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PERFORMANCE STANDARDS FOR STABLY TRANSFECTED TRANSACTIVATION IN VITRO ASSAYS TO DETECT ESTROGEN AGONISTS FOR TG 455

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IOMC

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organisations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. UNDP is an observer. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

This document includes Performance Standards (PS) for stably Transfected transactivation *in vitro* assays to detect estrogen agonists. These PS accompany the Performance-Based Test Guideline (PBTG) for Transfected Transactivation *In Vitro* Assays to Detect Estrogen Agonists (updated TG 455). They were discussed at length by a validation management group for non animal testing, during a meeting that was held on 30 November - 2 December 2011 and at a conference call held on 20 December 2011. Comments from the Working Group of National Coordinators of the Test Guidelines Programme (WNT) were requested on the draft PBTG and on these draft PSs in October and December 2011.

The number of chemicals, laboratories and runs for establishing the accuracy, within-laboratory reproducibility and between-laboratory reproducibility were further discussed at conference call of the validation management group in March 2012. The PS were approved by the WNT with a few changes at its meeting held on 24-27 April 2012, and declassified by the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides, and Biotechnology on 26 July 2012.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides, and Biotechnology.

INTRODUCTION

1. The following Performance Standards (PS) accompanies the Performance Based Test Guideline for Transfected Transactivation *In Vitro* Assays to Detect Estrogen Agonists (TG 455). This document is intended as a guide to developers of new test methods that are analogous to existing, fully validated test methods in that they are based on similar scientific principles and predict the same effect (colloquially referred to as "me too" tests) (1). Prior to the acceptance of a new test method for regulatory testing applications, validation studies are conducted using scientifically sound principles to establish its reliability (i.e., the extent of intra- and inter-laboratory reproducibility over time when performed using the standardized protocol), and its relevance (i.e., the ability of the test method to correctly predict or measure the biological effect of interest) (1) (2) (3) (4). The purpose of the PS is to communicate the basis by which new proprietary (i.e. copyrighted, trademarked, registered) or non-proprietary test methods can be determined to have sufficient accuracy (i.e., agreement between a test method result and an accepted reference value) and reliability (i.e., extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol) for a specific testing purpose. Thus, this provides an avenue to demonstrate that a newly developed test method based on similar scientific principles has comparable or better performance capabilities than those from which the existing PS were derived, and may allow a more timely use of the new test method. New test methods ("me too" tests) can be added to TG 455 after OECD review and agreement that performance standards are met. A new test method developed under this PS will be covered by TG 455 only after TG 455 has been updated to add the new test method.

2. Performance standards are based on an adequately validated test method(s) and provide a basis for evaluating the comparability of a proposed test method that is mechanistically and functionally similar (1) (2). The three elements of performance standards are:

- Essential test method components: These consist of essential structural, functional, and procedural elements of a validated test method that should be included in the protocol of a proposed test method that is considered to be mechanistically and functionally similar to the validated method. Essential test method components include unique characteristics of the test method, critical procedural details, and quality control measures.

- A list of reference chemicals: Reference chemicals are used to assess the accuracy and reliability of a proposed mechanistically and functionally similar test method. These chemicals are a representative subset of those used to demonstrate the reliability and the accuracy of the validated test method, and are the minimum number that should be used to evaluate the performance of a proposed mechanistically and functionally similar test method.

- Accuracy and reliability performance values: These are the standards for accuracy (i.e., sensitivity, specificity, false positive/negative rates) and reliability (i.e., degree to which the test method can be performed reproducibly within and among laboratories over time) that the proposed test method should meet or exceed when evaluated using the minimum list of reference chemicals.

3. The fully validated reference test methods that provide the basis for this PS are**:**

- The Stably Transfected TA assay (STTA) using the human (h) ERα-HeLa-9903 cell line (5) and
- The BG1Luc ER TA assay (6) using the BG1Luc4E2 cell line which predominately expresses hERα with some contribution from hERβ (7) (8).

ESSENTIAL TEST METHOD COMPONENTS AND OTHER VALIDATION CONSIDERATIONS

4. Certain principles are important in delineating the essential test method components that determine whether transactivation (TA) tests are functionally and mechanistically similar. *In vitro* estrogen receptor (ER) TA assays are designed to identify substances that might interfere with ER-mediated cellular processes *in vivo*. The interaction of estrogens with cellular ER initiates a cascade of events leading to the expression of specific genes in multiple target tissues.

5. The *following test method components may vary*, so this PBTG does apply to test methods that may differ in

- cell type (e.g. mammalian, fish, yeast)
- cell line (tissue type)
- characteristics of the cell line including presence of other receptors and metabolism
- culture conditions
- plating density
- plate layout (including how controls are incorporated)
- ERα characteristics (full length or partial, species of origin); if other ER proteins are present, ERα should predominate and the relative expression of each receptor should be known
- reporter gene construct (promoter, receptor binding elements, reporter)
- method of determining cytotoxicity.

These elements should be clearly described in each test method.

6. Essential test method components for *in vitro* ER TA protocols should include:

- The use of a strong reference estrogen, preferably 17ß-estradiol, to demonstrate the adequacy of the method for detecting ER agonists;

- A weak positive control with a potency (e.g., PC_{50}, EC_{50}) two to five orders of magnitude lower than the reference estrogen should be included to provide another quality control measure by which to judge the acceptability of the method for detecting a weak agonist, and by which to evaluate the reproducibility of the test method.

- A vehicle control (e.g., DMSO, EtOH, or H_2O) that is miscible with cell culture media at concentrations that are not cytotoxic and do not otherwise interfere with the test system.

- For initial range-finding, at least seven concentrations spaced at decadic logarithmic (log10) intervals should be tested up to the maximum concentration (see below). Based on these range-finding experiments, a suitable concentration range should then be used for testing the chemical in view of generating data on the possible potency of the substance and to derive categorical predictions (e.g., Positive, Negative).

- In the absence of solubility or cytotoxicity restraints, the maximum concentration may be 1 mM or even up to the limit of solubility, if appropriate.

- A qualitative or quantitative evaluation of cytotoxicity and how it is applied to the test method should be included in each study. Concentrations of test substances that clearly reduce viability should not be considered in the analysis of the data.

- All concentrations of the controls (e.g., vehicle, weak positive(s), or negative(s)), the reference estrogen, and the test substance should be tested in more than one replicate well.

7. No standardized statistical methods for analyzing data obtained from *in vitro* ER TA agonist assays have been developed. Each test method should establish a well-defined method for classifying a positive and a negative response. Positive results should be characterized by both the magnitude of the effect and the concentration at which the effect occurs (e.g., an EC_{50}, PC_{50}, % max, etc.) when possible.

8. To ensure that a proposed *in vitro* ER TA test method possesses characteristics similar to other validated test methods, the reference chemicals for testing ER agonists listed in Table 1 should be used to demonstrate the reliability and accuracy of the new test method. The 22 recommended Reference Chemicals, representing chemical classes commonly associated with ER agonist activity, have been classified as ER agonists or negatives based upon published reports, including *in vitro* assays for ER binding and TA, and the *in vivo* uterotrophic assay (6) (9) (10) (11) (12) (13) (14) (15). The Reference Chemicals were tested in both the STTA and BG1Luc ER TA test methods (6) (9); the classifications (16 positive, 6 negative) were 100% concordant between the two test methods and consistent with the classifications as ER agonists or negatives, and the group of chemicals cover the potency range of known ER agonists (i.e., EC_{50} 1×10^{-12} M) to very weak (i.e., PC_{10}, EC_{50} 1×10^{-5} M) to negative for ER agonist activity. If a Reference Chemical is no longer commercially available, a substance with the same classification and, comparable potency, mode of action and chemical class can be used. Supplementary information including the full listings of chemicals tested in both the STTA and the BG1Luc ER TAs, as well as additional chemicals tested in each test method during the respective validation studies, is provided in Annex 2 (Tables 1 - 10). Additional chemicals not included in the reference chemical list may be used to demonstrate an improvement (e.g., improved reproducibility and/or accuracy with regard to accepted reference data) of the new test method as compared with the fully validated test methods.

Table 1: List of Reference Chemicals (22) for Evaluation of ER Agonist Accuracy[1]

	Chemicals[1,2]	CASRN	Expected Reponse[1, 3]	Bg1Luc EC_{50} Value[3,4,5] (M)	STTA and BG1Luc ER TA Results[4,5,7]	STTA ER TA[6,7]	
						PC_{10} Value (M)	PC_{50} Value (M)
1	Ethyl paraben	120-47-8	POS	2.48×10^{-5}	POS	5.00×10^{-6}	-
2	Kaempferol	520-18-3	POS	3.99×10^{-6}	POS	1.36×10^{-7}	1.21×10^{-6}
3	Butylbenzyl phthalate	85-68-7	POS	1.98×10^{-6}	POS	1.14×10^{-6}	4.11×10^{-6}
4	*p,p*'- Methoxychlor	72-43-5	POS	1.92×10^{-6}	POS	1.23×10^{-6}	-
5	19-Nortestosterone	434-22-0	POS	1.80×10^{-6}	POS	9.64×10^{-9}	2.71×10^{-7}
6	Bisphenol A	80-05-7	POS	5.33×10^{-7}	POS	2.02×10^{-8}	2.94×10^{-7}
7	Kepone	143-50-0	POS	4.91×10^{-7}	POS	7.11×10^{-7}	7.68×10^{-6}
8	4-Cumylphenol	599-64-4	POS	3.20×10^{-7}	POS	1.49×10^{-7}	1.60×10^{-6}
9	Genistein	446-72-0	POS	2.71×10^{-7}	POS	2.24×10^{-9}	2.45×10^{-8}
10	Coumestrol	479-13-0	POS	1.32×10^{-7}	POS	1.23×10^{-9}	2.00×10^{-8}
11	4-*tert*-Octylphenol	140-66-9	POS	3.19×10^{-8}	POS	1.85×10^{-9}	7.37×10^{-8}
12	17α-Estradiol	57-91-0	POS	1.40×10^{-9}	POS	7.24×10^{-11}	6.44×10^{-10}
13	Norethynodrel	68-23-5	POS	9.39×10^{-10}	POS	1.11×10^{-10}	1.50×10^{-9}
14	Diethylstilbestrol	56-53-1	POS	3.34×10^{-11}	POS	$<1.00 \times 10^{-11}$	2.04×10^{-11}
15	*meso*-Hexestrol	84-16-2	POS	1.65×10^{-11}	POS	$<1.00 \times 10^{-11}$	2.75×10^{-11}
16	17α-Ethinyl estradiol	57-63-6	POS	7.31×10^{-12}	POS	$<1.00 \times 10^{-11}$	$<1.00 \times 10^{-11}$
17	Atrazine	1912-24-9	NEG	-	NEG	-	-
18	Corticosterone	50-22-6	NEG	-	NEG	-	-
19	Linuron	330-55-2	NEG	-	NEG	-	-
20	Spironolactone	52-01-7	NEG	-	NEG	-	-
21	Ketoconazole	65277-42-1	NEG	-	NEG	-	-
22	Reserpine	50-55-5	NEG	-	NEG	-	-

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; EC_{50} – half maximal effective concentration; NEG = negative; PC_{10} (and PC_{50}) = the concentration of a test chemical at which the response is 10% (or 50% for PC_{50}) of that induced by the positive controls (E2, 1nM); POS = positive.

[1]Chemicals, classified as ER agonists or negatives (6, 9, 10-15), were selected to represent the different chemical classes and the range of potency from strong (i.e., EC_{50} 1×10^{-12} M) to very weak (i.e., PC_{10}, EC_{50} 1×10^{-5} M) to negative for ER agonist activity.

[2]See Annex 2 (Table 1) for chemical and product classes as assigned using the U.S. National Library of Medicine's Medical Subject Headings (MeSH), an internationally recognized standardized classification scheme (available at: http://www.nlm.nih.gov/mesh), and the U.S. National Library of Medicine's Hazardous Substances Database (available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB).

[3]Expected responses and BG1Luc ER TA data compiled and reported in ICCVAM Test Method Evaluation Report on the LUMI-CELL® ER (BG1Luc ER TA) Test Method, An *In Vitro* Method for Identifying ER Agonists and Antagonists [6].

[4]Mean EC_{50} values were calculated with values reported by the laboratories of the BG1Luc ER TA validation study (XDS, ECVAM, and Hiyoshi).

[5]See draft proposal for new test guideline: BG1Luc Estrogen Receptor Transactivation Test Methods for identifying estrogen receptor agonist and antagonists, Table 4 for definitions of positive and negative classifications.

[6]See OECD TG 455: Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals, Table 5 for definitions of positive negative classifications (5).

[7]$PC10/PC_{50}$ values reported in Appendix 2 (Tables 1 and 2) and in the Draft Report of Pre-validation and Inter-laboratory Validation for Stably Transfected Transcriptional Activation (TA) Assay to Detect Estrogenic Activity - The Human Estrogen Receptor Alpha Mediated Reporter Gene Assay Using hER-HeLa-9903 Cell Line (9).

9. Metabolism of the reference chemicals in the cell system under development should be considered when assessing the results when testing the Reference Chemicals (Table 1). The degree of metabolic competence of the cell system may influence the qualitative (positive or negative) or quantitative (EC_{50}/PC_{10}) result. Metabolism of inactive chemicals to active chemicals, e.g., from DEHP (bis (2-ethylhexyl) phthalate) into MEHP (mono (2-ethylhexyl) phthalate), or from active chemicals to more active metabolites, e.g, metabolism of methoxychlor to HPTE (2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane), or the conversion of estrone into 17β-estradiol, may result in lower EC_{50}/PC_{10} values. However, the opposite may occur as well, e.g. inactivation of estradiol by hydroxylation, or in cell lines competent in Phase 2 metabolising enzymes the test chemicals may be metabolised to inactive glucuronide

or sulphate conjugates. Ideally, the metabolic capability of the cell line should be characterised. However, the metabolic capabilities of the STTA and BG1Luc cell lines have not been completely characterised, and therefore, this should also be considered. These considerations are extremely important when considering results of ER-TA test methods in the context of QSAR modeling approaches, as it may not be the compound under investigation that is actually responsible for the observed response, but rather the metabolites formed.

10. New similar test methods should not be developed solely on the basis of the 22 Reference Chemicals, but rather on a sufficiently large test development set. Reference Chemicals should be preferentially used to determine equivalence of performance compared to the validated reference test methods.

11. All chemicals should be tested in a coded/blinded manner. When evaluated using these reference chemicals, the reliability and accuracy (i.e. sensitivity, specificity, false positive rates, and false negative rates) of the proposed ER TA test method should approximate the following:

DEFINED RELIABILITY AND ACCURACY VALUES

12. For the purposes of establishing the reliability and accuracy of the proposed test method when transferred between laboratories, all 22 Reference Chemicals (Table 1) should be tested in two or (preferably) three laboratories. In each laboratory, all 22 Reference Chemicals should be tested in at least one run.

Within-laboratory reproducibility

13. For the assessment of within-laboratory reproducibility, the concordance of classifications (positive/negative) obtained in three independent consecutive test runs should be 100% for each laboratory when using the following subset of 8 chemicals selected from the 22 reference chemicals in Table 1 (weak positives: Genistein, Bisphenol A, Kaempferol, Butylbenzylphthalate, *p,p*'-Methoxyclor, Ethylparaben; negatives: Atrazine, Spironolactone). Three independent consecutive runs are required to fulfil the criteria for acceptance. If, for example, runs 2 and 3 are inconsistent with run 1, one additional run (run 4) will be sufficient to show within-lab reproducibility if run 4 is consistent with runs 2 and 3. If run 4 is consistent with run 1 instead, then at least two additional consecutive runs (runs 5 and 6) showing consistency with run 4 will be required to fulfil the requirement for three consecutive independent runs that have 100% concordance of classifications.

Between-laboratory reproducibility

14. To assess between-laboratory reproducibility, the remaining 14 Reference Chemicals should be tested at least once in a minimum of two laboratories. All the data available on the 22 chemicals (8 tested three times; the other 14 tested once in each laboratory) should be utilised. Concordance of classifications (negative/positive) between laboratories should be used as a measure to describe between-laboratory reproducibility. To be considered acceptable, a test method should show concordance of 83 % or greater.

Predictive capacity (accuracy)

15. The accuracy (sensitivity, specificity, and overall accuracy) of the proposed test method should be comparable to that demonstrated for the fully validated test methods, the STTA and BG1Luc ER TAs (6) (9). On the basis of the performance values (sensitivity / specificity) of the validated reference methods for chemicals in the validation test set as well as other empirical data from these methods (see Annex 2),

the target values for sensitivity, specificity, and overall accuracy to be obtained when testing the 22 Reference Chemicals (Table 1) are set to be greater or equal to 95%.

16. Although it is not realistic to expect test methods to perform identically, discordant results should be discussed in terms of the ability of the test method to detect a similar range of potencies and chemical/product classes, as demonstrated by the fully validated test methods (6) (9).

LITERATURE

1. OECD (2005), *Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment*, Series on Testing and Assessment No.34,,OECD, Paris. Available at: http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=ENV/JM/MONO(2005)14&doclanguage=en .

2. ICCVAM (1997), Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM),. NIH Publication No. 97-3981, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

3. ICCVAM (2003), ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods,NIH Publication No. 03-4508, Research Triangle Park, NC, National Institute of Environmental Health Sciences

4. OECD(1996), Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods, ENV/MC/CHEM/TG(96)9, OECD, Paris. Available at: http://www.oecd.org/document/24/0,3746,en_2649_37465_47858904_1_1_1_37465,00.html

5. OECD (2009), *Test No. 455: The Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing. doi: 10.1787/9789264076372-en]

6. ICCVAM (2011), *ICCVAM Test Method Evaluation Report on the LUMI-CELL® ER (BG1Luc ER TA) Test Method An In Vitro Method for Identifying ER Agonists and Antagonists*, National Institute of Environmental Health Sciences: Research Triangle Park, NC.

7. Pujol, P., *et al.,* (1998), Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. Cancer Res. 58(23): p. 5367-73.

8. Rogers, J.M. and M.S. Denison (2000), Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals. In Vitro and Molecular Toxicology: Journal of Basic and Applied Research, 13(1): p. 67-82.

9. Takeyoshi, M. (2006), *Draft Report of Pre-validation and Inter-laboratory Validation For Stably Transfected Transcriptional Activation (TA) Assay to Detect Estrogenic Activity - The Human Estrogen Receptor Alpha Mediated Reporter Gene Assay Using hER-HeLa-9903 Cell Line,*, Chemicals Evaluation and Research Institute (CERI): Japan. p. 1-188. [http://www.oecd.org/dataoecd/7/27/37504278.pdf]

10. ICCVAM (2003), ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays, Available at: [http://iccvam.niehs.nih.gov/docs/endo_docs/edfinalrpt0503/edfinrpt.pdf]

11. ICCVAM (2002), Background Review Document: Estrogen Receptor Binding. Appendix D, Substances Tested in the ER Binding Assay,. NIH Publication Report No. 03-4504. Available at: [http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erbndbrd/ERBd034504.pdf]

12. ICCVAM (2002), Background Review Document: Estrogen Receptor Transcriptional Activation (TA)

Assay. Appendix D, Substances Tested in the ER TA Assay,. NIH Publication Report No. 03-4505. Available at:
[http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erta_brd/ERTA034505.pdf]

13. Kanno, J, et al., (2001),The OECD program to validate the rat uterotrophic bioassay to screen compounds for in vivo estrogenic responses: Phase 1, Environ. Health Perspect. 109:785-94.

14. Kanno J, et al., (2003), The OECD program to validate the rat uterotrophic bioassay: Phase Two - Dose Response Studies, Environ. Health Persp. 111:1530-1549.

15. Kanno J, et al., (2003),The OECD program to validate the rat uterotrophic bioassay: Phase Two – Coded Single Dose Studies. Environ. Health Persp. 111:1550-1558.

ANNEX 1

Definitions and Abbreviations

Acceptability criteria: Minimum standards for the performance of experimental controls and reference standards. All acceptability criteria must be met for an experiment to be considered valid.

Accuracy: The closeness of agreement between a test method results and accepted reference values. It is a measure of test method performance and one aspect of relevance. The term is often used interchangeably with "concordance" to mean the proportion of correct outcomes of a test method (1).

Agonist: A substance that produces a response, e.g., transcription, when it binds to a specific receptor.

BG-1: An immortalized adenocarcinoma cell that endogenously express estrogen receptor.

BG1Luc4E2: The BG1Luc4E2 cell line was derived from BG-1 immortalized human-derived adenocarcinoma cells that endogenously express both forms of the estrogen receptor (ERα and ERβ) and have been stably transfected with the plasmid pGudLuc7.ERE. This plasmid contains four copies of a synthetic oligonucleotide containing the estrogen response element upstream of the mouse mammary tumor viral (MMTV) promoter and the firefly luciferase gene.

Cytotoxicity: Harmful effects to cell structure or function that can ultimately cause cell death and can be reflected by a reduction in the number of cells present in the well at the end of the exposure period or a reduction of the capacity for a measure of cellular function when compared to the concurrent vehicle control.

CV: Coefficient of variation

DMSO: Dimethyl sulfoxide

E2: 17β-estradiol

EC_{50}: The half maximal effective concentration of a test substance.

ER: Estrogen receptor

hERα: Human estrogen receptor alpha

hERß: Human estrogen receptor beta

ERE: Estrogen response element

Estrogenic activity: The capability of a chemical to mimic 17β-estradiol in its ability to bind to and activate estrogen receptors. hERα-mediated estrogenic activity can be detected with the PBTG.

HeLa: An immortal human cervical cell line

HeLa9903: A HeLa cell subclone into which hERα and a luciferase reporter gene have been stably transfected

Inter-laboratory reproducibility: A measure of the extent to which different qualified laboratories, using the same protocol and testing the same substances, can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes, and

indicates the extent to which a test method can be successfully transferred between laboratories, also referred to as between-laboratory reproducibility (1).

Intra-laboratory reproducibility: A determination of the extent that qualified people within the same laboratory can successfully replicate results using a specific protocol at different times. Also referred to as Within-laboratory reproducibility (1)

Me-too test: A colloquial expression for a test methods that is structurally and functionally similar to a validated and accepted reference test method. Interchangeably used with similar test method.

PBTG: Performance-Based Test Guideline.

PC: Positive control; a strongly active substance, preferably 17ß-estradiol, which is included in all tests to help ensure proper functioning of the assay.

PC_{10}: The concentration of a test substance at which the measured activity in an agonist assay is 10% of the maximum activity induced by the PC (E2 at 1nM for the STTA assay) in each plate.

PC_{50}: The concentration of a test substance at which the measured activity in an agonist assay is 50% of the maximum activity induced by the PC (E2 at the reference concentration specified in the test method) in each plate.

Performance standards: Standards, based on a validated test method, that provide a basis for evaluating the comparability of a proposed test method that is mechanistically and functionally similar. Included are (1) essential test method components; (2) a minimum list of reference chemicals selected from among the chemicals used to demonstrate the acceptable performance of the validated test method; and (3) the comparable levels of accuracy and reliability, based on what was obtained for the validated test method, that the proposed test method should demonstrate when evaluated using the minimum list of reference chemicals (1).

Proficiency chemicals (substances): A subset of the Reference Chemicals included in the Performance Standards that can be used by laboratories to demonstrate technical competence with a standardized test method. Selection criteria for these substances typically include that they represent the range of responses, are commercially available, and have high quality reference data available.

Proficiency: The demonstrated ability to properly conduct a test method prior to testing unknown substances.

Reference chemicals (substances): A set of chemicals to be used to demonstrate the ability of a new test method to meet the acceptability criteria demonstrated by the validated reference test method(s). These chemicals should be representative of the classes of chemicals for which the test method is expected to be used, and should represent the full range of responses that may be expected from the chemicals for which it may be used, from strong, to weak, to negative.

Reference estrogen (positive control, PC): 17ß-estradiol (E2, CASRN 50-28-2).

Reference test methods: The test methods upon which the PBTG is based.

Relevance: Description of relationship of the test to the effect of interest and whether it is meaningful and useful for a particular purpose. It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (concordance) of a test method (1).

Reliability: Measures of the extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility (1).

SD: Standard deviation

Sensitivity: The proportion of all positive/active substances that are correctly classified by the test. It is a measure of accuracy for a test method that produces categorical results, and is an important consideration in assessing the relevance of a test method (1).

Specificity: The proportion of all negative/inactive substances that are correctly classified by the test. It is a measure of accuracy for a test method that produces categorical results, and is an important consideration in assessing the relevance of attest method (1).

Stable transfection: When DNA is transfected into cultured cells in such a way that it is stably integrated into the cells genome, resulting in the stable expression of transfected genes. Clones of stably transfected cells are selected by stable markers (e.g., resistance to G418).

STTA: Stably Transfected Transactivation Assay, the ERα transactivation assay using the HeLa 9903 Cell Line.

Substance: Used in the context of the UN GHS as chemical elements and their compounds in the natural state or obtained by any production process, including any additive necessary to preserve the stability of the product and any impurities deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.

TA: Transactivation.

Transcription: mRNA synthesis

Transcriptional activation: The initiation of mRNA synthesis in response to a specific chemical signal, such as a binding of an estrogen to the estrogen receptor.

Validation: The process by which the reliability and relevance of a particular approach, method, process or assessment is established for a defined purpose.

VC: Vehicle control, the solvent that is used to dissolve test and control chemicals is tested solely as vehicle without dissolved chemical.

Weak positive control: A weakly active substance selected from the reference chemicals list that is included in all tests to help ensure proper functioning of the assay.

ANNEX 2

Supplementary Information

for the

Stably Transfected Human Estrogen Receptor-α TransActivation (STTA) Assay for Detection of Estrogenic Agonist-Activity of Chemicals using the hERα-HeLa-9903 cell line

And

BG1Luc Estrogen Receptor (ER) TransActivation (TA) Test Method for Identifying ER Agonists

Table 1: Comparison of Results from STTA and BG1Luc ER TA Assays for 34 Chemicals Tested in Both Assays and Classified as Positive or Negative ER Agonists

	Chemical	CASRN	STTA ER TA[1]			BG1Luc ER TA[2]		Data Source For Classification[4]			Chemical Class[5]	Product Class[6]
			ER TA Activity	PC_{10} Value (M)	PC_{50} Value[b] (M)	ER TA Activity	EC_{50} Value[b,3] (M)	Other ER TAs[c]	ER Binding	Uterotrophic		
1	17-ß Estradiol[a]	50-28-2	POS	$<1.00 \times 10^{-11}$	$<1.00 \times 10^{-11}$	POS	5.63×10^{-12}	POS (227/227)	POS	POS	Steroid	Pharmaceutical, Veterinary Agent
2	17-α Estradiol[a]	57-91-0	POS	7.24×10^{-11}	6.44×10^{-10}	POS	1.40×10^{-9}	POS (11/11)	POS	POS	Steroid	Pharmaceutical, Veterinary Agent
3	17-α Ethinyl estradiol[a]	57-63-6	POS	$<1.00 \times 10^{-11}$	$<1.00 \times 10^{-11}$	POS	7.31×10^{-12}	POS (22/22)	POS	POS	Steroid	Pharmaceutical, Veterinary Agent
4	17-β-Trenbolone	10161-33-8	POS	1.78×10^{-8}	2.73×10^{-7}	POS	4.20×10^{-8}	POS (2/2)	NT	NT	Steroid	Veterinary Agent
5	19-Nortestosterone[a]	434-22-0	POS	9.64×10^{-9}	2.71×10^{-7}	POS	1.80×10^{-6}	POS (4/4)	POS	POS	Steroid	Pharmaceutical, Veterinary Agent
6	4-Cumylphenol[a]	599-64-4	POS	1.49×10^{-7}	1.60×10^{-6}	POS	3.20×10^{-7}	POS (5/5)	POS	NT	Phenol	Chemical Intermediate
7	4-*tert*-Octylphenol[a]	140-66-9	POS	1.85×10^{-9}	7.37×10^{-8}	POS	3.19×10^{-8}	POS (21/24)	POS	POS	Phenol	Chemical Intermediate
8	Apigenin[a]	520-36-5	POS	1.31×10^{-7}	5.71×10^{-7}	POS	1.60×10^{-6}	POS (26/26)	POS	NT	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
9	Atrazine[a]	1912-24-9	NEG	-	-	NEG	-	NEG (30/30)	NEG	NT	Heterocyclic Compound	Herbicide
10	Bisphenol A[a]	80-05-7	POS	2.02×10^{-8}	2.94×10^{-7}	POS	5.33×10^{-7}	POS (65/65)	POS	POS	Phenol	Chemical Intermediate
11	Bisphenol B[a]	77-40-7	POS	2.36×10^{-8}	2.11×10^{-7}	POS	1.95×10^{-7}	POS (6/6)	POS	POS	Phenol	Chemical Intermediate
12	Butylbenzyl phthalate[a]	85-68-7	POS	1.14×10^{-6}	4.11×10^{-6}	POS	1.98×10^{-6}	POS (12/14)	POS	NEG	Carboxylic Acid, Ester, Phthalic Acid	Plasticizer, Industrial Chemical

	Chemical	CASRN	STTA ER TA[1]			BG1Luc ER TA[2]		Data Source For Classification[4]			Chemical Class[5]	Product Class[6]
			ER TA Activity	PC_{10} Value (M)	PC_{50} Value[b] (M)	ER TA Activity	EC_{50} Value[b,3] (M)	Other ER TAs[c]	ER Binding	Uterotrophic		
13	Corticosterone[a]	50-22-6	NEG	-	-	NEG	-	NEG (6/6)	NEG	NT	Steroid	Natural Hormone, Pharmaceutical
14	Coumestrol[a]	479-13-0	POS	1.23×10^{-9}	2.00×10^{-8}	POS	1.32×10^{-7}	POS (30/30)	POS	NT	Heterocyclic Compound	Natural Product
15	Daidzein[a]	486-66-8	POS	1.76×10^{-8}	1.51×10^{-7}	POS	7.95×10^{-7}	POS (39/39)	POS	POS	Flavonoid, Heterocyclic Compound	Natural Product
16	Diethylstilbestrol[a]	56-53-1	POS	$<1.00 \times 10^{-11}$	2.04×10^{-11}	POS	3.34×10^{-11}	POS (42/42)	POS	NT	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17	Di-n-butyl phthalate	84-74-2	POS	4.09×10^{-6}		POS	4.09×10^{-6}	POS (6/11)	POS	NEG	Ester, Phthalic Acid	Plasticizer, Chemical Intermediate
18	Ethyl paraben	120-47-8	POS	5.00×10^{-6}	(no PC_{50})	POS	2.48×10^{-5}	POS (6/6)	POS	NT	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
19	Estrone[a]	53-16-7	POS	3.02×10^{-11}	5.88×10^{-10}	POS	2.34×10^{-10}	POS (26/28)	POS	POS	Steroid	Pharmaceutical, Veterinary Agent
20	Genistein[a]	446-72-0	POS	2.24×10^{-9}	2.45×10^{-8}	POS	2.71×10^{-7}	POS (100/102)	POS	POS	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
21	Haloperidol	52-86-8	NEG	-	-	NEG	-	NEG (2/2)	NEG	NT	Butyrophenone	Pharmaceutical
22	Kaempferol[a]	520-18-3	POS	1.36×10^{-7}	1.21×10^{-6}	POS	3.99×10^{-6}	POS (23/23)	POS	NT	Flavonoid, Heterocyclic Compound	Natural Product
23	Kepone[a]	143-50-0	POS	7.11×10^{-7}	7.68×10^{-6}	POS	4.91×10^{-7}	POS (14/18)	POS	NT	Hydrocarbon, (Halogenated)	Pesticide
24	Ketoconazole	65277-42-1	NEG	-	-	NEG	-	NEG (2/2)	NEG	NT	Heterocyclic Compound	Pharmaceutical
25	Linuron[a]	330-55-2	NEG	-	-	NEG	-	NEG (8/8)	NEG	NT	Phenylurea	Herbicide
26	*meso*-Hexestrol[a]	84-16-2	POS	$<1.00 \times 10^{-11}$	2.75×10^{-11}	POS	1.65×10^{-11}	POS (4/4)	POS	NT	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
27	Methyl testosterone[a]	58-18-4	POS	1.73×10^{-7}	4.11×10^{-6}	POS	2.68×10^{-6}	POS (5/6)	POS	NT	Steroid	Pharmaceutical, Veterinary Agent

	Chemical	CASRN	STTA ER TA[1]			BG1Luc ER TA[2]		Data Source For Classification[4]			Chemical Class[5]	Product Class[6]
			ER TA Activity	PC_{10} Value (M)	PC_{50} Value[b] (M)	ER TA Activity	EC_{50} Value[b,3] (M)	Other ER TAs[c]	ER Binding	Uterotrophic		
28	Morin	480-16-0	POS	5.43×10^{-7}	4.16×10^{-6}	POS	2.37×10^{-6}	POS (2/2)	POS	NT	Flavonoid, Phenol	Natural product
29	Norethynodrel[a]	68-23-5	POS	1.11×10^{-11}	1.50×10^{-9}	POS	9.39×10^{-10}	POS (5/5)	POS	NT	Steroid	Pharmaceutical, Veterinary Agent
30	*p,p*'-Methoxychlor[a]	72-43-5	POS	1.23×10^{-6}	(no PC_{50})[b]	POS	1.92×10^{-6}	POS (24/27)	POS	POS	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
31	Phenobarbital[a]	57-30-7	NEG	-	-	NEG	-	NEG (2/2)	NEG	NT	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Analgesic
32	Reserpine	50-55-5	NEG	-	-	NEG	-	NEG (4/4)	NEG	NT	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
33	Spironolactone[a]	52-01-7	NEG	-	-	NEG	-	NEG (4/4)	NEG	NT	Lactone, Steroid	Pharmaceutical
34	Testosterone	58-22-0	POS	2.82×10^{-8}	9.78×10^{-6}	POS	1.75×10^{-5}	POS (5/10)	POS	NT	Steroid	Natural Hormone

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; M = molar; EC_{50} = half maximal effective concentration of test chemical; NEG = negative; NT = not tested; POS = positive; PC_{10} (and PC_{50}) = the concentration of a test chemical at which the response is 10% (or 50% for PC_{50}) of the response induced by the positive control (E2, 1nM) in each plate.

[a]Common chemicals tested in the STTA ER TA and BG1Luc ER TA that were designated as positive or negative ER Agonists and used to evaluate accuracy in the BG1 Luc ER TA validation study (ICCVAM BG1Luc ER TA Evaluation Report, Table 4-1 [6]).

[b]Maximum concentration tested in the absence of limitations due to cytotoxicity or insolubility was 1×10^{-5} M (STTA ER TA) and 1×10^{-3} M (BG1Luc ER TA).

[c]Number in parenthesis represents the test results classified as positive (POS) or negative (NEG) over the total number of referenced studies.

[1]Values reported in *Draft Report of Pre-validation and Inter-laboratory Validation For Stably Transfected Transcriptional Activation (TA) Assay to Detect Estrogenic Activity - The Human Estrogen Receptor Alpha Mediated Reporter Gene Assay Using hER-HeLa-9903 Cell Line* (9).
[2]ICCVAM Test *Method Evaluation Report (TMER) on the LUMI-CELL® ER (BG1Luc ER TA) Test Method: An In Vitro Method for Identifying ER Agonists and Antagonists* (6).
[3]Mean EC_{50} values were calculated with values reported by the laboratories of the BG1Luc ER TA validation study (XDS, ECVAM, and Hiyoshi) (6).
[4]Classification as an ER agonist or negative was based upon information in the ICCVAM Background Review Documents (BRD) for ER Binding and TA test methods (31) as well as information obtained from publications published and reviewed after the completion of the ICCVAM BRDs (6, 9-15).
[5]Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH), an internationally recognized standardized classification scheme (available at: http://www.nlm.nih.gov/mesh).
[6]Substances were assigned into one or more product classes using the U.S. National Library of Medicine's Hazardous Substances Database (available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB)

Table 2: Chemicals tested in the STTA ER TA Validation Study [9]

	Chemical	CASRN	PC10 (M)	PC50 (M)	PC10 based class	PC50 based class	Updated Classifications of Chemicals[a]			ER Binding Assay				Uterotrophic Assay		
							ICCVAM class[a]			ER-RBA E2=100%	ER-RBA class			Utero. Class		
1	Atrazine	1912-24-9	-	-	N	N	N			N.B.	N			no data		
2	Corticosterone	50-22-6	-	-	N	N	N			N.B.	N			no data		
3	Haloperidol	52-86-8	-	-	N	N	N			N.B.	N			no data		
4	Ketoconazole	65277-42-1	-	-	N	N	N			N.B.	N			no data		
5	Linuron	330-55-2	-	-	N	N	N			N.B.	N			no data		
6	Phenobarbital (Na salt)	57-30-7	-	-	N	N	N			no data				no data		
7	Reserpine	50-55-5	-	-	N	N	N			N.B.	N			no data		
8	Spironolactone	52-01-7	-	-	N	N	N			N.B.	N			no data		
9	Flutamide	13311-84-7	-	-	N	N	N			N.B.	N			no data		
10	Procymidone	32809-16-8	-	-	N	N	N			N.B.	N			no data		
11	Ethyl paraben	120-47-8	5.00E-06	PC10 (no PC50)	P	N	P			N.B.	N			no data		
12	p,p'-Methoxychlor	72-43-5	1.23E-06	PC10 (no PC50)	P	N	P			0.00238	P			N		
13	Tamoxifen	10540-29-1	1.49E-07	PC10 (no PC50)	P	N	P			47	P			no data		
14	Clomiphene citrate	50-41-9	3.68E-08	PC10 (no PC50)	P	N	P			37	P			no data		
15	Zearalenone [b]	17924-92-4	2.44E-11	6.44E-10	P	P	P			no data				P		
16	17β-Estradiol	50-28-2	<1.00E-11	<1.00E-11	P	P	P			126	P			no data		
17	17β-Trenbolone	10161-33-8	1.78E-08	2.73E-07	P	P	P			no data				no data		
18	17α-Estradiol	57-91-0	7.24E-11	6.44E-10	P	P	P			80.1	P			P		
19	17α-Ethinyl estradiol	57-63-6	<1.00E-11	<1.00E-11	P	P	P			142	P			P		
20	4-Cumylphenol	599-64-4	1.49E-07	1.60E-06	P	P	P			0.107	P			P		
21	4-tert-Octylphenol	140-66-9	1.85E-09	7.37E-08	P	P	P			0.124	P			P		
22	Apigenin	520-36-5	1.31E-07	5.71E-07	P	P	P			no data				no data		
23	Bisphenol A	80-05-7	2.02E-08	2.94E-07	P	P	P			0.195	P			P		
24	Bisphenol B	77-40-7	2.36E-08	2.11E-07	P	P	P			0.593	P			P		
25	Butylbenzyl phthalate	85-68-7	1.14E-06	4.11E-06	P	P	P			no data				no data		
26	Coumestrol	479-13-0	1.23E-09	2.00E-08	P	P	P			0.264	P			no data		
27	Daidzein	486-66-8	1.76E-08	1.51E-07	P	P	P			0.18	P			N		
28	Diethylstilbestrol	56-53-1	<1.00E-11	2.04E-11	P	P	P			no data				no data		
29	Estrone	53-16-7	3.02E-11	5.88E-10	P	P	P			44.2	P			P		
30	Genistein	446-72-0	2.24E-09	2.45E-08	P	P	P			0.12	P			P		
31	Kaempferol	520-18-3	1.36E-07	1.21E-06	P	P	P			0.029	P			no data		

	Chemical	CASRN	PC10 (M)	PC50 (M)	PC10 based class	PC50 based class	Updated Classifications of Chemicals[a]			ER Binding Assay				Uterotrophic Assay		
							ICCVAM class[a]			ER-RBA E2=100%	ER-RBA class			Utero. Class		
32	Kepone (Chlordecone)	143-50-0	7.11E-07	7.68E-06	P	P	P			no data				no data		
33	Methyl testosterone	58-18-4	1.73E-07	4.11E-06	P	P	P			N.D.	N			P		
34	Morin	480-16-0	5.43E-07	4.16E-06	P	P	P			0.0011	P			no data		
35	Norethynodrel	68-23-5	1.11E-10	1.50E-09	P	P	P			0.282	P			no data		
36	Phenolphthalin	81-90-3	-	-	N	N	PP			N.D.	N			no data		
37	Progesterone	57-83-0	-	-	N	N	PP			N.B.	N			no data		
38	Cyproterone acetate	427-51-0	-	-	N	N	PP			N.D.	N			no data		
39	Mifepristone	84371-65-3	-	-	N	N	PP			0.0594	P			no data		
40	Diethylhexyl phthalate	117-81-7	-	-	N	N	PP			0.071	P			N		
41	L-Thyroxine	51-48-9	1.32E-06	PC10 (no PC50)	P	N	PP			N.B.	N			no data		
42	4-Androstenedione	63-05-8	2.56E-07	PC10 (no PC50)	P	N	PP			N.B.	N			no data		
43	Testosterone	58-22-0	2.82E-08	9.78E-06	P	P	PP			N.D.	N			no data		
44	Vinclozolin	50471-44-8	1.33E-07	7.65E-06	P	P	PP			N.B.	N			no data		
45	Dibutyl phthalate	84-74-2	-	-	N	N				N.D.	N			N		
46	Nonylphenol	25154-52-3	1.37E-08	1.58E-07	P	P				0.143	P			P		
47	Phenobarbital	50-06-6	-	-	N	N				N.B.	N			no data		
48	Medroxyprogesterone	520-85-4	-	-	N	N				N.B.	N			no data		
49	DHT	521-18-6	1.04E-07	5.28E-07	P	P				0.0218	P			P		
50	Testosterone propionate	57-85-2	2.03E-09	2.91E-07	P	P				N.B.	N			no data		
51	Fenarimol	60168-88-9	-	-	N	N				0.00179	P			no data		
52	p,p'-DDE	72-55-9	-	-	N	N				N.B.	N			no data		
53	Hexestrol	84-16-2	<1.00E-11	2.75E-11	P	P				37.6	P			no data		
54	2,4,5-Trichlorophenoxyacetic acid	93-76-5	-	-	N	N				N.B.	N			no data		
55	para-sec-butylphenol	99-71-8	1.38E-06	PC10 (no PC50)	P	N				0.00177	P			no data		
56	Norgestrel	797-63-7	1.05E-07	PC10 (no PC50)	P	N				no data				P		
57	Norethindrone	68-22-4	1.01E-09	4.95E-08	P	P				no data				P		
58	Equilin	474-86-2	<1.00E-11	7.54E-11	P	P				no data				P		
59	Dicyclohexylphthalate	84-61-7	2.53E-06	PC10 (no PC50)	P	N				no data				N		
60	Diethyl phthalate	84-66-2	4.46E-06	PC10 (no PC50)	P	N				no data				N		
61	di(2-ethylhexyl)adipate	103-23-1	-	-	N	N				no data				N		
62	p-dodecyl-phenol	104-43-8	2.36E-08	4.10E-07	P	P				no data				P		
63	4-n-octylphenol	1806-26-4	1.26E-06	PC10 (no PC50)	P	N				no data				N		

	Chemical	CASRN	PC10 (M)	PC50 (M)	PC10 based class	PC50 based class	Updated Classifications of Chemicals[a]			ER Binding Assay				Uterotrophic Assay		
							ICCVAM class[a]			ER-RBA E2=100%	ER-RBA class			Utero. Class		
64	4-n-amylphenol	14938-35-3	1.78E-07	4.62E-06	P	P				no data				P		
65	Testosterone enanthate	315-37-7	1.71E-08	2.71E-07	P	P				no data				P		
66	p-(tert-pentyl)phenol	80-46-6	4.02E-07	3.46E-06	P	P				no data				P		
67	4-cyclohexylphenol	1131-60-8	6.43E-08	1.51E-06	P	P				no data				P		
68	4-(1-adamantyl)phenol	29799-07-3	1.25E-09	1.86E-08	P	P				no data				P		
69	4-(phenylmethyl)-phenol	101-53-1	1.20E-06	4.07E-06	P	P				no data				P		
70	2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane	6807-17-6	1.89E-09	1.99E-08	P	P				no data				P		
71	4,4'-exafluoroisopropylidene) diphenol	1478-61-1	6.91E-09	8.02E-08	P	P				no data				P		
72	4,4'-(octahydro-4,7-methano-5H-inden-5-lidene)bisphenol	1943-97-1	3.72E-08	PC10 (no PC50)	P	N				no data				P		
73	4,4'-dimethoxytriphenylmethane	7500-76-7	-	-	N	N				no data				N		
74	Benzophenone	119-61-9	-	-	N	N				no data				N		
75	4-hydroxybenzophenone	1137-42-4	1.10E-06	2.60E-06	P	P				no data				P		
76	4,4'-dihydroxybenzophenone	611-99-4	1.24E-07	1.65E-06	P	P				no data				P		
77	2,4,4'-trihydroxybenzophenone	1470-79-7	4.38E-08	3.75E-07	P	P				no data				P		
78	4,4'-dimethoxybenzophenone	90-96-0	2.50E-06	PC10 (no PC50)	P	N				no data				N		
79	2,2',4,4'-tetrahydroxybenzophenone	131-55-5	1.06E-07	3.28E-07	P	P				no data				P		
80	4-hydroxyazobenzene	1689-82-3	1.64E-07	1.08E-06	P	P				no data				P		
81	3,3,3',3'-tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol	77-08-7	1.43E-07	3.16E-06	P	P				no data				N		
82	4,4'-thiobis-phenol	2664-63-3	2.01E-08	2.14E-07	P	P				no data				P		
83	Diphenyl-p-phenylenediamine	74-31-7	2.30E-06	PC10 (no PC50)	P	N				no data				P		
84	Octachlorostyrene	29082-74-4	-	-	N	N				no data				N		
85	Hematoxylin	517-28-2	-	-	N	N				no data				N		
86	Tributyltin-chloride	1461-22-9	-	-	N	N				no data				N		

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; PC_{10} (and PC_{50}) = the concentration of a test chemical at which the response is 10% (or 50% for PC_{50}) of that induced by the positive controls (E2, 1nM); Classification of results: P = positive; N = negative; PP: presumed positive (positive in 50% or less of reported studies, or positive in the single study conducted); N.B. no ER binding; N.D: RBA was not determined though the displacement of hot ligand was 20-5%.

[a]Classifications of chemicals originally recommended by ICCVAM as ER agonists or negatives (10,11) have been updated based upon post-2003 published reports, including *in vitro* assays for ER binding and TA, and the *in vivo* uterotrophic assay (6, 9-15).

[b]Zeralenone can be classified here as clearly positive though this chemical was not included in the updated ICCVAM classifications as reported in the [1]ICCVAM Test Method Evaluation Report on the LUMI-CELL® ER (BG1Luc ER TA) Test Method an *In Vitro* Method for Identifying ER Agonists and Antagonists (9).

Table 3: Chemicals Tested during the BG1Luc ER TA Validation Study [6]

	Chemical[a]	CASRN	BG1Luc ER TA Classification[1]
1	17ß-Estradiol	50-28-2	POS
2	17ß-Trenbolone	10161-33-8	POS
3	17α-Estradiol	57-91-0	POS
4	17α-Ethinyl estradiol	57-63-6	POS
5	19-Nortestosterone	434-22-0	POS
6	2-sec-Butylphenol	89-72-5	POS
7	4-Cumylphenol	599-64-4	POS
8	4-Hydroxy-androstenedione	566-48-3	POS
9	4-tert-Octylphenol	140-66-9	POS
10	Apigenin	520-36-5	POS
11	Bisphenol A	80-05-7	POS
12	Bisphenol B	77-40-7	POS
13	Butylbenzyl phthalate	85-68-7	POS
14	Chrysin	480-40-0	POS
15	Coumestrol	479-13-0	POS
16	Daidzein	486-66-8	POS
17	Di-*n*-butyl phthalate	84-74-2	POS
18	Dicofol	115-32-2	POS
19	Diethylstilbestrol	56-53-1	POS
20	Estrone	53-16-7	POS
21	Ethyl paraben	120-47-8	POS
22	Fenarimol	60168-88-9	POS
23	Flavone	525-82-6	POS
24	Fluoranthene	206-44-0	POS
25	Fluoxymestrone	76-43-7	POS
26	Genistein	446-72-0	POS
27	Kaempferol	520-18-3	POS
28	Kepone	143-50-0	POS
29	meso-Hexestrol	84-16-2	POS
30	Methyl testosterone	58-18-4	POS
31	Morin	480-16-0	POS
32	Nilutamide	63612-50-0	POS
33	Norethynodrel	68-23-5	POS
34	*o,p*'-DDT	789-02-6	POS
35	p-*n*-Nonylphenol	104-40-5	POS
36	*p,p*'- Methoxychlor	72-43-5	POS
37	Phenolphthalin	81-90-3	POS
38	Progesterone	57-83-0	POS

	Chemical[a]	CASRN	BG1Luc ER TA Classification[1]
39	Testosterone	58-22-0	POS
40	12-*O*-Tetradecanoylphorbol-13-acetate	16561-29-8	NEG
41	2,4,5-Trichloro-phenoxyacetic acid	93-76-5	NEG
42	4-Hydroxytamoxifen	68047-06-3	NEG
43	Actinomycin D	50-76-0	NEG
44	Ammonium perchlorate	7790-98-9	NEG
45	Apomorphine	58-00-4	NEG
46	Atrazine	1912-24-9	NEG
47	Bicalutamide	90357-06-5	NEG
48	Corticosterone	50-22-6	NEG
49	Cyproterone acetate	427-51-0	NEG
50	Dibenzo[*a*,*h*] Anthracene	53-70-3	NEG
51	Finasteride	98319-26-7	NEG
52	Haloperidol	52-86-8	NEG
53	Hydroxy flutamide	52806-53-8	NEG
54	Ketoconazole	65277-42-1	NEG
55	L-Thyroxine	51-48-9	NEG
56	Linuron	330-55-2	NEG
57	Medroxyprogest-erone acetate	71-58-9	NEG
58	Mifepristone	84371-65-3	NEG
59	Phenobarbital	50-06-6	NEG
60	Pimozide	2062-78-4	NEG
61	Propylthiouracil	51-52-5	NEG
62	Raloxifene HCl	82640-04-8	NEG
63	Reserpine	50-55-5	NEG
64	Sodium azide	26628-22-8	NEG
65	Spironolactone	52-01-7	NEG
66	Vinclozolin	50471-44-8	NEG

[a]Table is sorted by classification and then alphabetically by chemical name.

[1]Classification based upon results reported in the ICCVAM Test Method Evaluation Report (TMER) on the LUMI-CELL® ER (BG1Luc ER TA) Test Method an *In Vitro* Method for Identifying ER Agonists and Antagonists [6].

Summary of the Reliability and Accuracy Values Obtained During the Validation Studies for the STTA and BG1Luc ER TAs (Agonists)

The respective validation study reports for the STTA and BG1Luc ER TAs provide comprehensive descriptions of the data used to develop the reliability and accuracy values for each of these assays (6, 9). The following is a summary of the estimates of intra- and inter-laboratory reproducibility and predictive capacity (accuracy) for each of these fully validated test methods.

I. **Intra-laboratory (within-laboratory) reproducibility:** The closeness of agreement between test results obtained within a single laboratory when the procedure is performed using the same substance under identical conditions within a given time period.

a. BG1Luc ER TA validation study: Intra-laboratory reproducibility was assessed using 8 substances that exhibited a wide range of estrogenic activity (positives) and 4 substances that were negatives. Each chemical was tested in at least 3 separate experiments by each of 3 laboratories and classified as positive or negative for estrogenic activity. There was 100% (12/12) agreement on substance classifications within each laboratory (6).

b. STTA ER TA validation study: Intra-laboratory reproducibility was assessed using 9 coded chemicals plus estradiol (7 positives, 3 negatives). Each was tested in at least 3 separate experiments in four laboratories, and chemicals were classified as positive or negative based upon the log [PC10] and log [PC50]. There was 100% (10/10) agreement on chemical classifications within each laboratory (Table 4). The standard deviations (SD) for the mean log [PC10 (M)], log [PC50 (M)], and log [EC50 (M)] for each chemical within each of the laboratories were also used as an assessment of intra-laboratory reproducibility. The maximum (max) SDs for the intra-laboratories were as follows: Max SD log [PC10 (M)]: 2.23; Max SD log [PC50 (M)]: 0.55; and Max SD log [EC50 (M)]: 4.41 (9).

Table 4: Intra-laboratory reproducibility for the STTA ER TA Assay (9)

		STTA (PC10 based)		
		Positive	Negative	Total
Updated classification of ICCVAM Chemicals	Positive	7	0	7
	Negative	0	3	3
	Total	7	3	10

Overall Accuracy	100%	10/10
Sensitivity	100%	7/7
Specificity	100%	3/3
False positive	0%	0/3
False negative	0%	0/7
Positive predictivity	100%	7/7
Negative predictivity	100%	3/3

Inter-laboratory (between laboratories) reproducibility: A measure of the extent to which different qualified laboratories using the same protocol and testing the same substances can produce qualitatively and quantitatively similar results. Inter-laboratory reproducibility is determine during the validation process, and indicates the extent to which a test method can be transferred successfully among laboratories.

II.

a. BG1Luc ER TA validation study: Inter-laboratory reproducibility was assessed using 36 chemicals that were tested at least once in each of 3 laboratories (24 positives, 12 negatives). There was 83% (30/36) agreement on the classifications for these chemicals among the laboratories (6). Three chemicals (3/36) had inadequate overall classifications (i.e., 1 positive, 1 negative and 1 inadequate call) and could not be used for the assessment of accuracy. For the 33 remaining chemicals, the accuracy is shown in Table 5.

Table 5: Inter-laboratory reproducibility for the BG1Luc ER TA Assay (6)

		BG1Luc ER TA		
		Positive	Negative	Total
Updated classification of ICCVAM Chemicals	Positive	21	0	21
	Negative	2	10	12
	Total	23	10	33

Overall accuracy	94%	30/33
Sensitivity	100%	21/21
Specificity	83%	10/12
False positive	17%	2/12
False negative	0%	0/21
Positive predictivity	88%	21/24
Negative predictivity	100%	10/10

b. STTA ER TA validation study: Inter-laboratory reproducibility was assessed using 9 coded chemicals plus estradiol (7 positives, 3 negatives). Each chemical was tested in at least 3 separate experiments in four laboratories, and chemicals were classified as positive or negative based upon the log [PC10 (M)] and log [PC50 (M)]. There was 100% (10/10) agreement on chemical classifications among the laboratories as well as concordance with all classifications as ER agonists or negatives based upon published reports (Table 6). The standard deviations (SD) for the mean log [PC10 (M)], log [PC50 (M)], and log [EC50 (M)] for each chemical within each of the laboratories were also reported as a quantitative assessment of inter-laboratory reproducibility. The range of SDs between laboratories were as follows: SD Log [PC10 (M)]: 0.14 – 0.41; SD log [PC50 (M)]: 0.14 – 0.32; SD log [EC50 (M)]: 0.14 – 1.49 (9).

Table 6: Inter-laboratory reproducibility for the STTA ER TA (9)

		STTA (PC10 based)		
		Positive	Negative	Total
Updated classification of ICCVAM Chemicals	Positive	7	0	7
	Negative	0	3	3
	Total	7	3	10

Overall Accuracy	100%	10/10
Sensitivity	100%	7/7
Specificity	100%	3/3
False positive	0%	0/3
False negative	0%	0/7
Positive predictivity	100%	7/7
Negative predictivity	100%	3/3

III. Predictive capacity (accuracy): Measures of accuracy (i.e., sensitivity, specificity, positive and negative predictivity), and overall accuracy provide a quantitative assessment of the closeness of agreement (e.g, the proportion of correct outcomes) between test methods results and the values obtained from reference chemicals.

a. BG1Luc ER TA validation study: The predictive capacity was assessed using 35 reference substances (28 positives, 7 negatives) that produced definitive results in the BG1Luc ER TA assay for agonist activity (See Section 3.4 in reference 6).

Table 7: Predictive capacity for the BG1Luc ER TA Assay (6)

		BG1Luc ER TA		
		Positive	Negative	Total
Updated classification of ICCVAM Chemicals	Positive	27	1	28
	Negative	0	7	7
	Total	27	8	35

Overall accuracy	97%	34/35
Sensitivity	96%	27/28
Specificity	100%	7/7
False positive	0%	0/7
False negative	4%	1/28
Positive predictivity	100%	27/27
Negative predictivity	88%	7/8

b. STTA ER TA validation study: The predictive capacity for the STTA ER TA was assessed using three sets of reference chemicals that were designated as ER agonists or negatives based upon available data from other assays that were designed to detect estrogenic activity (see Annex 2, Table 2). The positive/negative results reported as log [PC10 (M)] and log [PC50 (M)] were compared with 35 reference chemicals selected from an updated ICCVAM list of ER agonists (Table 8), a set of 48 chemicals that had been tested in the *in vitro* ERα binding assay (Table 9), and another set of 48 chemicals that had been tested in immature rat uterotrophic assay (Table 10) (9).

Table 8: Predictive capacity for the STTA ER TA Assay using 35 reference chemicals (25 positives, 10 negatives; see Annex 2, Table 2, chemicals 1-35).

		STTA (PC10 based)		
		Positive	Negative	Total
Updated classification of ICCVAM Chemicals	Positive	25	0	25
	Negative	0	10	10
	Total	25	10	35

Overall Accuracy	100%	35/35
Sensitivity	100%	25/25
Specificity	100%	10/10
False positive	0%	0/10
False negative	0%	0/25
Positive predictivity	100%	25/25
Negative predictivity	100%	10/10

Table 9: Predictive capacity for the STTA ER TA Assay using 48 substances (24 positives, 24 negatives) that had been classified as ER agonists or negatives based upon results from the ERα Binding Assay (See Table 14 in reference 9).

		STTA (PC10 based)		
		Positive	Negative	Total
ER binding assay	Positive	21	3	24
	Negative	7	17	24
	Total	28	20	48

Overall accuracy	79%	38/48
Sensitivity	88%	21/24
Specificity	71%	17/24
False positive	29%	7/24
False negative	13%	3/24
Positive predictivity	75%	21/28
Negative predictivity	85%	17/20

Table 10: Predictive capacity for the STTA ER TA Assay using 48 substances (32 positives, 16 negatives) that had been classified as ER agonists or negatives based upon results from the immature rat uterotrophic assays (See Table 16 in reference 9).

		STTA (PC10 based)		
		Positive	Negative	Total
Uterotrophic assay	Positive	32	0	32
	Negative	7	9	16
	Total	39	9	48

Overall accuracy	85%	41/48
Sensitivity	100%	32/32
Specificity	56%	9/16
False positive	44%	7/16
False negative	0%	0/32
Positive predictivity	82%	32/39
Negative predictivity	100%	9/9

Table 11: Template for Accuracy Analysis

		New Test Outcome		
		Positive	Negative	Total
Reference Test Classification	Positive	a	c	a + c
	Negative	b	d	b + d
	Total	a + b	c + d	a+b+c+d

a = positive in both new assay and by reference test classification
b = positive in new assay and negative by reference test classification
c = negative in new assay and positive by reference test classification
d = negative in both new assay and by reference test classification

Concordance = ([a+d]/[a+b+c+d])
Sensitivity = (a/[a+c])
Specificity = (d/[b+d])
False positive rate = (b/[b+d])
False negative rate = (c/[a+c])
Positive Predictivity = (a/[a+b])
Negative Predictivity = (d/[c+d])