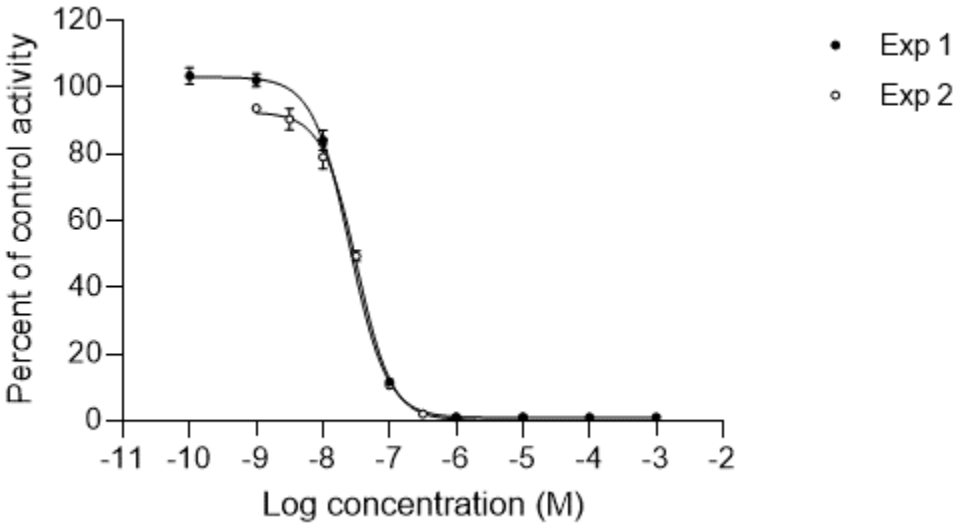

Figure 22
Dose Response Curves for IVA_532

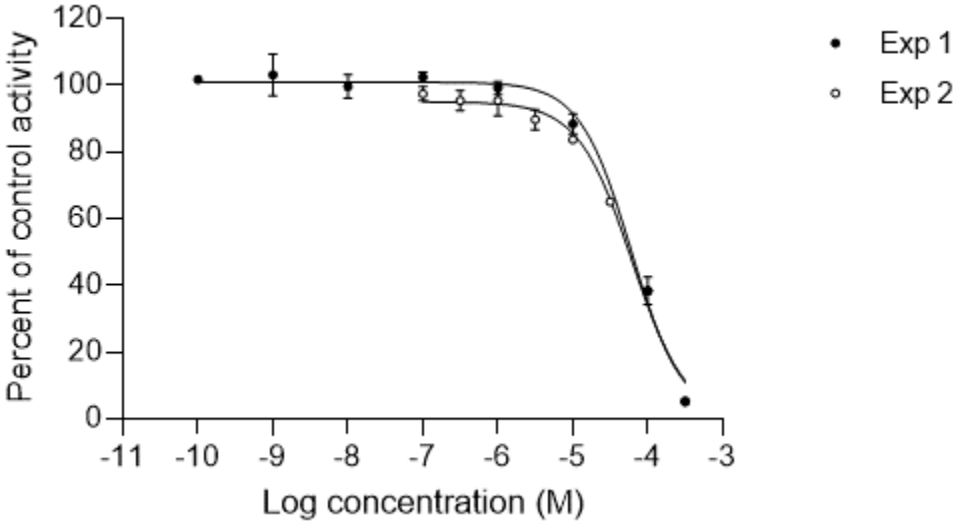

Figure 23
Dose Response Curves for IVA_550

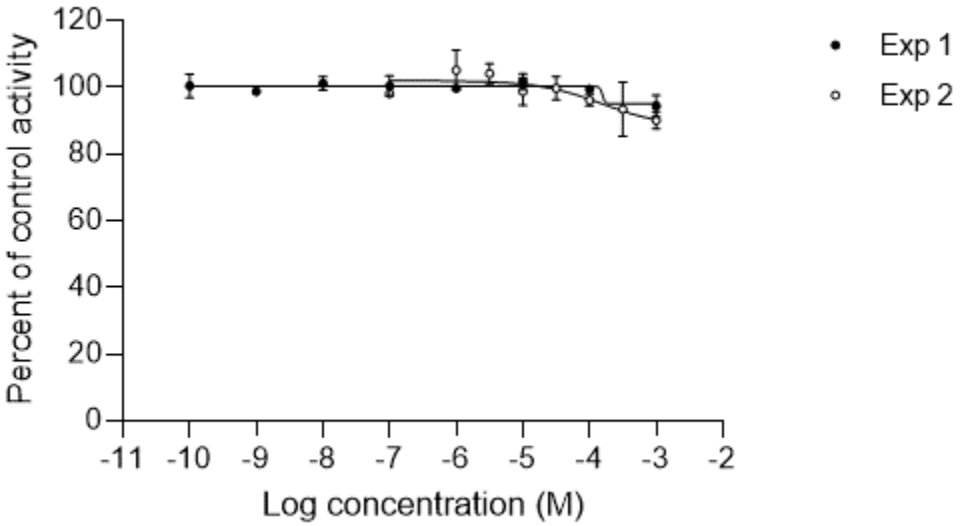

Figure 24
Dose Response Curves for IVA_563

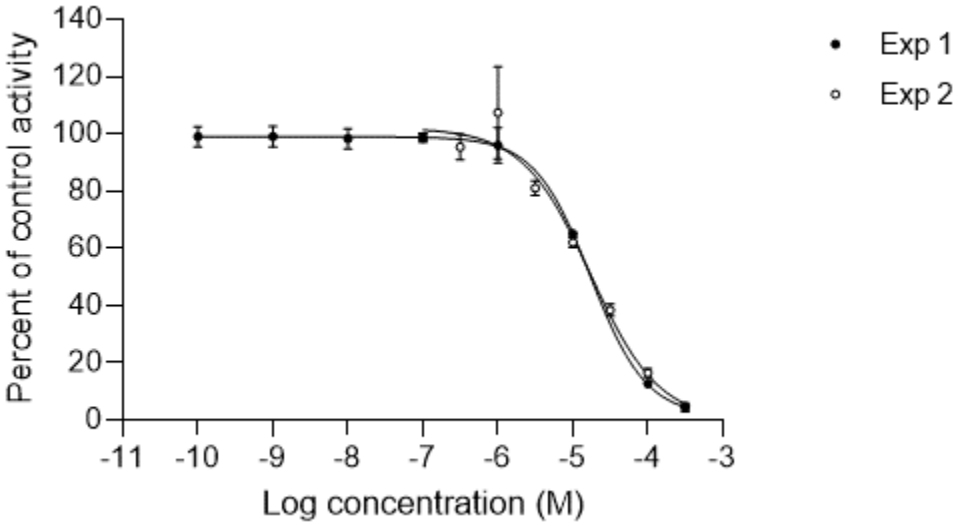

Figure 25
Dose Response Curves for IVA_597

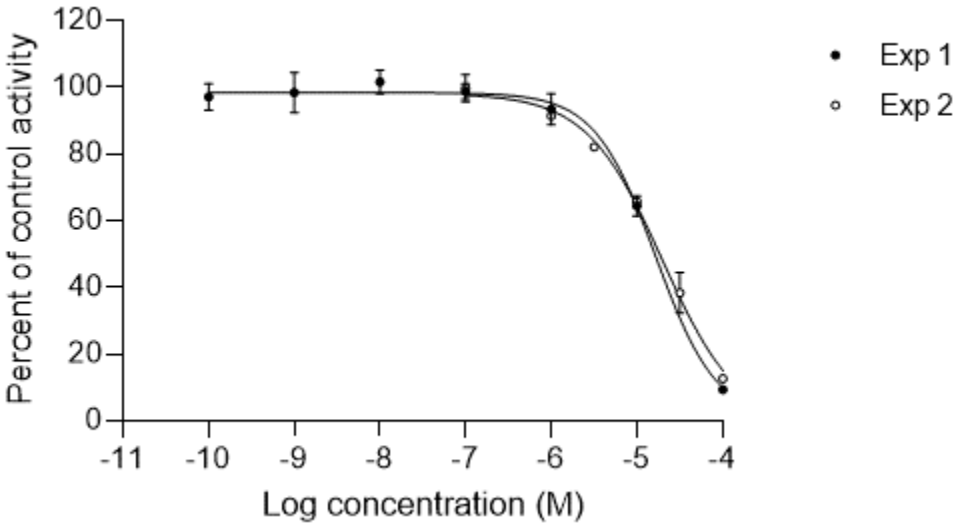

Figure 26
Dose Response Curves for IVA_661

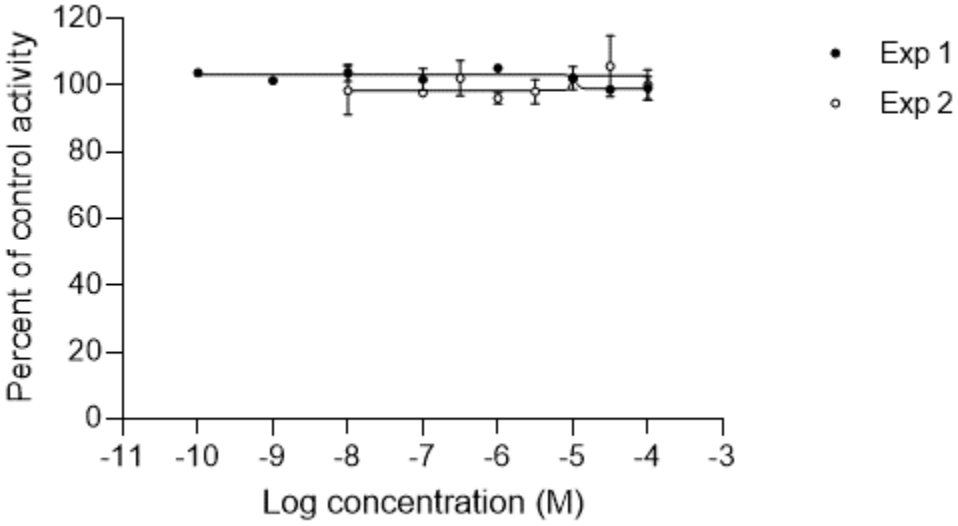

Figure 27
Dose Response Curves for IVA_696

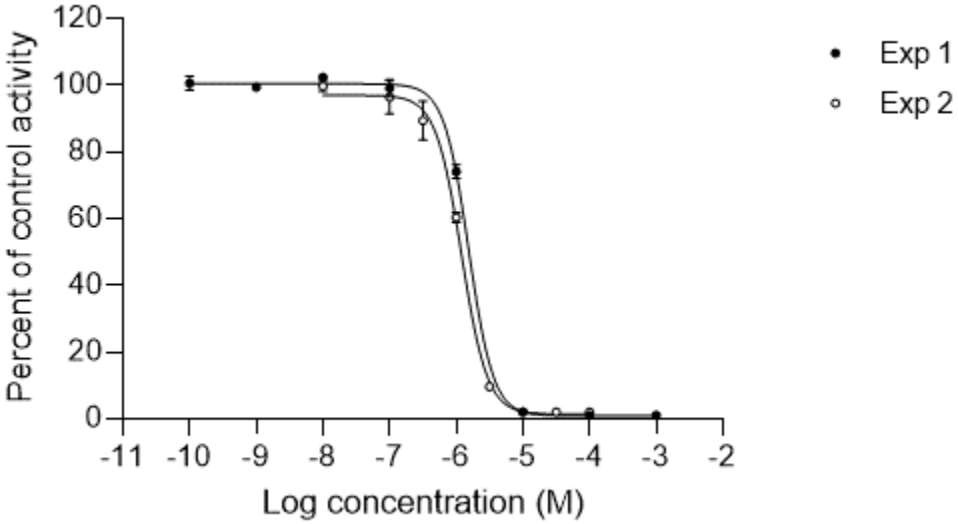

Figure 28
Dose Response Curves for IVA_779

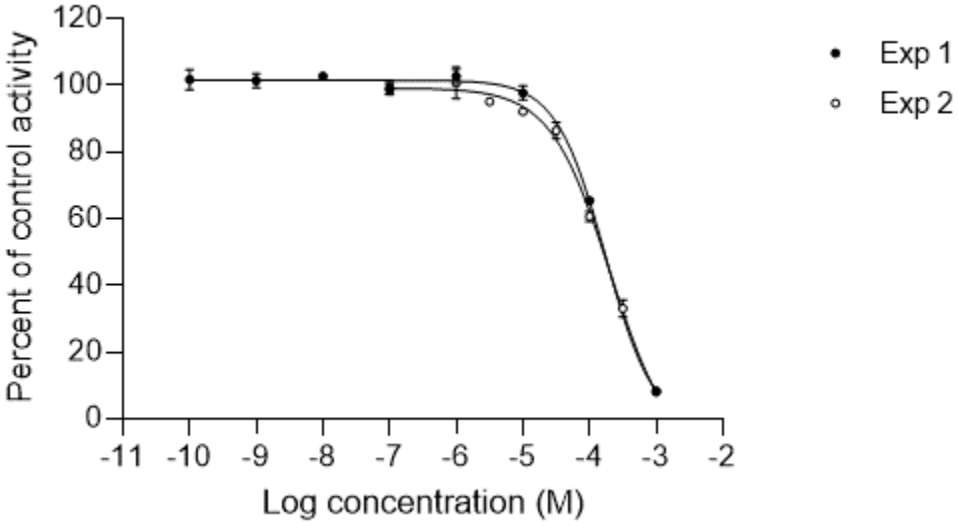

Figure 29
Dose Response Curves for IVA_805

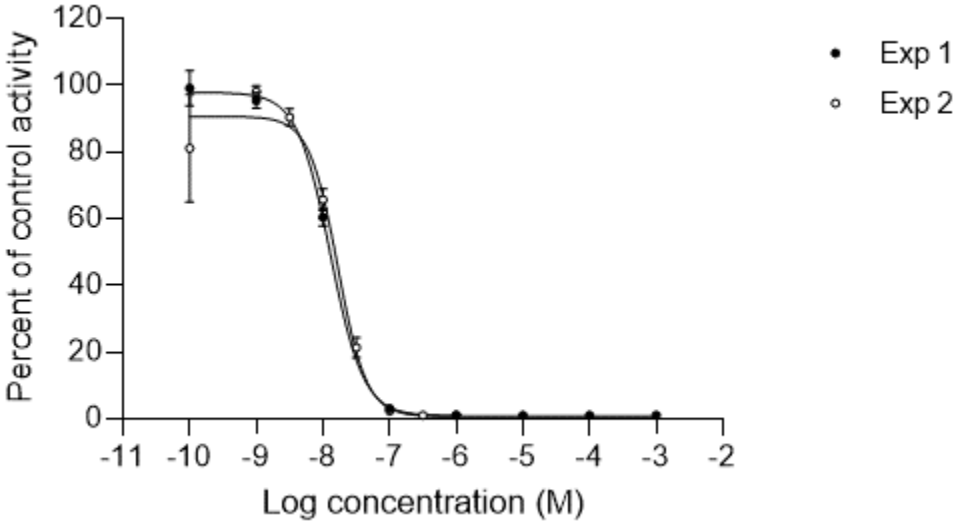

Figure 30
Dose Response Curves for IVA_820

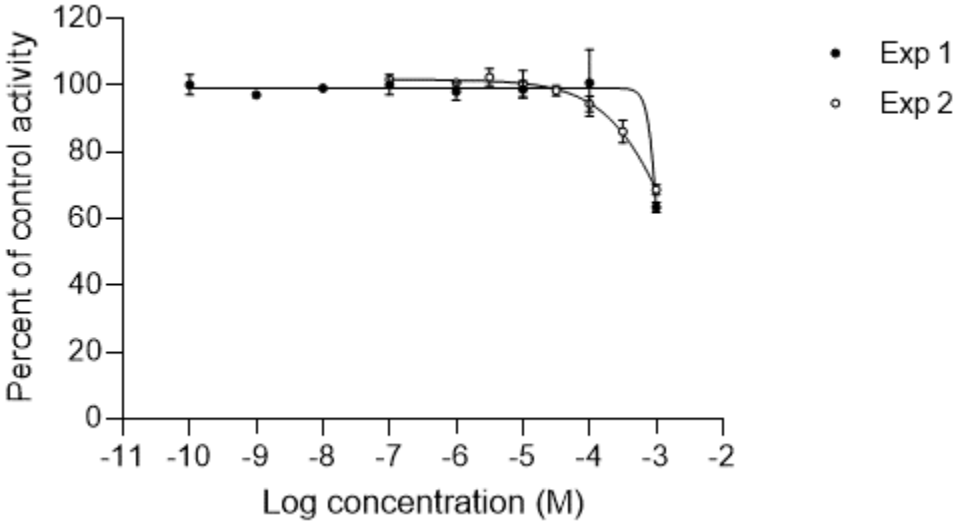

Figure 31
Dose Response Curves for IVA_834

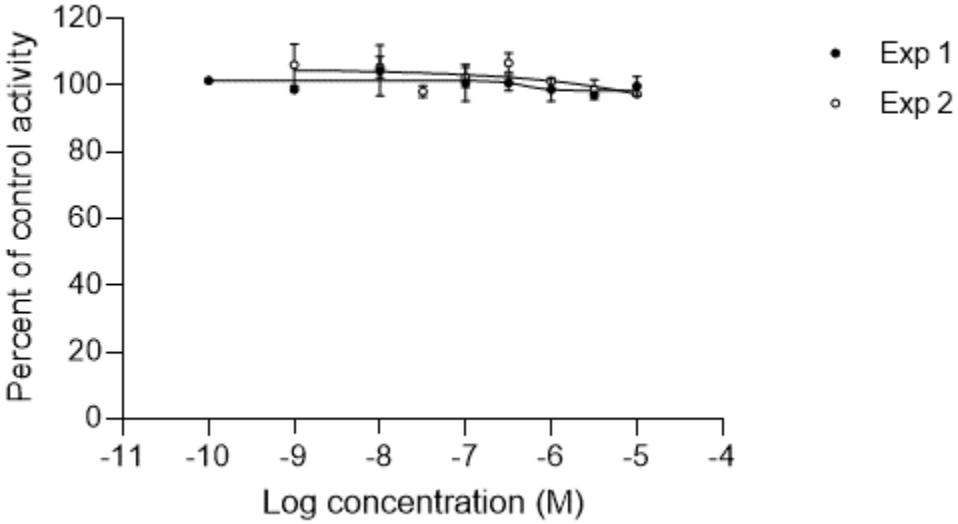

Figure 32
Dose Response Curves for IVA_920

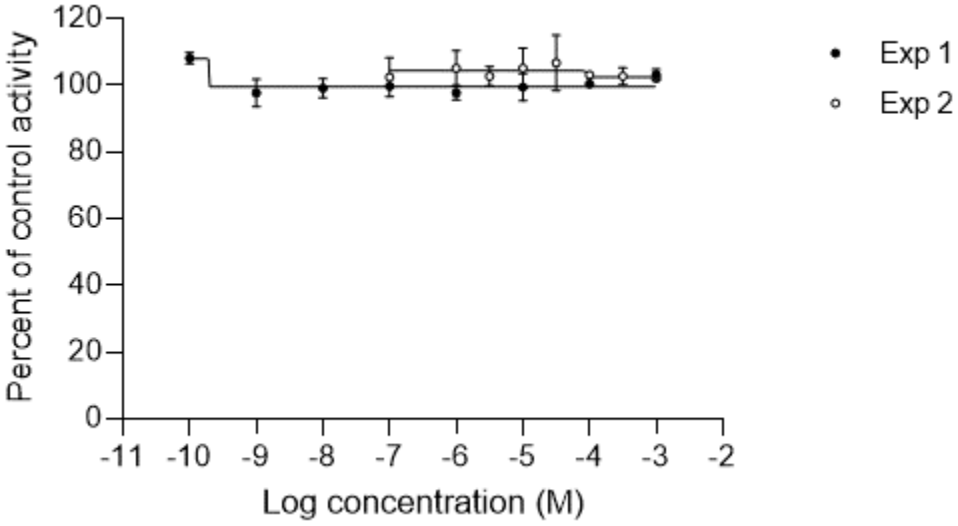

Figure 33
Dose Response Curves for IVA_992

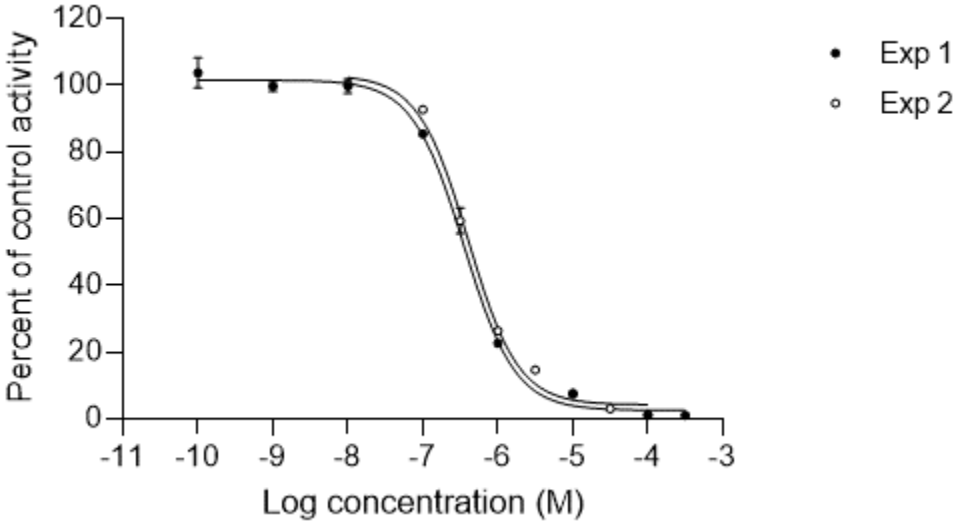

Table 1
Analytical Run History

Analytical Run	Run No.	Experimental Date [dd/Mmm/yyyy]	Remarks
20382705 Exp1 (IVA012-IVA071-IVA135-IVA136-IVA140)	MIT_1-1	14 Jun 2022	None
20382705 Exp1 (IVA151-IVA226-IVA246-IVA254-IVA301)	MIT_1-2	15 Jun 2022	None
20382705 Exp1 (IVA303-IVA310-IVA341-IVA387-IVA401)	MIT_1-3	15 Jun 2022	None
20382705 Exp1 (IVA431-IVA482-IVA515-IVA532-IVA550)	MIT_1-4	17 Jun 2022	None
20382705 Exp1 (IVA563-IVA597-IVA661-IVA696-IVA779)	MIT_1-5	17 Jun 2022	None
20382705 Exp1 (IVA805-IVA820-IVA834-IVA920-IVA992)	MIT_1-6	20 Jun 2022	None
20382705 Exp2 (IVA012-IVA071-IVA135-IVA136-IVA140)	MIT_2-1	28 Jun 2022	None
20382705 Exp2 (IVA151-IVA226-IVA246-IVA254-IVA301)	MIT_2-2	28 Jun 2022	None
20382705 Exp2 (IVA303-IVA310-IVA341-IVA387-IVA401)	MIT_2-3	29 Jun 2022	None
20382705 Exp2 (IVA431-IVA482-IVA515-IVA532-IVA550)	MIT_2-4	29 Jun 2022	None
20382705 Exp2 (IVA563-IVA597-IVA661-IVA696-IVA779)	MIT_2-5	30 Jun 2022	None
20382705 Exp2 (IVA805-IVA820-IVA834-IVA920-IVA992)	MIT_2-6	30 Jun 2022	None
20382705 Exp3 (IVA135-IVA834)	MIT_3	13 Jul 2022	None

Table 2
Statistical Parameters of the MIT Calibration Curves

Run No.	Range (µM)	Accepted points	Slope	Intercept	r^2
MIT_1-1	0.0761 – 75.0	20/20	2.37×10^{-1}	3.62×10^{-3}	0.998
MIT_1-2	0.0761 – 75.0	20/20	2.37×10^{-1}	3.68×10^{-3}	0.998
MIT_1-3	0.0761 – 75.0	20/20	2.36×10^{-1}	3.84×10^{-3}	0.998
MIT_1-4	0.0761 – 75.0	20/20	2.32×10^{-1}	4.17×10^{-3}	0.998
MIT_1-5	0.0761 – 75.0	20/20	2.30×10^{-1}	3.00×10^{-3}	0.998
MIT_1-6	0.0761 – 75.0	20/20	2.37×10^{-1}	3.76×10^{-3}	0.998
MIT_2-1	0.0761 – 75.0	20/20	2.51×10^{-1}	4.35×10^{-3}	0.9992
MIT_2-2	0.0761 – 75.0	20/20	2.51×10^{-1}	3.93×10^{-3}	0.9993
MIT_2-3	0.0761 – 75.0	20/20	2.48×10^{-1}	3.65×10^{-3}	0.9994
MIT_2-4	0.0761 – 75.0	20/20	2.47×10^{-1}	3.04×10^{-3}	0.9993
MIT_2-5	0.0761 – 75.0	20/20	2.44×10^{-1}	4.17×10^{-3}	0.9995
MIT_2-6	0.0761 – 75.0	20/20	2.47×10^{-1}	3.36×10^{-3}	0.9991
MIT_3	0.0761 – 75.0	20/20	2.34×10^{-1}	3.90×10^{-3}	0.996

Table 3
Accuracy and Repeatability of MIT QC Samples

Run No.	Range (µM)	Mean Accuracy (%)		
		QC-L (Accepted points)	QC-M (Accepted points)	QC-H (Accepted points)
MIT_1-1	0.0761 – 75.0	96 (4/4)	101 (4/4)	97 (4/4)
MIT_1-2	0.0761 – 75.0	96 (4/4)	101 (4/4)	97 (4/4)
MIT_1-3	0.0761 – 75.0	95 (4/4)	102 (4/4)	97 (4/4)
MIT_1-4	0.0761 – 75.0	96 (4/4)	102 (4/4)	98 (4/4)
MIT_1-5	0.0761 – 75.0	97 (4/4)	101 (4/4)	99 (4/4)
MIT_1-6	0.0761 – 75.0	97 (4/4)	102 (4/4)	99 (4/4)
MIT_2-1	0.0761 – 75.0	93 (4/4)	94 (4/4)	95 (4/4)
MIT_2-2	0.0761 – 75.0	93 (4/4)	93 (4/4)	94 (4/4)
MIT_2-3	0.0761 – 75.0	92 (4/4)	93 (4/4)	94 (4/4)
MIT_2-4	0.0761 – 75.0	95 (4/4)	95 (4/4)	94 (4/4)
MIT_2-5	0.0761 – 75.0	91 (4/4)	93 (4/4)	95 (4/4)
MIT_2-6	0.0761 – 75.0	92 (4/4)	92 (4/4)	94 (4/4)
MIT_3	0.0761 – 75.0	94 (4/4)	97 (4/4)	99 (4/4)

Table 4
Results obtained for Controls in Experiment 1-1

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	7.85	6.23	6.35	6.81	101
	Vehicle control (DMSO)	6.72	6.95	6.57	6.75	**100**
	No vehicle control	7.28	7.39	6.87	7.18	106
	Non-enzymatic iodination control	0.0538 *	0.0495 *	0.0517 *	0.0761	1
	No peroxide control	0.0083 *	0.0091 *	0.0000 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	6.82	6.50	6.64	6.65	97
	Vehicle control (DMSO)	6.86	6.86	6.83	6.85	**100**
	Vehicle control (Milli-Q)	7.00	6.83	6.78	6.87	100
	No vehicle control	7.11	6.93	6.92	6.99	102
	Non-enzymatic iodination control	0.0609 *	0.0586 *	0.0637 *	0.0761	1
	No peroxide control	0.0054 *	0.0022 *	0.0031 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.0860.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.0529.

Table 5
Results obtained for Controls in Experiment 1-2

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	5.49	5.32	5.70	5.50	97
	Vehicle control (DMSO)	5.65	5.70	5.64	5.66	**100**
	No vehicle control	5.65	5.64	5.78	5.69	101
	Non-enzymatic iodination control	0.0741 *	0.0722 *	0.0905	0.0809	1
	No peroxide control	0.0313 *	0.0371 *	0.0200 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	5.67	5.49	6.00	5.72	100
	Vehicle control (DMSO)	5.85	5.60	5.77	5.74	**100**
	No vehicle control	5.79	5.62	5.77	5.73	100
	Non-enzymatic iodination control	0.0700 *	0.0758 *	0.0750 *	0.0761	1
	No peroxide control	0.0155 *	0.0117 *	0.0140 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.521.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.243.

Table 6
Results obtained for Controls in Experiment 1-3

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	5.36	5.10	5.60	5.35	102
	Vehicle control (DMSO)	5.08	5.43	5.16	5.22	**100**
	No vehicle control	5.44	5.29	5.67	5.47	105
	Non-enzymatic iodination control	0.0749 *	0.0720 *	0.0711 *	0.0761	1
	No peroxide control	0.0066 *	0.0070 *	0.0115 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	5.32	5.02	5.25	5.20	97
	Vehicle control (DMSO)	5.25	5.47	5.32	5.35	**100**
	No vehicle control	5.55	5.39	5.39	5.44	102
	Non-enzymatic iodination control	0.0686 *	0.0615 *	0.0667 *	0.0761	1
	No peroxide control	0.0087 *	0.0004 *	0.0069 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.160.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.102.

Table 7
Results obtained for Controls in Experiment 1-4

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	6.18	6.15	6.22	6.18	93
	Vehicle control (DMSO)	6.67	6.75	6.51	6.64	**100**
	No vehicle control	6.82	7.03	7.79	7.21	109
	Non-enzymatic iodination control	0.0909	0.103	0.116	0.103	2
	No peroxide control	0.0347 *	0.0304 *	0.0116 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	7.80	6.44	6.34	6.86	104
	Vehicle control (DMSO)	6.59	6.44	6.74	6.59	**100**
	No vehicle control	6.59	6.89	6.49	6.66	101
	Non-enzymatic iodination control	0.116	0.102	0.114	0.111	2
	No peroxide control	0.0130 *	0.0210 *	0.0254 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.385.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.298.

Table 8
Results obtained for Controls in Experiment 1-5

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	5.64	5.50	5.76	5.63	94
	Vehicle control (DMSO)	6.05	5.91	5.99	5.99	**100**
	No vehicle control	6.27	6.08	6.32	6.22	104
	Non-enzymatic iodination control	0.0918	0.0888	0.115	0.0986	2
	No peroxide control	0.0067 *	0.102	0.0104 *	0.0848	1 [1)]
Plate 2	Negative control (1 mM DEHP)	5.82	5.83	5.97	5.88	98
	Vehicle control (DMSO)	5.93	5.89	6.08	5.97	**100**
	No vehicle control	6.14	6.14	6.19	6.16	103
	Non-enzymatic iodination control	0.114	0.0977	0.110	0.107	2
	No peroxide control	0.0079 *	0.0141 *	0.0113 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.664.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.185.

Table 9
Results obtained for Controls in Experiment 1-6

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	5.14	5.00	5.19	5.11	96
	Vehicle control (DMSO)	5.27	5.23	5.42	5.31	**100**
	No vehicle control	5.40	5.31	5.33	5.35	101
	Non-enzymatic iodination control	0.0670 *	0.0738 *	0.0767	0.0763	1
	No peroxide control	0.0056 *	0.0022 *	0.0074 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	5.42	5.29	5.40	5.37	100
	Vehicle control (DMSO)	5.45	5.35	5.37	5.39	**100**
	No vehicle control	5.44	5.71	5.37	5.51	102
	Non-enzymatic iodination control	0.0764	0.0695 *	0.0834	0.0786	1
	No peroxide control	0.0028 *	0.0040 *	0.0038 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.0955.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.0666.

Table 10
Results obtained for Controls in Experiment 2-1

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	4.63	4.57	4.69	4.63	98
	Vehicle control (DMSO)	4.65	4.70	4.87	4.74	**100**
	No vehicle control	4.61	4.78	4.91	4.77	101
	Non-enzymatic iodination control	0.0521 *	0.0507 *	0.0557 *	0.0761	2
	No peroxide control	0.0059 *	0.0000 *	0.0052 *	0.0761	2 [1)]
Plate 2	Negative control (1 mM DEHP)	4.71	4.71	4.69	4.70	100
	Vehicle control (DMSO)	4.78	4.65	4.67	4.70	**100**
	Vehicle control (Milli-Q)	4.73	4.81	4.82	4.79	102
	No vehicle control	5.08	4.71	4.90	4.90	104
	Non-enzymatic iodination control	0.0431 *	0.0485 *	0.0582 *	0.0761	2
	No peroxide control	0.0003 *	0.0000 *	0.0010 *	0.0761	2 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.0781.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.0091.

Table 11
Results obtained for Controls in Experiment 2-2

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	4.40 [1)]	6.70	6.93	6.81	94
	Vehicle control (DMSO)	7.36	7.36	6.96	7.23	**100**
	No vehicle control	7.44	7.34	7.32	7.37	102
	Non-enzymatic iodination control	0.0722 *	0.0730 *	0.0712 *	0.0761	1
	No peroxide control	0.0050 *	0.0052 *	0.0041 *	0.0761	1 [2)]
Plate 2	Negative control (1 mM DEHP)	6.37	6.92	7.14	6.81	99
	Vehicle control (DMSO)	6.66	6.99	6.98	6.88	**100**
	No vehicle control	6.67	6.89	6.95	6.83	99
	Non-enzymatic iodination control	0.0605 *	0.0703 *	0.0704 *	0.0761	1
	No peroxide control	0.0039 *	0.0005 *	0.0046 *	0.0761	1 [3)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Outlier based on other replicates and the data from Plate 2, excluded from further analysis.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.0659.
[3)] Based on the average of the analyzed MIT concentration the % of activity was 0.0415.

Table 12
Results obtained for Controls in Experiment 2-3

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	5.93	5.67	5.89	5.83	96
	Vehicle control (DMSO)	6.20	5.77	6.25	6.08	**100**
	No vehicle control	6.08	6.12	6.48	6.23	103
	Non-enzymatic iodination control	0.103	0.102	0.111	0.106	2
	No peroxide control	0.0059 *	0.0015 *	0.0063 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	6.14	6.27	6.15	6.19	96
	Vehicle control (DMSO)	6.39	6.44	6.43	6.42	**100**
	No vehicle control	6.67	6.37	6.39	6.47	101
	Non-enzymatic iodination control	0.106	0.109	0.119	0.111	2
	No peroxide control	0.0007 *	0.0022 *	0.0029 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.0752.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.0318.

Table 13
Results obtained for Controls in Experiment 2-4

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	6.70	6.84	6.84	6.79	99
	Vehicle control (DMSO)	6.91	7.24	6.47	6.87	**100**
	No vehicle control	7.12	6.70	7.85	7.22	105
	Non-enzymatic iodination control	0.134	0.147	0.158	0.146	2
	No peroxide control	0.0095 *	0.0111 *	0.0128 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	6.41	6.94	7.01	6.79	97
	Vehicle control (DMSO)	6.95	7.17	6.83	6.98	**100**
	No vehicle control	6.64	6.86	7.06	6.85	98
	Non-enzymatic iodination control	0.0000 *	0.0000 *	0.0000 *	0.0761	1
	No peroxide control	0.0082 *	0.0104 *	0.0127 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.162.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.152.

Table 14
Results obtained for Controls in Experiment 2-5

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 2 [1)]	Negative control (1 mM DEHP)	4.17	4.32	4.21	4.23	97
	Vehicle control (DMSO)	4.25	4.17	4.68	4.37	**100**
	No vehicle control	4.27	4.34	4.44	4.35	99
	Non-enzymatic iodination control	0.116	0.130	0.122	0.123	3
	No peroxide control	0.0046 *	0.0000 *	0.0083 *	0.0761	2 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Plate 2 was used for all test material calculations as the results of plate 1 were rejected due to a technical error.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.0984.

Table 15
Results obtained for Controls in Experiment 2-6

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	6.29	6.38	6.25	6.31	93
	Vehicle control (DMSO)	6.49	6.96	6.83	6.76	**100**
	No vehicle control	6.84	7.12	6.83	6.93	103
	Non-enzymatic iodination control	0.136	0.115	0.137	0.130	2
	No peroxide control	0.0046 *	0.0086 *	0.0000 *	0.0761	1 [1)]
Plate 2	Negative control (1 mM DEHP)	6.70	6.61	6.61	6.64	99
	Vehicle control (DMSO)	6.93	6.59	6.54	6.69	**100**
	No vehicle control	7.12	6.80	6.48	6.80	102
	Non-enzymatic iodination control	0.131	0.143	0.132	0.136	2
	No peroxide control	0.0128 *	0.0038 *	0.0346 *	0.0761	1 [2)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.0651.
[2)] Based on the average of the analyzed MIT concentration the % of activity was 0.252.

Table 16
Results obtained for Controls in Experiment 3

Sample Type		MIT Concentration (µM)				% Compared to Vehicle Control
		Replicate			Mean	Mean
		A	B	C		
Plate 1	Negative control (1 mM DEHP)	6.30	6.31	6.28	6.30	97
	Vehicle control (DMSO)	6.41	6.50	6.63	6.51	**100**
	No vehicle control	6.48	6.70	6.80	6.66	102
	Non-enzymatic iodination control	0.0633	0.0690	0.0757	0.0761	1
	No peroxide control	0.0021 *	0.0004 *	0.0020 *	0.0761	1 [1)]

The activity of the vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Based on the average of the analyzed MIT concentration the % of activity was 0.0230.

Table 17
Results obtained for the Reference Material PTU in Experiment 1-1

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	6.67	6.63	6.82	6.71	-9.0	99	98	101	99
PTU 10 nM	6.43	6.40	6.68	6.50	-8.0	95	95	99	96
PTU 100 nM	6.55	5.96	6.24	6.25	-7.0	97	88	93	93
PTU 316 nM	5.95	6.05	5.77	5.92	-6.5	88	90	86	88
PTU 1 µM	5.08	4.91	5.17	5.05	-6.0	75	73	77	75
PTU 3.16 µM	1.33	1.27	1.64	1.41	-5.5	20	19	24	21
PTU 10 µM	0.0170 *	0.0094 *	0.0289 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0048 *	0.0027 *	0.0043 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0060 *	0.0326 *	0.0063 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 18
Results obtained for the Reference Material PTU in Experiment 1-2

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	5.59	5.94	5.73	5.75	-9.0	99	105	101	102
PTU 10 nM	5.84	5.88	5.60	5.77	-8.0	103	104	99	102
PTU 100 nM	5.89	5.98	5.61	5.82	-7.0	104	106	99	103
PTU 316 nM	5.43	5.79	5.95	5.72	-6.5	96	102	105	101
PTU 1 µM	4.62	4.69	4.90	4.74	-6.0	82	83	86	84
PTU 3.16 µM	0.988	1.81	1.86	1.55	-5.5	17	32	33	27
PTU 10 µM	0.0178 *	0.0229 *	0.0159 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0141 *	0.0062 *	0.0469 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0134 *	0.0211 *	0.0489 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 19
Results obtained for the Reference Material PTU in Experiment 1-3

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	4.97	5.29	5.21	5.15	-9.0	95	101	100	99
PTU 10 nM	5.12	5.08	5.16	5.12	-8.0	98	97	99	98
PTU 100 nM	5.04	5.07	5.08	5.07	-7.0	96	97	97	97
PTU 316 nM	4.96	4.89	4.88	4.91	-6.5	95	94	93	94
PTU 1 µM	4.41	4.20	4.56	4.39	-6.0	84	80	87	84
PTU 3.16 µM	0.959	0.679	0.853	0.830	-5.5	18	13	16	16
PTU 10 µM	0.0230 *	0.0163 *	0.0394 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0095 *	0.0139 *	0.0101 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0030 *	0.0054 *	0.0070 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 20
Results obtained for the Reference Material PTU in Experiment 1-4

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	6.63	7.15	6.69	6.82	-9.0	100	108	101	103
PTU 10 nM	6.35	7.04	6.67	6.69	-8.0	96	106	100	101
PTU 100 nM	6.38	6.24	6.43	6.35	-7.0	96	94	97	96
PTU 316 nM	6.27	6.17	6.08	6.17	-6.5	94	93	91	93
PTU 1 µM	5.66	5.16	5.38	5.40	-6.0	85	78	81	81
PTU 3.16 µM	1.78	1.66	1.63	1.69	-5.5	27	25	24	25
PTU 10 µM	0.0408 *	0.0219 *	0.0172 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0205 *	0.0046 *	0.0123 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0326 *	0.0180 *	0.0233 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 21
Results obtained for the Reference Material PTU in Experiment 1-5

Reference Material	MIT Concentration (µM)					% Activity of Vehicle Control			
	Replicate			Mean	Log Conc. (M)	Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	5.90	5.93	6.17	6.00	-9.0	99	99	103	100
PTU 10 nM	5.58	5.93	5.93	5.81	-8.0	93	99	99	97
PTU 100 nM	5.94	6.15	5.75	5.95	-7.0	99	103	96	99
PTU 316 nM	5.36	5.92	5.69	5.66	-6.5	90	99	95	94
PTU 1 µM	4.78	4.95	4.72	4.82	-6.0	80	83	79	80
PTU 3.16 µM	0.892	1.36	1.31	1.19	-5.5	15	23	22	20
PTU 10 µM	0.0223 *	0.0227 *	0.0200 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0317 *	0.0280 *	0.0110 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0304 *	0.0276 *	0.0138 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 22
Results obtained for the Reference Material PTU in Experiment 1-6

Reference Material	MIT Concentration (µM)					% Activity of Vehicle Control			
	Replicate			Mean	Log Conc. (M)	Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	4.77	5.06	5.28	5.04	-9.0	90	95	99	95
PTU 10 nM	4.80	5.38	5.24	5.14	-8.0	90	101	99	97
PTU 100 nM	4.79	4.96	4.95	4.90	-7.0	90	94	93	92
PTU 316 nM	4.76	5.00	4.83	4.86	-6.5	90	94	91	92
PTU 1 µM	4.04	4.01	3.84	3.96	-6.0	76	76	72	75
PTU 3.16 µM	0.230	0.500	0.718	0.483	-5.5	4	9	14	9
PTU 10 µM	0.0240 *	0.0072 *	0.0116 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0121 *	0.0087 *	0.0114 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0324 *	0.0026 *	0.0076 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 23
Results obtained for the Reference Material PTU in Experiment 2-1

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	5.12	4.58	4.65	4.78	-9.0	108	97	98	101
PTU 10 nM	4.82	4.55	4.71	4.69	-8.0	102	96	99	99
PTU 100 nM	4.65	4.36	4.43	4.48	-7.0	98	92	93	95
PTU 316 nM	4.49	4.13	4.17	4.27	-6.5	95	87	88	90
PTU 1 µM	3.40	3.17	3.36	3.31	-6.0	72	67	71	70
PTU 3.16 µM	0.240	0.141	0.183	0.188	-5.5	5	3	4	4
PTU 10 µM	0.0043 *	0.0030 *	0.0057 *	0.0761	-5.0	2	2	2	2
PTU 31.6 µM	0.0000 *	0.0318 *	0.0022 *	0.0761	-4.5	2	2	2	2
Plate 2 PTU 31.6 µM	0.0185 *	0.0476 *	0.0014 *	0.0761	-4.5	2	2	2	2

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 24
Results obtained for the Reference Material PTU in Experiment 2-2

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	7.01	7.46	7.34	7.27	-9.0	97	103	102	101
PTU 10 nM	7.02	7.53	7.21	7.25	-8.0	97	104	100	100
PTU 100 nM	6.99	7.62	7.05	7.22	-7.0	97	105	97	100
PTU 316 nM	6.76	6.87	6.96	6.86	-6.5	94	95	96	95
PTU 1 µM	6.08	6.48	6.33	6.30	-6.0	84	90	88	87
PTU 3.16 µM	2.58	2.67	2.44	2.56	-5.5	36	37	34	35
PTU 10 µM	0.0119 *	0.0115 *	0.0111 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0019 *	0.0021 *	0.0042 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0071 *	0.0042 *	0.0002 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 25
Results obtained for the Reference Material PTU in Experiment 2-3

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	5.78	6.25	5.90	5.98	-9.0	95	103	97	98
PTU 10 nM	5.85	6.34	6.17	6.12	-8.0	96	104	102	101
PTU 100 nM	6.31	5.96	5.92	6.06	-7.0	104	98	97	100
PTU 316 nM	5.67	5.82	6.23	5.91	-6.5	93	96	103	97
PTU 1 µM	4.56	4.82	4.70	4.69	-6.0	75	79	77	77
PTU 3.16 µM	0.831	0.795	0.734	0.787	-5.5	14	13	12	13
PTU 10 µM	0.0190 *	0.0177 *	0.0232 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0172 *	0.0000 *	0.0038 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0000 *	0.0009 *	0.0020 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 26
Results obtained for the Reference Material PTU in Experiment 2-4

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	7.94	7.18	6.98	7.37	-9.0	115	104	102	107
PTU 10 nM	6.70	7.20	6.87	6.92	-8.0	97	105	100	101
PTU 100 nM	6.49	6.99	7.06	6.84	-7.0	94	102	103	100
PTU 316 nM	6.69	6.75	6.27	6.57	-6.5	97	98	91	96
PTU 1 µM	5.51	5.81	5.67	5.66	-6.0	80	85	82	82
PTU 3.16 µM	1.16	1.78	1.93	1.62	-5.5	17	26	28	24
PTU 10 µM	0.0258 *	0.0372 *	0.0210 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0209 *	0.0000 *	0.0354 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0228 *	0.0234 *	0.0126 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 27
Results obtained for the Reference Material PTU in Experiment 2-5

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	4.16	4.27	4.21	4.21	-9.0	95	98	96	96
PTU 10 nM	4.08	4.34	4.48	4.30	-8.0	93	99	103	98
PTU 100 nM	4.38	3.87	4.17	4.14	-7.0	100	89	96	95
PTU 316 nM	3.94	4.20	3.85	4.00	-6.5	90	96	88	91
PTU 1 µM	2.77	3.31	3.01	3.03	-6.0	63	76	69	69
PTU 3.16 µM	0.0610 *	0.206	0.180	0.154	-5.5	2	5	4	4
PTU 10 µM	0.0070 *	0.0127 *	0.0030 *	0.0761	-5.0	2	2	2	2
PTU 31.6 µM	0.0000 *	0.0000 *	0.0049 *	0.0761	-4.5	2	2	2	2
Plate 2 PTU 31.6 µM	0.0040 *	0.0073 *	0.0340 *	0.0761	-4.5	2	2	2	2

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 28
Results obtained for the Reference Material PTU in Experiment 2-6

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	6.21	6.53	6.66	6.47	-9.0	92	97	99	96
PTU 10 nM	6.46	6.80	6.58	6.61	-8.0	96	101	97	98
PTU 100 nM	6.37	7.36	6.10	6.61	-7.0	94	109	90	98
PTU 316 nM	6.03	6.65	6.64	6.44	-6.5	89	98	98	95
PTU 1 µM	5.25	5.75	5.62	5.54	-6.0	78	85	83	82
PTU 3.16 µM	1.85	2.07	2.39	2.10	-5.5	27	31	35	31
PTU 10 µM	0.0299 *	0.0185 *	0.0128 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0162 *	0.0136 *	0.0049 *	0.0761	-4.5	1	1	1	1
Plate 2 PTU 31.6 µM	0.0022 *	0.0185 *	0.0194 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 29
Results obtained for the Reference Material PTU in Experiment 3

Reference Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
PTU 1 nM	6.31	6.45	6.69	6.48	-9.0	97	99	103	100
PTU 10 nM	6.33	6.43	6.93	6.56	-8.0	97	99	106	101
PTU 100 nM	6.43	6.39	6.41	6.41	-7.0	99	98	98	98
PTU 316 nM	6.16	6.11	6.01	6.09	-6.5	95	94	92	94
PTU 1 µM	5.26	5.09	5.30	5.22	-6.0	81	78	81	80
PTU 3.16 µM	1.55	1.52	1.52	1.53	-5.5	24	23	23	23
PTU 10 µM	0.0385 *	0.0094 *	0.0051 *	0.0761	-5.0	1	1	1	1
PTU 31.6 µM	0.0170 *	0.0041 *	0.0000 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 30
Results obtained for IVA_012 in Experiment 1-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_012 100 pM	6.46	6.23	6.35	6.35	-10.0	96	92	94	94
TIIVA_012 1 nM	6.19	6.07	6.07	6.11	-9.0	92	90	90	91
TIIVA_012 10 nM	6.33	6.16	6.35	6.28	-8.0	94	91	94	93
TIIVA_012 100 nM	5.93	6.37	6.29	6.20	-7.0	88	94	93	92
TIIVA_012 316 nM	6.04	6.05	5.98	6.03	-6.5	90	90	89	89
TIIVA_012 1 µM	6.19	6.07	5.99	6.08	-6.0	92	90	89	90
TIIVA_012 3.16 µM	5.52	5.37	5.20	5.36	-5.5	82	80	77	80
TIIVA_012 10 µM	4.38	4.49	4.22	4.36	-5.0	65	67	63	65

The activity of the corresponding vehicle control was set at 100%.

Table 31
Results obtained for IVA_012 in Experiment 2-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_012 1 nM	4.78	4.99	4.58	4.78	-9.0	101	105	97	101
TIIVA_012 10 nM	4.63	4.51	4.68	4.60	-8.0	98	95	99	97
TIIVA_012 31.6 nM	4.76	4.66	4.71	4.71	-7.5	100	98	99	99
TIIVA_012 100 nM	4.63	4.54	4.75	4.64	-7.0	98	96	100	98
TIIVA_012 316 nM	4.89	4.65	4.38	4.64	-6.5	103	98	92	98
TIIVA_012 1 µM	4.47	4.52	4.44	4.48	-6.0	94	95	94	95
TIIVA_012 3.16 µM	4.13	4.13	4.26	4.17	-5.5	87	87	90	88
TIIVA_012 10 µM	3.44	3.36	3.45	3.42	-5.0	73	71	73	72

The activity of the corresponding vehicle control was set at 100%.

Table 32
Results obtained for IVA_071 in Experiment 1-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_071 100 pM	6.19	6.22	6.35	6.26	-10.0	92	92	94	93
TIIVA_071 1 nM	6.36	6.54	6.29	6.40	-9.0	94	97	93	95
TIIVA_071 10 nM	6.42	6.29	6.05	6.25	-8.0	95	93	90	93
TIIVA_071 100 nM	5.79	5.74	5.98	5.84	-7.0	86	85	89	87
TIIVA_071 1 µM	4.01	3.80	4.13	3.98	-6.0	59	56	61	59
TIIVA_071 10 µM	1.21	1.79	1.38	1.46	-5.0	18	26	20	22
TIIVA_071 31.6 µM	0.266	0.550	0.470	0.429	-4.5	4	8	7	6
TIIVA_071 100 µM	0.0437 *	0.0492 *	0.0518 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 33
Results obtained for IVA_071 in Experiment 2-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_071 10 nM	4.68	4.75	4.83	4.75	-8.0	99	100	102	100
TIIVA_071 100 nM	4.42	4.31	4.37	4.36	-7.0	93	91	92	92
TIIVA_071 316 nM	4.05	3.99	3.86	3.97	-6.5	85	84	81	84
TIIVA_071 1 µM	3.03	3.00	3.26	3.10	-6.0	64	63	69	65
TIIVA_071 3.16 µM	1.94	1.74	1.87	1.85	-5.5	41	37	39	39
TIIVA_071 10 µM	0.896	0.926	0.940	0.921	-5.0	19	20	20	19
TIIVA_071 31.6 µM	0.304	0.289	0.295	0.296	-4.5	6	6	6	6
TIIVA_071 100 µM	0.0409 *	0.0559 *	0.0443 *	0.0761	-4.0	2	2	2	2

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 34
Results obtained for IVA_135 in Experiment 1-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_135 100 pM	6.35	6.49	6.69	6.51	-10.0	93	95	98	95
TIIVA_135 1 nM	6.26	6.27	6.15	6.23	-9.0	91	92	90	91
TIIVA_135 10 nM	6.42	6.13	6.33	6.29	-8.0	94	89	92	92
TIIVA_135 100 nM	6.37	6.47	6.08	6.31	-7.0	93	94	89	92
TIIVA_135 1 µM	6.39	6.36	6.34	6.36	-6.0	93	93	93	93
TIIVA_135 10 µM	6.27	6.40	6.29	6.32	-5.0	92	93	92	92
TIIVA_135 100 µM	5.71	5.91	5.90	5.84	-4.0	83	86	86	85
TIIVA_135 316 µM	5.12	5.16	4.91	5.06	-3.5	75	75	72	74

The activity of the corresponding vehicle control was set at 100%.

Table 35
Results obtained for IVA_135 in Experiment 2-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_135 10 nM	4.90	4.90	4.86	4.89	-8.0	104	104	103	104
TIIVA_135 100 nM	4.82	4.75	4.70	4.76	-7.0	102	101	100	101
TIIVA_135 1 µM	4.78	4.83	4.72	4.78	-6.0	102	103	100	102
TIIVA_135 3.16 µM	4.79	4.75	4.69	4.74	-5.5	102	101	100	101
TIIVA_135 10 µM	4.72	4.58	4.67	4.66	-5.0	100	98	99	99
TIIVA_135 31.6 µM	4.65	4.51	4.58	4.58	-4.5	99	96	98	97
TIIVA_135 100 µM	4.59	4.21	4.16	4.32	-4.0	98	90	88	92
TIIVA_135 316 µM	3.79	3.79	3.78	3.79	-3.5	81	81	81	81

The activity of the corresponding vehicle control was set at 100%.

Table 36
Results obtained for IVA_135 in Experiment 3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_135 10 nM	6.45	6.28	6.34	6.36	-8.0	99	96	97	98
TIIVA_135 100 nM	6.30	6.37	6.40	6.36	-7.0	97	98	98	98
TIIVA_135 1 µM	6.34	6.07	6.41	6.27	-6.0	97	93	98	96
TIIVA_135 3.16 µM	6.42	6.36	6.68	6.49	-5.5	99	98	102	100
TIIVA_135 10 µM	6.27	6.44	6.43	6.38	-5.0	96	99	99	98
TIIVA_135 31.6 µM	6.13	6.22	6.56	6.30	-4.5	94	95	101	97
TIIVA_135 100 µM	5.58	5.81	5.90	5.76	-4.0	86	89	91	88
TIIVA_135 316 µM	5.26	5.28	5.11	5.22	-3.5	81	81	78	80

The activity of the corresponding vehicle control was set at 100%.

Table 37
Results obtained for IVA_136 in Experiment 1-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_136 100 pM	6.59	6.38	6.47	6.48	-10.0	96	93	94	95
TIIVA_136 1 nM	6.57	6.65	6.86	6.69	-9.0	96	97	100	98
TIIVA_136 10 nM	7.27	6.91	6.29	6.82	-8.0	106	101	92	100
TIIVA_136 100 nM	6.79	6.19	6.63	6.54	-7.0	99	90	96	95
TIIVA_136 1 µM	7.23	6.14	6.31	6.56	-6.0	105	89	92	96
TIIVA_136 10 µM	6.70	6.78	6.67	6.72	-5.0	98	99	97	98
TIIVA_136 31.6 µM	7.24	7.20	7.43	7.29	-4.5	105	105	108	106
TIIVA_136 100 µM	11.2	11.6	11.2	11.3	-4.0	163	169	163	165

The activity of the corresponding vehicle control was set at 100%.

Table 38
Results obtained for IVA_136 in Experiment 2-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_136 10 nM	4.91	5.19	4.81	4.97	-8.0	103	108	100	106
TIIVA_136 100 nM	4.78	4.97	4.81	4.85	-7.0	100	104	100	103
TIIVA_136 316 nM	4.65	4.80	4.85	4.77	-6.5	97	100	101	101
TIIVA_136 1 µM	4.65	4.99	4.89	4.84	-6.0	97	104	102	103
TIIVA_136 3.16 µM	4.76	5.13	4.98	4.96	-5.5	99	107	104	105
TIIVA_136 10 µM	5.18	6.40 [1)]	5.15	5.17	-5.0	108	134 [1)]	108	110
TIIVA_136 31.6 µM	5.99	7.77	6.72	6.83	-4.5	125	162	140	145
TIIVA_136 100 µM	8.71	5.41 [1)]	9.48	9.09	-4.0	182	113 [1)]	198	194

The activity of the corresponding vehicle control was set at 100%.
[1)] Outlier based on other replicates, excluded from further analysis.

Table 39
Results obtained for IVA_140 in Experiment 1-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_140 100 pM	6.59	6.49	6.59	6.56	-10.0	96	95	96	96
TIIVA_140 1 nM	6.43	6.47	6.15	6.35	-9.0	94	94	90	93
TIIVA_140 10 nM	6.26	7.05	6.30	6.54	-8.0	91	103	92	95
TIIVA_140 100 nM	6.25	6.45	6.47	6.39	-7.0	91	94	94	93
TIIVA_140 1 µM	5.80	5.94	5.75	5.83	-6.0	85	87	84	85
TIIVA_140 10 µM	3.17	3.34	3.08	3.19	-5.0	46	49	45	47
TIIVA_140 100 µM	0.289	0.327	0.236	0.284	-4.0	4	5	3	4
TIIVA_140 1 mM	0.0245 *	0.0500 *	0.102	0.0848	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 40
Results obtained for IVA_140 in Experiment 2-1

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_140 10 nM	4.72	4.96	4.92	4.87	-8.0	100	106	105	104
TIIVA_140 100 nM	4.73	4.71	4.71	4.71	-7.0	101	100	100	100
TIIVA_140 1 µM	4.38	4.34	4.33	4.35	-6.0	93	92	92	93
TIIVA_140 3.16 µM	3.57	3.70	3.56	3.61	-5.5	76	79	76	77
TIIVA_140 10 µM	2.24	2.31	2.26	2.27	-5.0	48	49	48	48
TIIVA_140 31.6 µM	0.902	0.937	0.841	0.893	-4.5	19	20	18	19
TIIVA_140 100 µM	0.240	0.205	0.235	0.227	-4.0	5	4	5	5
TIIVA_140 1 mM	0.0227 *	0.0264 *	0.0255 *	0.0761	-3.0	2	2	2	2

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 41
Results obtained for IVA_151 in Experiment 1-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_151 100 pM	5.62	5.38	5.63	5.55	-10.0	99	95	99	98
TIIVA_151 1 nM	5.45	5.69	5.35	5.50	-9.0	96	101	95	97
TIIVA_151 10 nM	5.75	5.59	5.66	5.67	-8.0	102	99	100	100
TIIVA_151 100 nM	5.64	5.65	5.43	5.57	-7.0	100	100	96	98
TIIVA_151 1 µM	5.34	5.23	5.74	5.44	-6.0	94	92	101	96
TIIVA_151 10 µM	4.62	4.49	4.49	4.53	-5.0	82	79	79	80
TIIVA_151 31.6 µM	3.96	3.83	3.54	3.78	-4.5	70	68	63	67
TIIVA_151 100 µM	2.74	2.56	2.59	2.63	-4.0	48	45	46	46

The activity of the corresponding vehicle control was set at 100%.

Table 42
Results obtained for IVA_151 in Experiment 2-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_151 10 nM	7.36	7.14	7.03	7.18	-8.0	102	99	97	99
TIIVA_151 100 nM	7.25	7.36	7.40	7.33	-7.0	100	102	102	101
TIIVA_151 316 nM	6.87	7.16	7.38	7.14	-6.5	95	99	102	99
TIIVA_151 1 µM	6.61	7.35	7.07	7.01	-6.0	91	102	98	97
TIIVA_151 3.16 µM	6.37	6.74	7.15	6.76	-5.5	88	93	99	93
TIIVA_151 10 µM	6.18	6.33	6.09	6.20	-5.0	85	88	84	86
TIIVA_151 31.6 µM	4.87	5.09	4.92	4.96	-4.5	67	70	68	69
TIIVA_151 100 µM	3.40	3.46	3.57	3.48	-4.0	47	48	49	48

The activity of the corresponding vehicle control was set at 100%.

Table 43
Results obtained for IVA_226 in Experiment 1-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_226 100 pM	5.59	5.63	5.74	5.65	-10.0	99	99	101	100
TIIVA_226 1 nM	5.34	5.50	5.76	5.53	-9.0	94	97	102	98
TIIVA_226 10 nM	5.52	5.61	5.58	5.57	-8.0	97	99	99	98
TIIVA_226 100 nM	5.35	5.80	5.47	5.54	-7.0	95	102	97	98
TIIVA_226 1 µM	4.55	4.27	4.67	4.49	-6.0	80	75	82	79
TIIVA_226 10 µM	0.0125 *	0.0112 *	0.0155 *	0.0761	-5.0	1	1	1	1
TIIVA_226 100 µM	0.0037 *	0.0187 *	0.0042 *	0.0761	-4.0	1	1	1	1
TIIVA_226 1 mM	0.0493 *	0.0402 *	0.0067 *	0.0761	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 44
Results obtained for IVA_226 in Experiment 2-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_226 10 nM	7.43	7.15	7.02	7.20	-8.0	103	99	97	100
TIIVA_226 31.6 nM	7.07	8.08	7.21	7.45	-7.5	98	112	100	103
TIIVA_226 100 nM	7.31	7.30	6.77	7.13	-7.0	101	101	94	99
TIIVA_226 316 nM	7.42	7.09	7.01	7.18	-6.5	103	98	97	99
TIIVA_226 1 µM	6.07	6.72	6.49	6.43	-6.0	84	93	90	89
TIIVA_226 3.16 µM	3.24	3.11	3.48	3.28	-5.5	45	43	48	45
TIIVA_226 10 µM	0.0185 *	0.0278 *	0.0183 *	0.0761	-5.0	1	1	1	1
TIIVA_226 100 µM	0.0073 *	0.0051 *	0.0008 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 45
Results obtained for IVA_246 in Experiment 1-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_246 100 pM	5.68	5.60	3.93 [1)]	5.64	-10.0	99	97	68 [1)]	98
TIIVA_246 1 nM	5.85	5.52	5.54	5.64	-9.0	102	96	97	98
TIIVA_246 10 nM	5.61	5.69	5.84	5.71	-8.0	98	99	102	99
TIIVA_246 100 nM	5.95	5.81	5.87	5.87	-7.0	104	101	102	102
TIIVA_246 1 µM	5.88	5.66	5.74	5.76	-6.0	102	99	100	100
TIIVA_246 10 µM	6.00	5.91	5.93	5.95	-5.0	104	103	103	104
TIIVA_246 31.6 µM	5.96	5.78	5.69	5.81	-4.5	104	101	99	101
TIIVA_246 100 µM	5.67	5.72	5.72	5.70	-4.0	99	100	100	99

The activity of the corresponding vehicle control was set at 100%.
[1)] Outlier based on other replicates, excluded from further analysis.

Table 46
Results obtained for IVA_246 in Experiment 2-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_246 10 nM	4.85	6.77	5.56	5.73	-8.0	70	99	81	83
TIIVA_246 100 nM	5.74	6.68	4.55	5.66	-7.0	83	97	66	82
TIIVA_246 316 nM	6.40	6.66	6.84	6.64	-6.5	93	97	99	96
TIIVA_246 1 µM	7.03	6.97	6.88	6.96	-6.0	102	101	100	101
TIIVA_246 3.16 µM	7.10	6.63	5.55	6.42	-5.5	103	96	81	93
TIIVA_246 10 µM	7.09	6.97	7.03	7.03	-5.0	103	101	102	102
TIIVA_246 31.6 µM	6.71	6.78	6.91	6.80	-4.5	98	99	101	99
TIIVA_246 100 µM	6.55	6.78	7.01	6.78	-4.0	95	99	102	99

The activity of the corresponding vehicle control was set at 100%.

Table 47
Results obtained for IVA_254 in Experiment 1-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_254 100 pM	5.75	6.19	5.70	5.88	-10.0	100	108	99	102
TIIVA_254 1 nM	5.62	5.67	6.05	5.78	-9.0	98	99	105	101
TIIVA_254 10 nM	5.70	5.78	5.83	5.77	-8.0	99	101	102	100
TIIVA_254 100 nM	5.29	5.52	5.44	5.42	-7.0	92	96	95	94
TIIVA_254 1 µM	1.68	1.86	1.64	1.73	-6.0	29	32	29	30
TIIVA_254 10 µM	0.0538 *	0.0525 *	0.0462 *	0.0761	-5.0	1	1	1	1
TIIVA_254 31.6 µM	0.0215 *	0.0313 *	0.0290 *	0.0761	-4.5	1	1	1	1
TIIVA_254 100 µM	0.0288 *	0.0235 *	0.0146 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 48
Results obtained for IVA_254 in Experiment 2-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_254 10 nM	4.70	5.63	6.26	5.53	-8.0	68	82	91	80
TIIVA_254 31.6 nM	5.92	6.78	5.42	6.04	-7.5	86	99	79	88
TIIVA_254 100 nM	4.76	5.37	5.09	5.07	-7.0	69	78	74	74
TIIVA_254 316 nM	4.49	3.76	5.16	4.47	-6.5	65	55	75	65
TIIVA_254 1 µM	2.62	2.95	2.85	2.81	-6.0	38	43	41	41
TIIVA_254 3.16 µM	0.475	0.427	0.441	0.447	-5.5	7	6	6	7
TIIVA_254 10 µM	0.0554 *	0.0436 *	0.0547 *	0.0761	-5.0	1	1	1	1
TIIVA_254 31.6 µM	0.0166 *	0.0300 *	0.0157 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 49
Results obtained for IVA_301 in Experiment 1-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_301 100 pM	5.59	5.49	5.62	5.57	-10.0	97	96	98	97
TIIVA_301 1 nM	5.68	5.79	5.58	5.68	-9.0	99	101	97	99
TIIVA_301 10 nM	5.72	5.64	5.67	5.68	-8.0	100	98	99	99
TIIVA_301 100 nM	5.53	5.61	5.58	5.57	-7.0	96	98	97	97
TIIVA_301 1 µM	2.35	2.21	2.39	2.32	-6.0	41	39	42	40
TIIVA_301 10 µM	0.0584 *	0.0606 *	0.0402 *	0.0761	-5.0	1	1	1	1
TIIVA_301 100 µM	0.0034 *	0.0099 *	0.0026 *	0.0761	-4.0	1	1	1	1
TIIVA_301 500 µM	0.0092 *	0.0090 *	0.0063 *	0.0761	-3.3	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 50
Results obtained for IVA_301 in Experiment 2-2

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_301 10 nM	5.63	5.63	6.99	6.08	-8.0	82	82	102	88
TIIVA_301 31.6 nM	6.78	6.95	5.40	6.37	-7.5	99	101	78	93
TIIVA_301 100 nM	6.10	7.02	7.17	6.76	-7.0	89	102	104	98
TIIVA_301 316 nM	6.61	6.65	5.55	6.27	-6.5	96	97	81	91
TIIVA_301 1 µM	2.78	3.31	3.90	3.33	-6.0	40	48	57	48
TIIVA_301 3.16 µM	0.475	0.472	0.470	0.472	-5.5	7	7	7	7
TIIVA_301 10 µM	0.0666 *	0.0693 *	0.0621 *	0.0761	-5.0	1	1	1	1
TIIVA_301 100 µM	0.0024 *	0.0073 *	0.0088 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 51
Results obtained for IVA_303 in Experiment 1-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_303 100 pM	5.38	5.22	5.24	5.28	-10.0	103	100	100	101
TIIVA_303 1 nM	5.09	5.24	5.20	5.17	-9.0	97	100	99	99
TIIVA_303 10 nM	5.36	4.98	5.01	5.11	-8.0	103	95	96	98
TIIVA_303 100 nM	4.45	4.44	4.59	4.49	-7.0	85	85	88	86
TIIVA_303 1 µM	1.14	1.29	1.10	1.18	-6.0	22	25	21	22
TIIVA_303 10 µM	0.0471 *	0.0611 *	0.0502 *	0.0761	-5.0	1	1	1	1
TIIVA_303 31.6 µM	0.0226 *	0.0332 *	0.0425 *	0.0761	-4.5	1	1	1	1
TIIVA_303 100 µM	0.0052 *	0.0083 *	0.0110 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 52
Results obtained for IVA_303 in Experiment 2-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_303 10 nM	6.00	6.02	5.90	5.97	-8.0	99	99	97	98
TIIVA_303 31.6 nM	5.78	5.60	5.45	5.61	-7.5	95	92	90	92
TIIVA_303 100 nM	5.19	5.16	5.24	5.20	-7.0	85	85	86	86
TIIVA_303 316 nM	3.80	3.80	3.76	3.78	-6.5	63	62	62	62
TIIVA_303 1 µM	1.50	1.48	1.55	1.51	-6.0	25	24	26	25
TIIVA_303 3.16 µM	0.247	0.245	0.240	0.244	-5.5	4	4	4	4
TIIVA_303 10 µM	0.0506 *	0.0500 *	0.0513 *	0.0761	-5.0	1	1	1	1
TIIVA_303 31.6 µM	0.0181 *	0.0198 *	0.0291 *	0.0761	-4.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 53
Results obtained for IVA_310 in Experiment 1-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_310 100 pM	5.17	5.30	5.53	5.33	-10.0	99	101	106	102
TIIVA_310 1 nM	5.18	5.19	4.88	5.08	-9.0	99	99	93	97
TIIVA_310 10 nM	5.44	5.42	5.24	5.37	-8.0	104	104	100	103
TIIVA_310 100 nM	5.07	5.29	5.26	5.21	-7.0	97	101	101	100
TIIVA_310 1 µM	5.33	5.33	5.00	5.22	-6.0	102	102	96	100
TIIVA_310 10 µM	4.86	4.88	5.18	4.97	-5.0	93	93	99	95
TIIVA_310 100 µM	3.24	3.19	3.25	3.22	-4.0	62	61	62	62
TIIVA_310 1 mM	0.522	0.428	0.417	0.456	-3.0	10	8	8	9

The activity of the corresponding vehicle control was set at 100%.

Table 54
Results obtained for IVA_310 in Experiment 2-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_310 100 nM	6.32	6.11	6.08	6.17	-7.0	104	101	100	102
TIIVA_310 1 µM	6.02	6.03	6.09	6.05	-6.0	99	99	100	100
TIIVA_310 3.16 µM	5.72	6.26	5.89	5.96	-5.5	94	103	97	98
TIIVA_310 10 µM	5.76	5.97	5.75	5.83	-5.0	95	98	95	96
TIIVA_310 31.6 µM	5.13	5.35	5.47	5.31	-4.5	84	88	90	87
TIIVA_310 100 µM	4.22	4.24	4.21	4.22	-4.0	69	70	69	69
TIIVA_310 316 µM	2.31	2.31	2.48	2.37	-3.5	38	38	41	39
TIIVA_310 1 mM	0.764	0.847	0.723	0.778	-3.0	13	14	12	13

The activity of the corresponding vehicle control was set at 100%.

Table 55
Results obtained for IVA_341 in Experiment 1-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_341 100 pM	5.37	5.53	5.35	5.42	-10.0	100	103	100	101
TIIVA_341 1 nM	5.35	5.15	5.34	5.28	-9.0	100	96	100	99
TIIVA_341 10 nM	5.69	6.08	5.12	5.63	-8.0	106	114	96	105
TIIVA_341 100 nM	5.32	5.34	5.14	5.26	-7.0	99	100	96	98
TIIVA_341 1 µM	4.43	4.24	4.07	4.25	-6.0	83	79	76	79
TIIVA_341 10 µM	0.0064 *	0.0031 *	0.0213 *	0.0761	-5.0	1	1	1	1
TIIVA_341 100 µM	0.0003 *	0.0079 *	0.0000 *	0.0761	-4.0	1	1	1	1
TIIVA_341 1 mM	0.0000 *	0.0049 *	0.0008 *	0.0761	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 56
Results obtained for IVA_341 in Experiment 2-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_341 10 nM	5.94	6.64	6.06	6.21	-8.0	92	103	94	97
TIIVA_341 31.6 nM	5.98	6.19	6.21	6.13	-7.5	93	96	97	95
TIIVA_341 100 nM	6.13	6.11	6.28	6.17	-7.0	95	95	98	96
TIIVA_341 316 nM	5.75	5.89	5.98	5.87	-6.5	90	92	93	91
TIIVA_341 1 µM	4.92	5.02	4.96	4.97	-6.0	77	78	77	77
TIIVA_341 3.16 µM	0.171	0.225	0.242	0.213	-5.5	3	4	4	3
TIIVA_341 10 µM	0.0091 *	0.0099 *	0.0076 *	0.0761	-5.0	1	1	1	1
TIIVA_341 100 µM	0.0075 *	0.0069 *	0.0000 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 57
Results obtained for IVA_387 in Experiment 1-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_387 100 pM	5.25	5.32	5.52	5.36	-10.0	98	99	103	100
TIIVA_387 1 nM	5.38	4.89	5.42	5.23	-9.0	101	91	101	98
TIIVA_387 10 nM	5.45	5.19	5.30	5.31	-8.0	102	97	99	99
TIIVA_387 100 nM	5.34	5.22	5.46	5.34	-7.0	100	98	102	100
TIIVA_387 1 µM	5.44	5.31	5.32	5.35	-6.0	102	99	99	100
TIIVA_387 10 µM	5.36	5.32	5.48	5.39	-5.0	100	100	103	101
TIIVA_387 100 µM	5.17	5.29	5.10	5.19	-4.0	97	99	95	97
TIIVA_387 1 mM	3.29	3.22	3.14	3.22	-3.0	62	60	59	60

The activity of the corresponding vehicle control was set at 100%.

Table 58
Results obtained for IVA_387 in Experiment 2-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_387 100 nM	6.27	6.35	6.14	6.25	-7.0	98	99	96	97
TIIVA_387 1 µM	6.49	6.22	6.23	6.31	-6.0	101	97	97	98
TIIVA_387 3.16 µM	6.08	6.09	6.11	6.09	-5.5	95	95	95	95
TIIVA_387 10 µM	6.07	6.09	6.00	6.05	-5.0	95	95	93	94
TIIVA_387 31.6 µM	5.76	6.14	6.07	5.99	-4.5	90	96	95	93
TIIVA_387 100 µM	5.89	5.96	5.68	5.84	-4.0	92	93	88	91
TIIVA_387 316 µM	5.21	5.21	5.31	5.24	-3.5	81	81	83	82
TIIVA_387 1 mM	3.94	3.88	3.96	3.93	-3.0	61	60	62	61

The activity of the corresponding vehicle control was set at 100%.

Table 59
Results obtained for IVA_401 in Experiment 1-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_401 100 pM	5.48	5.42	5.43	5.44	-10.0	102	101	102	102
TIIVA_401 1 nM	5.40	5.31	5.22	5.31	-9.0	101	99	98	99
TIIVA_401 10 nM	5.57	5.49	4.81	5.29	-8.0	104	103	90	99
TIIVA_401 100 nM	5.38	5.37	5.24	5.33	-7.0	101	100	98	100
TIIVA_401 1 µM	4.90	5.09	4.36	4.78	-6.0	92	95	81	89
TIIVA_401 10 µM	0.832	0.820	0.920	0.857	-5.0	16	15	17	16
TIIVA_401 100 µM	0.0420 *	0.0449 *	0.0408 *	0.0761	-4.0	1	1	1	1
TIIVA_401 316 µM	0.0247 *	0.0224 *	0.0273 *	0.0761	-3.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 60
Results obtained for IVA_401 in Experiment 2-3

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_401 100 nM	6.37	5.92	6.50	6.26	-7.0	99	92	101	98
TIIVA_401 316 nM	6.02	4.67	6.42	5.70	-6.5	94	73	100	89
TIIVA_401 1 µM	5.25	4.63	5.01	4.96	-6.0	82	72	78	77
TIIVA_401 3.16 µM	3.51	3.44	3.38	3.44	-5.5	55	54	53	54
TIIVA_401 10 µM	1.22	1.07	1.18	1.15	-5.0	19	17	18	18
TIIVA_401 31.6 µM	0.157	0.159	0.163	0.159	-4.5	2	2	3	2
TIIVA_401 100 µM	0.0616 *	0.0544 *	0.0482 *	0.0761	-4.0	1	1	1	1
TIIVA_401 316 µM	0.0315 *	0.0267 *	0.0229 *	0.0761	-3.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 61
Results obtained for IVA_431 in Experiment 1-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_431 100 pM	6.50	6.78	5.62	6.30	-10.0	98	102	85	95
TIIVA_431 1 nM	6.67	6.47	6.25	6.47	-9.0	100	97	94	97
TIIVA_431 10 nM	6.53	6.66	6.50	6.56	-8.0	98	100	98	99
TIIVA_431 100 nM	6.68	6.60	6.65	6.64	-7.0	101	99	100	100
TIIVA_431 1 µM	6.52	6.72	6.56	6.60	-6.0	98	101	99	99
TIIVA_431 10 µM	6.35	6.37	6.20	6.31	-5.0	96	96	93	95
TIIVA_431 100 µM	1.81	1.65	1.65	1.70	-4.0	27	25	25	26
TIIVA_431 316 µM	1.31	1.21	1.25	1.26	-3.5	20	18	19	19

The activity of the corresponding vehicle control was set at 100%.

Table 62
Results obtained for IVA_431 in Experiment 2-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_431 10 nM	7.14	6.81	6.88	6.94	-8.0	104	99	100	101
TIIVA_431 100 nM	7.08	6.94	6.97	7.00	-7.0	103	101	101	102
TIIVA_431 1 µM	7.10	6.89	6.75	6.91	-6.0	103	100	98	101
TIIVA_431 3.16 µM	7.35	6.92	6.68	6.98	-5.5	107	101	97	102
TIIVA_431 10 µM	6.54	6.36	6.32	6.41	-5.0	95	93	92	93
TIIVA_431 31.6 µM	4.67	4.79	4.81	4.76	-4.5	68	70	70	69
TIIVA_431 100 µM	1.70	1.81	1.60	1.70	-4.0	25	26	23	25
TIIVA_431 316 µM	1.08	1.22	1.22	1.17	-3.5	16	18	18	17

The activity of the corresponding vehicle control was set at 100%.

Table 63
Results obtained for IVA_482 in Experiment 1-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_482 100 pM	6.77	6.80	6.79	6.79	-10.0	102	102	102	102
TIIVA_482 1 nM	6.91	6.74	6.57	6.74	-9.0	104	101	99	101
TIIVA_482 10 nM	6.61	6.81	6.88	6.77	-8.0	100	103	104	102
TIIVA_482 100 nM	6.71	6.62	6.69	6.67	-7.0	101	100	101	100
TIIVA_482 1 µM	5.08	4.85	5.37	5.10	-6.0	77	73	81	77
TIIVA_482 10 µM	0.0279 *	0.0323 *	0.0310 *	0.0761	-5.0	1	1	1	1
TIIVA_482 100 µM	0.0004 *	0.0000 *	0.0000 *	0.0761	-4.0	1	1	1	1
TIIVA_482 1 mM	0.0000 *	0.0000 *	0.0000 *	0.0761	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 64
Results obtained for IVA_482 in Experiment 2-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_482 10 nM	7.17	7.48	7.99	7.55	-8.0	104	109	116	110
TIIVA_482 31.6 nM	7.62	7.19	6.64	7.15	-7.5	111	105	97	104
TIIVA_482 100 nM	6.88	7.72	7.79	7.47	-7.0	100	112	113	109
TIIVA_482 316 nM	6.73	6.60	6.58	6.64	-6.5	98	96	96	97
TIIVA_482 1 µM	5.57	5.63	5.51	5.57	-6.0	81	82	80	81
TIIVA_482 3.16 µM	1.73	2.12	1.76	1.87	-5.5	25	31	26	27
TIIVA_482 10 µM	0.0365 *	0.0362 *	0.0382 *	0.0761	-5.0	1	1	1	1
TIIVA_482 100 µM	0.0014 *	0.0021 *	0.0021 *	0.0761	-4.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 65
Results obtained for IVA_515 in Experiment 1-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_515 100 pM	6.99	6.78	6.65	6.81	-10.0	106	103	101	103
TIIVA_515 1 nM	6.71	6.59	6.83	6.71	-9.0	102	100	104	102
TIIVA_515 10 nM	5.76	5.32	5.51	5.53	-8.0	87	81	84	84
TIIVA_515 100 nM	0.800	0.743	0.767	0.770	-7.0	12	11	12	12
TIIVA_515 1 µM	0.0270 *	0.0313 *	0.0302 *	0.0761	-6.0	1	1	1	1
TIIVA_515 10 µM	0.0147 *	0.0077 *	0.0059 *	0.0761	-5.0	1	1	1	1
TIIVA_515 100 µM	0.0126 *	0.0000 *	0.0008 *	0.0761	-4.0	1	1	1	1
TIIVA_515 1 mM	0.0101 *	0.0000 *	0.0034 *	0.0761	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 66
Results obtained for IVA_515 in Experiment 2-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_515 1 nM	6.48	6.56	6.60	6.55	-9.0	93	94	94	94
TIIVA_515 3.16 nM	6.22	6.11	6.59	6.31	-8.5	89	88	94	90
TIIVA_515 10 nM	5.24	5.69	5.62	5.52	-8.0	75	82	80	79
TIIVA_515 31.6 nM	3.58	3.42	3.32	3.44	-7.5	51	49	48	49
TIIVA_515 100 nM	0.756	0.698	0.775	0.743	-7.0	11	10	11	11
TIIVA_515 316 nM	0.121	0.105	0.117	0.114	-6.5	2	2	2	2
TIIVA_515 1 µM	0.0546 *	0.0471 *	0.0446 *	0.0761	-6.0	1	1	1	1
TIIVA_515 10 µM	0.0151 *	0.0118 *	0.0176 *	0.0761	-5.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 67
Results obtained for IVA_532 in Experiment 1-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_532 100 pM	6.79	6.69	6.67	6.71	-10.0	103	101	101	102
TIIVA_532 1 nM	7.25	6.63	6.47	6.78	-9.0	110	101	98	103
TIIVA_532 10 nM	2.69 [1)]	6.72	6.42	6.57	-8.0	41 [1)]	102	97	100
TIIVA_532 100 nM	6.85	6.76	6.64	6.75	-7.0	104	102	101	102
TIIVA_532 1 µM	6.52	6.67	6.38	6.52	-6.0	99	101	97	99
TIIVA_532 10 µM	6.01	5.86	5.59	5.82	-5.0	91	89	85	88
TIIVA_532 100 µM	2.44	2.56	2.55	2.52	-4.0	37	39	39	38
TIIVA_532 316 µM	0.335	0.359	0.360	0.351	-3.5	5	5	5	5

The activity of the corresponding vehicle control was set at 100%.
[1)] Outlier based on other replicates and overall curve profile, excluded from further analysis.

Table 68
Results obtained for IVA_532 in Experiment 2-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_532 100 nM	6.86	6.62	6.90	6.79	-7.0	98	95	99	97
TIIVA_532 316 nM	6.68	6.45	6.82	6.65	-6.5	96	92	98	95
TIIVA_532 1 µM	7.01	6.33	6.67	6.67	-6.0	100	91	95	96
TIIVA_532 3.16 µM	6.06	6.46	6.20	6.24	-5.5	87	93	89	89
TIIVA_532 10 µM	5.93	5.82	5.76	5.84	-5.0	85	83	83	84
TIIVA_532 31.6 µM	4.57	4.63	4.50	4.57	-4.5	65	66	64	65
TIIVA_532 100 µM	2.46	2.97	2.62	2.68	-4.0	35	43	37	38
TIIVA_532 316 µM	0.422	0.446	0.309	0.392	-3.5	6	6	4	6

The activity of the corresponding vehicle control was set at 100%.

Table 69
Results obtained for IVA_550 in Experiment 1-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_550 100 pM	6.87	6.39	6.56	6.61	-10.0	104	97	100	100
TIIVA_550 1 nM	6.62	6.47	6.43	6.51	-9.0	100	98	98	99
TIIVA_550 10 nM	6.67	6.56	6.78	6.67	-8.0	101	99	103	101
TIIVA_550 100 nM	6.79	6.37	6.65	6.60	-7.0	103	97	101	100
TIIVA_550 1 µM	6.65	6.51	6.55	6.57	-6.0	101	99	99	100
TIIVA_550 10 µM	6.81	6.82	6.55	6.72	-5.0	103	103	99	102
TIIVA_550 100 µM	6.44	6.57	6.59	6.53	-4.0	98	100	100	99
TIIVA_550 1 mM	6.47	6.11	6.08	6.22	-3.0	98	93	92	94

The activity of the corresponding vehicle control was set at 100%.

Table 70
Results obtained for IVA_550 in Experiment 2-4

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_550 100 nM	6.83	6.87	6.85	6.85	-7.0	98	98	98	98
TIIVA_550 1 µM	6.84	7.61	7.54	7.33	-6.0	98	109	108	105
TIIVA_550 3.16 µM	7.03	7.25	7.46	7.25	-5.5	101	104	107	104
TIIVA_550 10 µM	7.00	7.11	6.54	6.88	-5.0	100	102	94	99
TIIVA_550 31.6 µM	7.19	6.73	7.00	6.97	-4.5	103	96	100	100
TIIVA_550 100 µM	6.87	6.66	6.63	6.72	-4.0	98	95	95	96
TIIVA_550 316 µM	6.43	7.09	6.03	6.52	-3.5	92	102	86	93
TIIVA_550 1 mM	6.52	6.12	6.23	6.29	-3.0	93	88	89	90

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 71
Results obtained for IVA_563 in Experiment 1-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_563 100 pM	5.71	6.07	6.05	5.94	-10.0	95	101	101	99
TIIVA_563 1 nM	6.12	5.67	5.98	5.92	-9.0	102	95	100	99
TIIVA_563 10 nM	5.84	6.08	5.68	5.87	-8.0	98	102	95	98
TIIVA_563 100 nM	5.90	5.96	5.83	5.90	-7.0	99	100	97	98
TIIVA_563 1 µM	5.46	6.18	5.61	5.75	-6.0	91	103	94	96
TIIVA_563 10 µM	3.81	3.95	3.88	3.88	-5.0	64	66	65	65
TIIVA_563 100 µM	0.766	0.744	0.780	0.763	-4.0	13	12	13	13
TIIVA_563 316 µM	0.230	0.339	0.201	0.256	-3.5	4	6	3	4

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 72
Results obtained for IVA_563 in Experiment 2-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_563 100 nM	4.22	4.34	4.37	4.31	-7.0	97	99	100	99
TIIVA_563 316 nM	4.35	3.99	4.13	4.16	-6.5	100	91	95	95
TIIVA_563 1 µM	5.35	4.79	3.93	4.69	-6.0	122	110	90	107
TIIVA_563 3.16 µM	3.43	3.64	3.59	3.55	-5.5	78	83	82	81
TIIVA_563 10 µM	2.63	2.75	2.74	2.71	-5.0	60	63	63	62
TIIVA_563 31.6 µM	1.59	1.70	1.74	1.68	-4.5	36	39	40	38
TIIVA_563 100 µM	0.639	0.778	0.692	0.703	-4.0	15	18	16	16
TIIVA_563 316 µM	0.236	0.188	0.214	0.213	-3.5	5	4	5	5

The activity of the corresponding vehicle control was set at 100%.

Table 73
Results obtained for IVA_597 in Experiment 1-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_597 100 pM	6.02	5.83	5.57	5.81	-10.0	101	97	93	97
TIIVA_597 1 nM	5.95	6.21	5.51	5.89	-9.0	99	104	92	98
TIIVA_597 10 nM	5.90	6.20	3.10 [1)]	6.05	-8.0	99	104	52 [1)]	101
TIIVA_597 100 nM	6.00	5.96	5.73	5.90	-7.0	100	100	96	99
TIIVA_597 1 µM	5.83	5.69	5.27	5.60	-6.0	97	95	88	94
TIIVA_597 10 µM	4.02	3.90	3.63	3.85	-5.0	67	65	61	64
TIIVA_597 100 µM	0.581	0.569	0.503	0.551	-4.0	10	10	8	9
TIIVA_597 1 mM	0.111	0.0863	0.0725 *	0.0912	-3.0	2	1	1	2

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Outlier based on other replicates and overall curve profile, excluded from further analysis.

Table 74
Results obtained for IVA_597 in Experiment 2-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_597 100 nM	4.21	4.32	4.56	4.36	-7.0	96	99	104	100
TIIVA_597 1 µM	4.03	4.01	3.92	3.99	-6.0	92	92	90	91
TIIVA_597 3.16 µM	3.62	3.60	3.53	3.58	-5.5	83	82	81	82
TIIVA_597 10 µM	2.84	2.88	2.88	2.87	-5.0	65	66	66	66
TIIVA_597 31.6 µM	1.44	1.98	1.62	1.68	-4.5	33	45	37	38
TIIVA_597 100 µM	0.534	0.506	0.593	0.544	-4.0	12	12	14	12
TIIVA_597 316 µM	0.184	0.153	0.154	0.164	-3.5	4	3	4	4
TIIVA_597 1 mM	0.0513 *	0.0558 *	0.0598 *	0.0761	-3.0	2	2	2	2

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 75
Results obtained for IVA_661 in Experiment 1-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_661 100 pM	6.20	6.13	6.21	6.18	-10.0	104	103	104	104
TIIVA_661 1 nM	6.04	6.00	6.08	6.04	-9.0	101	101	102	101
TIIVA_661 10 nM	6.18	6.05	6.31	6.18	-8.0	104	101	106	104
TIIVA_661 100 nM	6.18	6.15	5.82	6.05	-7.0	104	103	98	101
TIIVA_661 1 µM	6.19	6.24	6.30	6.24	-6.0	104	105	106	105
TIIVA_661 10 µM	6.12	6.11	6.11	6.11	-5.0	102	102	102	102
TIIVA_661 31.6 µM	5.90	5.83	5.92	5.88	-4.5	99	98	99	99
TIIVA_661 100 µM	6.00	6.05	5.67	5.91	-4.0	101	101	95	99

The activity of the corresponding vehicle control was set at 100%.

Table 76
Results obtained for IVA_661 in Experiment 2-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_661 10 nM	3.95	4.49	4.44	4.29	-8.0	90	103	102	98
TIIVA_661 100 nM	4.25	4.29	4.28	4.27	-7.0	97	98	98	98
TIIVA_661 316 nM	4.70	4.39	4.29	4.46	-6.5	108	100	98	102
TIIVA_661 1 µM	4.11	4.24	4.25	4.20	-6.0	94	97	97	96
TIIVA_661 3.16 µM	4.32	4.13	4.42	4.29	-5.5	99	94	101	98
TIIVA_661 10 µM	4.56	4.28	4.55	4.46	-5.0	104	98	104	102
TIIVA_661 31.6 µM	5.06	4.44	4.32	4.61	-4.5	116	102	99	105
TIIVA_661 100 µM	4.58	4.22	4.31	4.37	-4.0	105	96	99	100

The activity of the corresponding vehicle control was set at 100%.

Table 77
Results obtained for IVA_696 in Experiment 1-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_696 100 pM	10.1 [1)]	5.92	6.09	6.01	-10.0	170 [1)]	99	102	101
TIIVA_696 1 nM	5.99	5.89	5.90	5.93	-9.0	100	99	99	99
TIIVA_696 10 nM	6.10	6.08	6.12	6.10	-8.0	102	102	103	102
TIIVA_696 100 nM	5.83	6.06	5.77	5.89	-7.0	98	102	97	99
TIIVA_696 1 µM	4.52	4.28	4.43	4.41	-6.0	76	72	74	74
TIIVA_696 10 µM	0.132	0.133	0.123	0.130	-5.0	2	2	2	2
TIIVA_696 100 µM	0.0221 *	0.0247 *	0.0130 *	0.0761	-4.0	1	1	1	1
TIIVA_696 1 mM	0.0122 *	0.0232 *	0.0060 *	0.0761	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
[*] Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.
[1)] Outlier based on other replicates and overall curve profile, excluded from further analysis.

Table 78
Results obtained for IVA_696 in Experiment 2-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_696 10 nM	4.35	4.40	4.29	4.35	-8.0	100	101	98	99
TIIVA_696 100 nM	4.08	4.10	4.46	4.21	-7.0	93	94	102	96
TIIVA_696 316 nM	3.74	4.20	3.78	3.90	-6.5	85	96	87	89
TIIVA_696 1 µM	2.71	2.59	2.63	2.64	-6.0	62	59	60	60
TIIVA_696 3.16 µM	0.385	0.376	0.465	0.409	-5.5	9	9	11	9
TIIVA_696 10 µM	0.0990	0.0978	0.104	0.100	-5.0	2	2	2	2
TIIVA_696 31.6 µM	0.0228 *	0.0227 *	0.0211 *	0.0761	-4.5	2	2	2	2
TIIVA_696 100 µM	0.0009 *	0.0011 *	0.0000 *	0.0761	-4.0	2	2	2	2

The activity of the corresponding vehicle control was set at 100%.
[*] Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 79
Results obtained for IVA_779 in Experiment 1-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_779 100 pM	5.92	6.25	6.02	6.06	-10.0	99	105	101	102
TIIVA_779 1 nM	5.88	6.12	6.06	6.02	-9.0	99	103	102	101
TIIVA_779 10 nM	6.17	6.08	6.13	6.13	-8.0	103	102	103	103
TIIVA_779 100 nM	5.76	5.96	5.92	5.88	-7.0	97	100	99	99
TIIVA_779 1 µM	6.10	6.24	6.02	6.12	-6.0	102	105	101	103
TIIVA_779 10 µM	6.00	5.78	5.73	5.83	-5.0	100	97	96	98
TIIVA_779 100 µM	3.86	3.90	3.91	3.89	-4.0	65	65	66	65
TIIVA_779 1 mM	0.450	0.530	0.504	0.494	-3.0	8	9	8	8

The activity of the corresponding vehicle control was set at 100%.

Table 80
Results obtained for IVA_779 in Experiment 2-5

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_779 100 nM	4.22	4.38	4.38	4.33	-7.0	97	100	100	99
TIIVA_779 1 µM	4.32	4.64	4.22	4.39	-6.0	99	106	97	101
TIIVA_779 3.16 µM	4.15	4.15	4.17	4.16	-5.5	95	95	95	95
TIIVA_779 10 µM	4.05	4.01	3.99	4.02	-5.0	93	92	91	92
TIIVA_779 31.6 µM	3.66	3.87	3.76	3.77	-4.5	84	89	86	86
TIIVA_779 100 µM	2.56	2.64	2.70	2.63	-4.0	59	61	62	60
TIIVA_779 316 µM	1.38	1.36	1.56	1.43	-3.5	32	31	36	33
TIIVA_779 1 mM	0.341	0.346	0.338	0.342	-3.0	8	8	8	8

The activity of the corresponding vehicle control was set at 100%.

Table 81
Results obtained for IVA_805 in Experiment 1-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_805 100 pM	5.57	5.06	5.14	5.26	-10.0	105	95	97	99
TIIVA_805 1 nM	5.00	5.19	4.99	5.06	-9.0	94	98	94	95
TIIVA_805 10 nM	3.32	3.20	3.09	3.20	-8.0	63	60	58	60
TIIVA_805 100 nM	0.136	0.152	0.134	0.140	-7.0	3	3	3	3
TIIVA_805 1 µM	0.0206 *	0.0261 *	0.0173 *	0.0761	-6.0	1	1	1	1
TIIVA_805 10 µM	0.0166 *	0.0156 *	0.0065 *	0.0761	-5.0	1	1	1	1
TIIVA_805 100 µM	0.0119 *	0.0045 *	0.0083 *	0.0761	-4.0	1	1	1	1
TIIVA_805 1 mM	0.0085 *	0.0037 *	0.0049 *	0.0761	-3.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 82
Results obtained for IVA_805 in Experiment 2-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_805 100 pM	6.69	5.12	4.59	5.47	-10.0	99	76	68	81
TIIVA_805 1 nM	6.79	6.53	6.59	6.64	-9.0	100	97	97	98
TIIVA_805 3.16 nM	6.05	6.27	5.94	6.09	-8.5	90	93	88	90
TIIVA_805 10 nM	4.53	4.60	4.18	4.44	-8.0	67	68	62	66
TIIVA_805 31.6 nM	1.21	1.64	1.51	1.45	-7.5	18	24	22	21
TIIVA_805 100 nM	0.171	0.167	0.169	0.169	-7.0	3	2	2	3
TIIVA_805 316 nM	0.0427 *	0.0425 *	0.0481 *	0.0761	-6.5	1	1	1	1
TIIVA_805 1 µM	0.0257 *	0.0241 *	0.0328 *	0.0761	-6.0	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 83
Results obtained for IVA_820 in Experiment 1-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_820 100 pM	5.32	5.17	5.45	5.31	-10.0	100	97	103	100
TIIVA_820 1 nM	5.14	5.13	5.17	5.14	-9.0	97	97	97	97
TIIVA_820 10 nM	5.23	5.33	5.23	5.26	-8.0	98	100	99	99
TIIVA_820 100 nM	5.31	5.46	5.16	5.31	-7.0	100	103	97	100
TIIVA_820 1 µM	5.34	5.13	5.10	5.19	-6.0	101	97	96	98
TIIVA_820 10 µM	5.37	5.27	5.11	5.25	-5.0	101	99	96	99
TIIVA_820 100 µM	4.94	5.94	5.12	5.33	-4.0	93	112	97	101
TIIVA_820 1 mM	3.35	3.30	3.43	3.36	-3.0	63	62	65	63

The activity of the corresponding vehicle control was set at 100%.

Table 84
Results obtained for IVA_820 in Experiment 2-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_820 100 nM	6.81	6.82	6.99	6.88	-7.0	101	101	103	102
TIIVA_820 1 µM	6.75	6.82	6.82	6.79	-6.0	100	101	101	101
TIIVA_820 3.16 µM	6.92	7.11	6.78	6.93	-5.5	102	105	100	103
TIIVA_820 10 µM	6.85	7.06	6.48	6.80	-5.0	101	104	96	101
TIIVA_820 31.6 µM	6.58	6.63	6.73	6.65	-4.5	97	98	100	98
TIIVA_820 100 µM	6.52	6.27	6.29	6.36	-4.0	97	93	93	94
TIIVA_820 316 µM	5.52	5.97	5.96	5.82	-3.5	82	88	88	86
TIIVA_820 1 mM	4.55	4.64	4.70	4.63	-3.0	67	69	70	69

The activity of the corresponding vehicle control was set at 100%.

Table 85
Results obtained for IVA_834 in Experiment 1-6

Test Material	MIT Concentration (µM)				% Activity of Vehicle Control				
	Replicate			Mean	Log Conc. (M)	Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_834 100 pM	5.46	5.46	5.48	5.46	-10.0	101	101	102	101
TIIVA_834 1 nM	5.29	5.39	5.29	5.33	-9.0	98	100	98	99
TIIVA_834 10 nM	6.07	5.31	5.45	5.61	-8.0	113	99	101	104
TIIVA_834 100 nM	5.45	5.68	5.11	5.42	-7.0	101	106	95	101
TIIVA_834 316 nM	5.49	5.49	5.28	5.42	-6.5	102	102	98	101
TIIVA_834 1 µM	5.50	5.35	5.12	5.33	-6.0	102	99	95	99
TIIVA_834 3.16 µM	5.23	5.15	5.26	5.22	-5.5	97	96	98	97
TIIVA_834 10 µM	5.53	5.28	5.30	5.37	-5.0	103	98	98	100

The activity of the corresponding vehicle control was set at 100%.

Table 86
Results obtained for IVA_834 in Experiment 2-6

Test Material	MIT Concentration (µM)				% Activity of Vehicle Control				
	Replicate			Mean	Log Conc. (M)	Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_834 1 nM	7.57	6.74	6.92	7.08	-9.0	113	101	104	106
TIIVA_834 10 nM	6.86	6.97	7.26	7.03	-8.0	103	104	109	105
TIIVA_834 31.6 nM	6.48	6.69	6.51	6.56	-7.5	97	100	97	98
TIIVA_834 100 nM	6.62	6.87	6.99	6.83	-7.0	99	103	105	102
TIIVA_834 316 nM	7.02	6.99	7.33	7.11	-6.5	105	105	110	106
TIIVA_834 1 µM	6.66	6.74	6.83	6.74	-6.0	100	101	102	101
TIIVA_834 3.16 µM	6.47	6.49	6.84	6.60	-5.5	97	97	102	99
TIIVA_834 10 µM	6.47	6.55	6.49	6.50	-5.0	97	98	97	97

The activity of the corresponding vehicle control was set at 100%.

Table 87
Results obtained for IVA_920 in Experiment 1-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_920 100 pM	5.87	5.87	5.68	5.81	-10.0	109	109	106	108
TIIVA_920 1 nM	5.44	5.34	5.02	5.27	-9.0	101	99	93	98
TIIVA_920 10 nM	5.42	5.22	4.19 [1)]	5.32	-8.0	101	97	78 [1)]	99
TIIVA_920 100 nM	5.41	5.31	5.38	5.37	-7.0	100	99	100	100
TIIVA_920 1 µM	5.17	5.20	5.41	5.26	-6.0	96	97	100	98
TIIVA_920 10 µM	5.59	5.19	5.30	5.36	-5.0	104	96	98	100
TIIVA_920 100 µM	5.45	5.33	5.44	5.41	-4.0	101	99	101	100
TIIVA_920 1 mM	5.64	5.49	5.47	5.53	-3.0	105	102	102	103

The activity of the corresponding vehicle control was set at 100%.
[1)] Outlier based on other replicates, excluded from further analysis.

Table 88
Results obtained for IVA_920 in Experiment 2-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_920 100 nM	7.31	6.55	6.72	6.86	-7.0	109	98	100	103
TIIVA_920 1 µM	7.44	6.86	6.77	7.03	-6.0	111	103	101	105
TIIVA_920 3.16 µM	6.79	6.76	7.05	6.87	-5.5	101	101	106	103
TIIVA_920 10 µM	7.21	7.31	6.55	7.02	-5.0	108	109	98	105
TIIVA_920 31.6 µM	7.74	6.94	6.71	7.13	-4.5	116	104	100	107
TIIVA_920 100 µM	6.87	6.93	6.81	6.87	-4.0	103	104	102	103
TIIVA_920 316 µM	7.02	6.69	6.90	6.87	-3.5	105	100	103	103
TIIVA_920 1 mM	6.80	6.77	6.91	6.83	-3.0	102	101	103	102

The activity of the corresponding vehicle control was set at 100%.

Table 89
Results obtained for IVA_992 in Experiment 1-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_992 100 pM	5.60	5.83	5.35	5.59	-10.0	104	108	99	104
TIIVA_992 1 nM	5.44	5.26	5.40	5.37	-9.0	101	98	100	100
TIIVA_992 10 nM	5.27	5.33	5.48	5.36	-8.0	98	99	102	100
TIIVA_992 100 nM	4.60	4.64	4.59	4.61	-7.0	85	86	85	86
TIIVA_992 1 µM	1.25	1.21	1.22	1.22	-6.0	23	22	23	23
TIIVA_992 10 µM	0.403	0.460	0.404	0.422	-5.0	7	9	7	8
TIIVA_992 100 µM	0.0720 *	0.0626 *	0.0726 *	0.0761	-4.0	1	1	1	1
TIIVA_992 316 µM	0.0211 *	0.0331 *	0.0191 *	0.0761	-3.5	1	1	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Table 90
Results obtained for IVA_992 in Experiment 2-6

Test Material	MIT Concentration (µM)				Log Conc. (M)	% Activity of Vehicle Control			
	Replicate			Mean		Replicate			Mean
	#1	#2	#3			#1	#2	#3	
TIIVA_992 10 nM	6.72	6.70	6.75	6.72	-8.0	100	100	101	101
TIIVA_992 100 nM	6.12	6.23	6.20	6.18	-7.0	92	93	93	92
TIIVA_992 316 nM	4.17	3.65	4.11	3.97	-6.5	62	55	61	59
TIIVA_992 1 µM	1.75	1.71	1.83	1.76	-6.0	26	26	27	26
TIIVA_992 3.16 µM	1.00	0.970	0.947	0.973	-5.5	15	15	14	15
TIIVA_992 10 µM	0.474	0.494	0.518	0.496	-5.0	7	7	8	7
TIIVA_992 31.6 µM	0.206	0.210	0.210	0.209	-4.5	3	3	3	3
TIIVA_992 100 µM	0.0809	0.143	0.0317 *	0.0999	-4.0	1	2	1	1

The activity of the corresponding vehicle control was set at 100%.
* Concentration below LLOQ (0.0761 µM). LLOQ used for calculation of % compared to vehicle control.

Appendix 1
Study Plan

FINAL STUDY PLAN

Test Facility Study No. 20382705

In Vitro Determination of the Potential of Thirty Test Items to Suppress the Thyroid Peroxidase (TPO)-Catalyzed Iodination using FTC-238-hrTPO Cell Homogenates

Non-GLP

SPONSOR:
European Commission (DG-JRC)
Directorate F - Health, Consumers and Reference Materials
Unit F3 - Chemical Safety and Alternative Methods / The European Union Reference Laboratory for Alternatives to Animal Testing
(EURL ECVAM)
Via E. Fermi, 2749. TP126
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TEST FACILITY:
Charles River Laboratories Den Bosch BV
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5231 DD ‘s-Hertogenbosch
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1. OBJECTIVE

This study is performed for PART 2 of the EURL ECVAM coordinated Thyroid Validation Study. After the full description of method 2C (Tyrosine iodination using liquid chromatography) in standard operating procedures (SOPs) and successful assessment of the robustness and reliability of the in vitro method to determine the suppression of human thyroid peroxidase (TPO)-catalyzed iodination during PART 1 of the Thyroid Validation Study (Charles River Test Facility Study No. 20309164), in PART 2 the effect of in total 30 randomized test items will be assessed in a blinded manner.

TPO is an enzyme which is present on the apical membrane of thyroid follicular cells where it reduces hydrogen peroxide (H_2O_2), thereby elevating the oxidation state of iodide to an iodinating species (often considered to be hypoiodous acid) and iodinates tyrosyl residues in the thyroglobulin (Tg) glycoprotein. Initial iodination of Tg produces monoiodotyrosine (MIT) and diiodotyrosine (DIT) while subsequent oxidation of MIT and DIT by TPO to radical species couples two residues of DIT, both still linked to the Tg, to produce thyroxine (T4) and couples one residue of MIT and one residue of DIT to produce triiodothyronine (T3). When thyroid hormones are needed, hormone-rich Tg is taken up into thyroid epithelial cells by endocytosis and digested by proteases which results in the release of T4 and T3 into the blood circulation through the action of their transporters. Chemicals potentially can inhibit TPO-catalyzed iodination and/or coupling and in that way alter thyroid hormone homeostasis in vivo.

FTC-238-hrTPO cells are human thyroid carcinoma cells stably transfected with an expression clone coding for human recombinant (hr) TPO and can be used to prepare cell lysates containing the hrTPO enzyme. To evaluate potential interference with TPO-catalyzed iodination, FTC-238-hrTPO cell lysates containing TPO enzymes will be incubated together with L-tyrosine, potassium iodide and H_2O_2 in the presence or absence of the individual test items, the negative control bis-(2-ethylhexyl)-phthalate (DEHP) or the reference item 6-propyl-2-thiouracil (PTU). During incubation, TPO enzymatically converts L-tyrosine into MIT and formation of this metabolite can be monitored by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as a direct measurement of TPO-catalyzed iodination. A small amount of MIT may also be formed non-enzymatically, its formation will be assessed in separate incubations.

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual dates will be included in the Final Report.

Experimental Start Date:	30 May 2022 (First date of study-specific data collection)
Experimental Completion Date:	30 Sep 2022 (Last date data are collected from the study)
Unaudited Draft Report:	07 Oct 2022

3. SPONSOR

Role	Name	Contact Information
Sponsor Representative/ Study Monitor	Ingrid Langezaal	Address as cited for Sponsor E-mail: EU-Thyroid@ec.europa.eu

4. RESPONSIBLE PERSONNEL

Role/Phase	Quality Assurance Unit	Name	Contact Information
Study Director	Charles River	Jelle Reinen, PhD, ERT	Address as cited for Test Facility
Test Facility Management	Charles River	Beppy van de Waart, MSc, ERT	Address as cited for Test Facility

5. TEST ITEMS

5.1. Test Item Identification

The test item information as provided by EURL ECVAM is summarized in the table below.

Test Item No.	Appearance	Storage Conditions	Approx. Molecular Weight (g/mol)
IVA_012	Solid	-20°C	475
IVA_071	Solid	-20°C	750
IVA_135	Solid	4°C	325
IVA_136	Solid	RT	200
IVA_140	Solid	RT	350
IVA_151	Solid	4°C	550
IVA_226	Solid	RT	175
IVA_246	Solid	RT, inert gas	200
IVA_254	Solid	RT	550
IVA_301	Solid	4°C	500
IVA_303	Solid	4°C	275
IVA_310	Solid	4°C	350
IVA_341	Solid	RT	175
IVA_387	Solid	RT	275
IVA_401	Solid	RT	300
IVA_431	Liquid	RT	1 M solution
IVA_482	Solid	4°C	150
IVA_515	Solid	RT	250
IVA_532	Solid	RT	275
IVA_550	Solid	RT	125
IVA_563	Solid	RT	250
IVA_597	Solid	-20°C	350
IVA_661	Liquid	RT	300
IVA_696	Solid	RT	125

Test Item No.	Appearance	Storage Conditions	Approx. Molecular Weight (g/mol)
IVA_779	Solid	RT	325
IVA_805	Solid	RT	125
IVA_820	Solid	RT, inert gas	300
IVA_834	Solid	4°C	700
IVA_920	Solid	RT	200
IVA_992	Solid	RT, inert gas	375

-20°C: Freezer set to maintain -20°C; 4°C: Refrigerator set to maintain 4°C; RT: Room temperature (15 to 25°C).

5.1.1. Control Materials

5.1.1.1. Vehicle

Dimethyl sulfoxide (DMSO) or another appropriate solvent will be used as vehicle.

5.1.1.2. Substrate

L-tyrosine (CAS# 60-18-4) will be used as substrate for the TPO inhibition assay. Details on the supplier and batch will be included in the Final Report.

5.1.1.3. Metabolite

Monoiodotyrosine (MIT) (CAS# 70-78-0, AS2084) is the metabolite that will be evaluated in the TPO-catalyzed iodination assay. Details on the supplier and batch will be included in the Final Report.

5.1.1.4. Internal Standard

Monoiodotyrosine-$^{13}C_6$ (MIT-$^{13}C_6$) (AS2083) will be used as internal standard (IS) in the TPO-catalyzed iodination assay. Details on the supplier and batch will be included in the Final Report.

5.1.1.5. Negative Control

Bis-(2-ethylhexyl)-phthalate (DEHP, CAS# 117-81-7, RS493) will be used as a negative control. Details on the supplier will be included in the Final Report.

5.1.1.6. Reference Material

6-propyl-2-thiouracil (PTU, CAS# 51-52-5, RS506) will be used as a reference material. Details on the supplier and batch will be included in the Final Report.

5.2. Reserve Samples

For each batch (lot) of test item, if practically possible, a reserve sample will be collected and maintained under the appropriate storage conditions by the Test Facility.

5.3. Test and Control Material Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test items will be maintained.

5.4. Safety

The following safety instruction(s) apply to this study:

- Standard safety precautions specified in Charles River Den Bosch procedures

6. DOSE FORMULATION AND ANALYSIS

Test item amounts may be pre-weighed for a part of- or even the entire study at the discretion of the Study Director when stored under the same conditions as prescribed for the bulk container.

6.1. Preparation of Test items

No correction for the purity/composition of the test items will be performed.

Before performing the first TPO-catalyzed iodination experiment, a solubility test will be performed for each test item to determine the appropriate concentration ranges to be tested (see Section 8.1). Each test item will be tested at eight concentrations in the TPO-catalyzed iodination assay.

6.1.1. Stock Solutions of the Test items

For IVA_301, a 50 mM stock solution will be prepared in DMSO by dissolving the entire vial containing 10 mg of the test item into 400 µL of DMSO. The resulting solution will be transferred to another glass vial in steps of 100 µL and by rinsing the vial during transfer. IVA_301 stock solutions will be stored in the freezer set to maintain -20°C for a maximum of one month.

For IVA_597, a 100 mM stock solution will be prepared in DMSO by weighing up to 10 mg of the test item and dissolving this in the appropriate volume of DMSO. IVA_597 stock solutions will be stored in the freezer set to maintain -20°C for a maximum of one month.

Stock solutions of the other test items will be prepared in DMSO or another appropriate solvent on the day of use.

The selection of the solvent will be approved by the Study Director in the study files. Any residual volumes will be discarded unless otherwise requested by the Study Director.

6.1.2. Spiking Solutions of the Test items

For each test item, the stock solution will be further diluted in DMSO or another appropriate solvent in a log or half-log fashion to obtain eight 100× spiking solutions. The spiking solutions will be further diluted in the incubation mixtures. The final exposure concentrations in the TPO-catalyzed iodination assay will be e.g., 100 pM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM and 1 mM. These target concentrations may change if solubility, or other limitations, are encountered or if the results from the first assay run indicate an alternate concentration range would be better suited for an improved dose-response assessment. The actual final test item concentrations used in the TPO-catalyzed iodination assay experiments will be included in the Final Report. The final concentration of the solvent in the incubation mixtures will be the same for all incubations and will not exceed 1% (v/v).

Any residual volumes will be discarded unless otherwise requested by the Study Director.

6.1.3. Preparation of Control Material Solutions

6.1.3.1. Preparation of DEHP Solutions

A 100 mM stock solution will be prepared in DMSO or another appropriate solvent and will be stored in the freezer set to maintain -20°C for a maximum of one month. The 100 mM

stock solution will be further diluted in the incubation mixture. The final concentration in the incubation mixtures will be 1 mM.

Any residual volumes will be discarded unless otherwise requested by the Study Director.

6.1.3.2. Preparation of PTU Solutions

A 3.16 mM stock solution will be prepared in DMSO or another appropriate solvent on the day of use. The stock solution will be diluted to achieve eight 100× concentrations in solvent. The resulting spike solutions will be further diluted in the incubation mixture. The final concentrations in the incubation mixture will be 1 nM, 10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 31.6 µM.

PTU spiking solutions will be prepared freshly on the day of use. Any residual volumes will be discarded unless otherwise requested by the Study Director.

6.1.4. Preparation of Internal Standard Solutions

No correction will be made for the purity/composition of the internal standard (IS).

A stock solution of the IS will be prepared in 0.1 M HCl at a target concentration of 1 mg/mL. The stock solution will be aliquoted in glass vials and stored in the freezer set to maintain -20°C.

The IS stock solution will be diluted in methanol containing 300 µM sodium thiosulfate to obtain a solution with a final IS concentration of 3000 ng/mL (IS working solution). If applicable, additional IS working solutions with a different final IS concentration may be prepared.

IS working solutions will be prepared freshly on the day of use. Any residual volumes will be discarded unless otherwise requested by the Study Director.

6.2. Sample Collection and Analysis

Analysis of test item in vehicle for concentration, stability, homogeneity will not be performed, however, to limit the impact, the test item preparation will be performed with approved procedures and documented in detail. Formulations will be visually inspected for homogeneity prior to use.

7. TEST SYSTEM

Test system	FTC-238-hrTPO cell lysates.
Rationale	The FTC-238 human follicular thyroid carcinoma cell line was established from a lung metastasis of a follicular thyroid carcinoma from a 42-year-old male. The cells are polymorphic showing flat polygonal to spindle-like morphologies. The FTC-238 cells are genetically modified to incorporate human recombinant TPO and a neomycin resistance gene. Prepared cell lysates of hematin-stimulated FTC-238-hrTPO cells contain active human thyoid peroxidase.
Source	The FTC-238-hrTPO cell line was provided by the study Sponsor EURL ECVAM, who obtained it from Charité.

Storage	FTC-238-hrTPO cell lysates are stored in an ultra-low freezer set to maintain -80°C.

8. EXPERIMENTAL DESIGN

Incubations will be performed with FTC-238-hrTPO cell lysates and the test items to evaluate the possible inhibitory effect of each test item on the TPO-catalyzed iodination by measuring the formation of MIT.

At least two independent and valid runs will be performed for each test item. If results between the two independent and valid runs differ, additional runs may be performed for selected test items.

During the first (range finding) experiment, it is recommended to use the highest possible test item concentration, however, the highest (nominal) concentration to be tested should be at least 10^{-5} M irrespective of the test item solubility. When concentrations equal or lower than 10^{-5} M are insoluble, this will be noted in the raw data files of the study and mentioned in the Final Report. The lowest concentration to be tested is generally 10^{-10} M. If the highest concentration to be tested is lower than $10^{-3.5}$ M, mid-log concentration(s) will be added near the lower end of the curve to obtain a total of eight test item concentrations.

If during the first experiment the top of the curve (control iodination activity) is not reached at the lowest test item concentration, lower concentrations will be selected for the second experiment and, only if applicable, subsequent experiment(s).

8.1. Solubility Test

For each test item, a preliminary test will be performed to determine whether the test item may have any solubility problems, i.e., the presence of cloudiness or precipitate will be evaluated. The test item will be dissolved in an appropriate vehicle (e.g., DMSO) to prepare a stock solution at an initial concentration of 100 mM. The presence of cloudiness or precipitate will be evaluated by visual inspection and under the microscope. If necessary, the test item stock solution will be further diluted (e.g., ½ log lower) to define the highest soluble concentration of test item in vehicle.

If the test item is soluble in vehicle, a 100-fold dilution of the stock solution will be prepared in the incubation buffer and it will be determined whether the test item may have any solubility problems in the incubation buffer, i.e., the presence of cloudiness or precipitate will be evaluated visually and using a light microscope. If necessary, the test item stock solution in vehicle will be further diluted (e.g., ½ log lower) to define the highest soluble concentration of test item in the incubation buffer.

8.2. TPO-Catalyzed Iodination Assay

In the TPO-catalyzed iodination assay, incubations will be performed with FTC-238-hrTPO cell lysates to determine the possible suppressive effect of the test item on the TPO-catalyzed iodination by measuring the formation of the metabolite MIT.

Incubation mixtures will be prepared on ice by mixing phosphate buffer (0.1 M, pH 7.4), potassium iodide (final concentration 150 µM), L-tyrosine (final concentration 500 µM) FTC-238-hrTPO cell lysate (fixed concentration, details will be specified in the Final Report) and vehicle, test or control items. After shaking, the samples will be pre-incubated for

5 minutes at 37±1°C in a water bath and the reaction will be started by the addition of 20 µL H_2O_2 (final concentration 250 µM). The total volume will be 300 µL. Incubations will be stopped after the appropriate incubation time (fixed time, details will be specified in the Final Report) by transferring the reaction tubes to ice and addition of a half reaction volume of the appropriate IS working solution (fixed IS concentration in the IS working solution, details will be specified in the Final Report). After vortex-mixing of the containers, the samples will be kept on ice until centrifugation (2000 *g* for at least 5 minutes at 4°C) and prepared for MIT analysis by UPLC-MS/MS.

When one incubation plate will be prepared for an experiment, vehicle controls, assay buffer controls (no vehicle controls), no peroxide controls, non-enzymatic iodination controls, the negative control DEHP at a single concentration (1 mM), the reference item PTU at eight concentrations (1 nM, 10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 31.6 µM) and incubations with the appropriate test items will be included.

When two incubation plates will be prepared for an experiment, vehicle controls, assay buffer controls (no vehicle controls), no peroxide controls, non-enzymatic iodination controls, the negative control DEHP at a single concentration (1 mM) and incubations with the appropriate test items will be included on each incubation plate in triplicate. The first incubation plate will contain the reference item PTU which will be tested at eight concentrations (1 nM, 10 nM, 100 nM, 316 nM, 1 µM, 3.16 µM, 10 µM and 31.6 µM) in triplicate. The second incubation plate will also contain the reference item PTU which will only be tested at the highest concentration (31.6 µM) in triplicate.

An overview of the incubations included for each independent experiment is presented in the table below.

Composition of Incubations for Each Independent Experiment

Constituent (final concentration)	No peroxide control	Vehicle control	No vehicle control	Non-enzymatic iodination control	Test item, reference item or negative control material incubations
Phosphate buffer (0.1 M, pH 7.4)	X	X	X	X	X
Tyrosine (500 µM)	X	X	X	X	X
Potassium Iodide (150 µM)	X	X	X	X	X
H_2O_2 (250 µM)	---	X	X	X	X
FTC-238-hrTPO cell lysate	X	X	X	---	X
Inactivated FTC-238-hrTPO cell lysate	---	---	---	X	---
Vehicle (1%)	X	X	---	X	---
Test item or PTU (in triplicate) and DEHP (in triplicate)	---	---	---	---	X

8.3. Analysis of MIT

8.3.1. MIT Stock and Spiking Solutions

Duplicate MIT stock solutions (stocks A and B) will be prepared at a 10 mM concentration in 0.1 M HCl in glass vials. Stock solutions will be prepared freshly on the first day of use and any residual volumes will be discarded unless otherwise requested by the Study Director.

MIT stock solutions will be diluted in 0.1 M potassium phosphate buffer pH 7.4 to obtain spiking solutions for the preparation of calibration standards and quality control (QC) samples. Spiking solutions will be prepared as presented in the tables below.

Preparation of MIT Spiking Solutions used for the Preparation of Calibration Standards

Code	Applied solution	Volume applied (µL)	Volume buffer added (µL) [1)]	Target concentration (µM)
Spike 0.0761 µM	Spike 0.352 µM	108	392	0.0761
Spike 0.163 µM	Spike 0.756 µM	108	392	0.163
Spike 0.352 µM	Spike 1.63 µM	108	392	0.352
Spike 0.756 µM	Spike 3.50 µM	108	392	0.756
Spike 1.63 µM	Spike 7.55 µM	108	392	1.63
Spike 3.50 µM	Spike 16.2 µM	108	392	3.50
Spike 7.55 µM	Spike 35.0 µM	108	392	7.55
Spike 16.2 µM	Spike 75.0 µM	108	392	16.2
Spike 35.0 µM	150 µM – B	116.5	383.5	35.0
Spike 75.0 µM	150 µM – A	300	300	75.0
150 µM – B	Stock B	15	985	150
150 µM – A	Stock A	15	985	150

[1)] The buffer will consist of 0.1 M potassium phosphate buffer pH 7.4.

Preparation of MIT Spiking Solutions used for the Preparation of QC Samples

Code	Applied solution	Volume applied (µL)	Volume buffer added (µL) [1)]	Target concentration (µM)
Spike 15 µM	Spike 75 µM	200	800	0.150
Spike 75 µM	Spike 600 µM	125	875	0.750
Spike 600 µM	Spike 6 mM	100	900	6.00
Spike 6 mM	Stock A	600	400	60.0

[1)] The buffer will consist of 0.1 M potassium phosphate buffer pH 7.4.

If applicable, other volumes may be used as long as the ratios between components remain the same.

8.3.1.1. Calibration Standards

Ten calibration standards will be prepared from MIT spiking solutions which will be prepared from two MIT stock solutions (A and B as described in Section 8.3.1). An aliquot of the appropriate spiking solution will be added to a 3000 ng/mL IS working solution as described in the table below. Samples will be vortex-mixed and prepared for UPLC-MS/MS analysis by diluting them 100-fold in phosphate buffer (0.1 M, pH 7.4). Calibration standards will be prepared freshly on the first day of use.

Preparation of MIT Calibration Standards

Code	Applied solution	Volume Applied		Target concentration (µM) [2)]
		Spiking solution (µL)	IS Working Solution (µL)	
MIT 0.0 µM	Buffer [1)]	300	150	0
MIT 0.0761 µM	Spike 0.0761 µM	300	150	0.0761
MIT 0.163 µM	Spike 0.163 µM	300	150	0.163
MIT 0.352 µM	Spike 0.352 µM	300	150	0.352
MIT 0.756 µM	Spike 0.756 µM	300	150	0.756
MIT 1.63 µM	Spike 1.63 µM	300	150	1.63
MIT 3.50 µM	Spike 3.50 µM	300	150	3.50
MIT 7.55 µM	Spike 7.55 µM	300	150	7.55
MIT 16.2 µM	Spike 16.2 µM	300	150	16.2
MIT 35.0 µM	Spike 35.0 µM	300	150	35.0
MIT 75.0 µM	Spike 75.0 µM	300	150	75.0

[1)] The buffer will consist of 0.1 M potassium phosphate buffer pH 7.4.
[2)] Concentration in the sample before addition of the IS working solution (see Section 6.1.4).

If applicable, other volumes may be used as long as the ratios between components remain the same.

8.3.1.2. Preparation of Quality Control (QC) Samples

MIT spiking solutions will be applied to prepare quality control (QC)-0, low (L), -middle (M), -high (H) samples. First, a blank matrix working solution will be prepared by mixing the following components:

(1) 4631 µL 0.1 M potassium phosphate buffer pH 7.4
(2) 300 µL 10 mM L-Tyrosine dissolved in 0.1 M HCl
(3) 9 µL 100 mM potassium iodide prepared in buffer (1)
(4) 600 µL of heat-inactivated FTC-238-hrTPO cell lysate solution (concentration to be specified in the Final Report) solution in buffer (1)

An aliquot of the appropriate spiking solution will be added to the blank matrix working solution as described in the table below. The QC samples will be prepared freshly on the first day of use.

Preparation of MIT QC-0, QC-L, QC-M and QC-H Samples

Code	Applied solution	Volume Applied			Target concentration (µM) [2)]
		Spiking solution (µL)	Blank Matrix Working Solution (µL)	IS Working Solution(µL)	
QC-0	Buffer [1)]	3	277	150	0
QC-L	Spike 15 µM	3	277	150	0.150
QC-M	Spike 75 µM	3	277	150	0.750
QC-H	Spike 6 mM	3	277	150	60.0

[1)] The buffer will consist of 0.1 M phosphate buffer pH 7.4.
[2)] Concentration in the sample before addition of the IS working solution (see Section 6.1.4).

QC-0, QC-L, QC-M and QC-H samples will be prepared in polypropylene tubes in triplicate as described in the table above and will be vortex-mixed after which 3.75 µL of H_2O_2 will be added to each tube. After vortex-mixing of the containers one by one, the samples will be kept on ice until centrifugation (2000 *g* for at least 5 minutes at 4°C) and prepared for UPLC-MS/MS analysis by diluting them 100-fold in potassium phosphate buffer (0.1 M, pH 7.4).

If applicable, other volumes may be used as long as the ratios between components remain the same.

8.3.1.3. UPLC-MS/MS Analysis

MIT and IS peak areas in the samples will be measured by UPLC-MS/MS using the method validated in Charles River Test Facility Study No. 20278296 and the following system:

- Acquity UPLC I-Class system (Waters, Milford, MA, USA)
- Xevo TQ-(X)S mass spectrometer (Waters)

Data will be acquired and interpreted with MassLynx software (Waters).

9. ACCEPTABILITY CRITERIA

9.1. Sample Analysis

An UPLC-MS/MS analytical run is considered acceptable if the criteria for the calibration curve and the QC samples are met.

9.1.1. Calibration Curve

The response of the calibration standards will be correlated with the nominal MIT concentration of the calibration solutions using linear regression analysis with a $1/x^2$ weighting factor. Calibration curves with back calculated accuracies within the criterion range of 80-120% of the nominal concentration for the lowest calibration standard and 85-115% of the nominal concentration for the remaining calibration standards will be accepted.

When a back calculated accuracy (once established) does not comply with the criterion range, the response of the calibration standard with the highest deviation will be rejected and the calibration curve will be re-evaluated. Calibration curves will be accepted when ≥ 75% of the calibration standards fulfill the acceptance criteria.

Zero will not be part of the calibrated range. Blank samples will not be taken into account in the fitting procedure.

9.1.2. QC Samples

The analytical method will be considered applicable for the quantitative analysis of MIT in the samples when the accuracy of the QC samples is in the criterion range of 85-115%. Results outside the criterion range may be discarded as long as per run 2/3 of the QC samples are accepted with ≥ 50% of each level.

9.2. TPO-Catalyzed Iodination Assay

An independent TPO-catalyzed iodination experiment will be considered acceptable if the following criteria are met:

- The final curve for the reference item PTU is composed of a minimum of six concentrations obtained from the average of three replicates after excluding samples on the basis of insolubility, operator errors or other information.
- The final curve for a test item is composed of a minimum of six concentrations obtained from the average of three replicates after excluding samples on the basis of insolubility, operator errors or other information.
- The percentage of TPO-catalyzed iodination of the lowest test item concentration is within the range of 80%-120% when compared to the averaged activity in the vehicle control samples.
- A complete sigmoidal curve for the reference item PTU is obtained.
- The calculated IC_{50} for PTU is within the range of 0.5 – 5 µM.
- The percentage of TPO-catalyzed iodination compared to the vehicle control for the negative control DEHP is > 80%.
- The averaged percentage of TPO-catalyzed iodination of the no-peroxide control samples is < 1% when compared to the averaged activity in the vehicle control samples.
- The mean percentage of TPO-catalyzed iodination of the no-vehicle controls is within the range of 80%-120% when compared to the averaged TPO-catalyzed iodination in the vehicle control samples.

9.3. Data Interpretation Criteria

For each run, a test item will be considered negative when the percentage of TPO-catalyzed iodination compared to the average activity in the vehicle control samples remains above 80% for all concentrations tested.

For each run, a test item will be considered positive when the percentage of TPO-catalyzed iodination compared to the average activity in the vehicle control samples is less or equal to 80% for at least one concentration and is showing a dose-dependent effect.

A run will be considered inconclusive in all other cases.

If (one of) the acceptability criteria are not met and the Study Director decides that this has a critical effect on the study, the test will be rejected and repeated.

10. ANALYSIS

10.1. MIT Analysis

Response (R)

Peak area of the analyte × (IS Conc./ IS peak area) [units]

Calibration curve

$R = a \times C_N + b$

where:

a = linear regression factor

b = intercept

C_N = nominal concentration

Regression analysis will be performed using the least squares method.

Analyzed concentration (C_A)

$$C_A = \frac{(R - b)}{a} \text{ [µM]}$$

Please note that if a different dilution factor for the study samples has been used than the 100-fold dilution factor used for the calibration standards and QC samples, MIT concentrations of the study samples need to be corrected for this. For example, if a 20-fold dilution factor was used for the study samples (and a 100-fold dilution factor for the calibration standards and QC samples), the calculated MIT concentration in the study samples needs to be divided by a factor 5.

Accuracy of analytical QC samples

$$\frac{C_A - C_B}{C_N} \times 100 \text{ [\%]}$$

where:

C_B = analyzed concentration in QC-0 sample

10.2. TPO-Catalyzed Iodination

The percentage of TPO-catalyzed iodination will be calculated compared to the average TPO-catalyzed iodination in the vehicle control samples (= full activity) for each individual sample (vehicle control, no vehicle control, no peroxide control, DEHP, PTU and test item samples) using the following equation:

$$\%\text{TPO catalyzed iodination} = \frac{C_A \text{ in sample}}{\text{Average } C_A \text{ of vehicle control samples}} \times 100\%$$

If applicable, the IC_{50} value will be calculated by plotting the percentage of control activity versus the logarithm of the concentration fitted by the Hill curve model (variable slope,

4 parameters) using GraphPad Prism (GraphPad Software, San Diego, USA) and the following equation:

$$y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{\left(1 + 10^{((\text{LogIC}_{50} - x) * \text{HillSlope})}\right)}$$

In which the variables will be defined as follows:

Y = Percent of the control activity
X = Logarithm (base 10) of the concentration
Top = Top of the curve in same units as Y
Bottom = Bottom of the curve in same units as Y
Log IC_{50} = Logarithm of concentration at which 50% of maximum response is observed
HillSlope = Slope factor of the Hill curve

11. COMPUTERIZED SYSTEMS

The following computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Computerized Systems

System Name	Description of Data Collected and/or Analyzed
Share Document Management System	Reporting
M-Files®	Reporting and collection of 21 CFR Part 11 compliant signature
Magellan Tracker	Optical Density Measurement System control and data acquisition
MassLynx	System control, data acquisition and integration
REES Centron	Temperature, relative humidity and/or atmospheric pressure monitoring

Data for parameters not required by Study Plan, which are automatically generated by analytical devices used will be retained on file but not reported. Statistical analysis results that are generated by the program but are not required by Study Plan and/or are not scientifically relevant will be retained on file but will not be included in the tabulations.

12. REGULATORY COMPLIANCE

This study is not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

13. AMENDMENTS AND DEVIATIONS

Changes to the approved Study Plan shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary Study Plan changes in advance with the Sponsor. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

14. RETENTION AND DISPOSITION OF RECORDS

All applicable study-specific raw data, electronic data, documentation, Study Plan and Final Report will be archived at finalization of the report. All materials generated by Charles River from this study will be transferred to a Charles River archive.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study Plan, Study Plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt
- Test item receipt, identification and preparation
- Measurements and observations

Disposition of residual/retained analytical samples will be as described in the table below.

Disposition of Residual/Retained Samples

Sample Type	Disposition	Schedule
Analytical (and Test item used in analysis)	Discard	After completion of the measurements

15. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft Report. The Final Report will be provided in Adobe Acrobat PDF format (hyperlinked and searchable). The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

16. JUSTIFICATIONS AND GUIDELINES

16.1. Guidelines for Study

This study is not within the scope of regulations governing the conduct of nonclinical laboratory studies and is not intended to comply with such regulations.

17. REFERENCES

1. Doerge, D.L., Chang, H.C., Divi, R.L., Churchwell, M.I., Mechanism for inhibition of thyroid peroxidase by Leucomalachite Green. Chemical Research in Toxicology 11, p. 1098-1104 (1998).

2. Freyberger, A., Ahr, H.-J., Studies on the goitrogenic mechanism of action of *N,N,N'N'*-tetramethylthiorea. Toxicology, p. 169-175 (2006).

3. Price, R.J., Burch, R., Chatham, L.R., Higgins, L.G., Currie, R.A., Lake, B.G., An assay for screening xenobiotics for inhibition of rat thyroid gland peroxidase activity. Xenobiotica, DOI: 10.1080/00498254.2019.1629044.

TEST FACILITY APPROVAL

All electronic signatures appear at the end of the document upon finalization.

SPONSOR APPROVAL

The Study Plan was approved by the Sponsor by e-mail on the date designated below. The correspondence giving approval will be archived, as appropriate with other Sponsor communications.

30 May 2022
Date of Sponsor Approval

ATTACHMENT A

Distribution List

Electronic copies will be supplied unless otherwise specified below.

Version	**Recipient**
Original	Study Director
1 Copy	Sponsor Representative / Study Monitor
1 Copy	Management

SIGNATURE(S) FOR DOCUMENT: 20382705 - 49149 EU NETVAL TPO Inhibition assay Part 2 Final Study Plan

TFM Approval-NGLP:	I approve the Study Director identified in this document and acknowledge the study.
Name:	**Wenker, Mira**
	Wenker, Mira
Electronically Signed in M-Files	Timestamp: 30-May-2022 09:02:38 (UTC+00:00)

Study Director Approval:	I approve this document.
Name:	**Reinen, Jelle**
	Reinen, Jelle
Electronically Signed in M-Files	Timestamp: 30-May-2022 14:43:26 (UTC+00:00)

Appendix 2
UPLC-MS/MS Conditions for the Analysis of MIT

Table 91
UPLC-MS/MS Conditions for the Analysis of MIT

Mobile Phase:	A: 0.1% FA in MQ B: 0.1% FA in ACN	
	Gradient: 0.0-0.2 min 0.2-1.2 min 1.2-1.5 min 1.5-1.9 min 1.9-2.0 min 2.0-2.5 min	95% A - 5% B 50% A - 50% B 5% A - 95% B 5% A - 95% B 95% A - 5% B 95% A - 5% B
Flow Rate :	0.4 mL/min	
Column:	Acquity UPLC BEH C18 50 mm × 2.1 mm ID, 1.7 µm particle size	
Column oven temperature:	Set at 40ºC	
Sample tray temperature:	Set at 8ºC	
Injection volume:	3 µL	
Detection:	Ionisation source: Capillary voltage: Source temperature: Source parameters:	ESI+ 1.0 kV 120ºC x = 6 z = 6
	Collision Gas Flow Desolvation Temp. Cone Gas Flow Desolvation Gas Flow	0.18 mL/min 500ºC 150 L/Hr 1000 L/Hr
	MRM monitored MIT Dwell Time	 *m/z* 307.75 > *m/z* 134.9 Collision energy: 26 eV Cone voltage: 20 V Measured from 0.6 – 2.50 min 100 ms.
	MIT-$^{13}C_6$ Dwell Time	*m/z* 313.75 > *m/z* 140.9 Collision energy: 26 eV Cone voltage: 20 V Measured from 0.6 – 2.50 min 50 ms.
	Divert Valve:	0.0 min to waste / 0.5 min to MS / 1.5 min to waste.

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Study Director Approval:	I approve this Report.
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