- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated within and among the participating laboratories

4.4 Phase III

In Phase III, a subset of 41 substances from the ICCVAM list of 78 recommended reference substances for validation of ER TA assays will be tested in each laboratory to evaluate interlaboratory reproducibility. Reference standard and control data collected during Phase IIb will be added to the historical database compiled in Phases I and IIa and this combined historical database will be used to establish acceptance criteria for Phase III.

4.4.1 Phase III Testing

After a range-finding assay is completed for each of the 41 coded test substances, recommended starting concentrations for the comprehensive concentration-response experiment and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each substance should not begin until the starting concentrations have been approved and should not be modified without approval of the SMT. The comprehensive concentration-response experiment for each coded test substance should be performed once. Laboratories will calculate EC_{50} values for the agonist reference standard or IC_{50} values for the antagonist reference standard (in µg/mL). Laboratories will also calculate EC_{50} or IC_{50} values (in µg/mL), when possible, for coded test substances. These data, along with all quality control, raw, derived and supporting data, will be reported to the SMT through the designated contacts. If there is excessive variation among participating laboratories, the SMT will work with the laboratories to determine the cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable interlaboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.4.2 Criteria for Advancing to Phase IV

The SMT will decide when XDS will advance to Phase IV of the validation study, based on the following criteria:

- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Data, reviewed by QA, has been received by the SMT
- Acceptable interlaboratory reproducibility has been demonstrated among the participating laboratories

4.5 Phase IV

In Phase IV, the U.S, participating laboratory, Xenobiotic Detection Systems, Inc. (XDS) only will test the remaining 25 substances from the ICCVAM list of 78 recommended reference substances for validation of ER TA assays.

4.5.1 Phase IV Testing of Remaining ICCVAM Substances

After a range-finding assay is completed for each of the remaining 25 coded test substances, recommended starting concentrations for the comprehensive concentration response experiments and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each substance should not begin until the starting concentrations have been approved and should not be modified without approval of the SMT. The comprehensive concentration-response experiment for each coded test substance should be performed once. XDS will calculate EC_{50} or IC_{50} values (in µg/mL) for reference standards and coded test substances, and report this and all raw, derived, and supporting data to the SMT Project Coordinator.

4.5.2 Criteria for Completion of Phase IV

Phase IV will be considered complete once all of the remaining 25 coded substances have been tested, data reviewed by QA has been received by the SMT, and the Study Director provides a final report to the SMT Project Coordinator.

5.0 REFERENCE STANDARDS, CONTROLS AND TEST SUBSTANCES

Substance Inventory and Distribution Management (see **Section 2.2.2**) will supply all reference standards and control substances for the validation study, which will be shipped prior to initiation of testing. Phase IIa coded test substances will be shipped as a unit of eight (four substances for testing in the agonist protocol and four substances for testing in the antagonist protocol). Phase IIb coded test substances will be shipped as a unit of 16 (eight substances for

testing in the agonist protocol and eight substances for testing in the antagonist protocol). Phase III coded test substances will be shipped as a unit of 82 (41 substances for testing in the agonist protocol and 41 substances for testing in the antagonist protocol) and Phase IV coded test substances will be shipped as a unit of 50 (25 substances for testing in the agonist protocol and 25 substances for testing in antagonist protocol). The SMT and Substance Inventory and Distribution Management will have all descriptive information for each substance (e.g., purity, Chemical Abstracts Service Registry Number® [CASRN], supplier, etc.).

5.1 Reference Substances

5.1.1 Range of Responses

The substances proposed for the validation study are representative of a range of ER TA responses, chemical classes, and physico-chemical properties.

5.1.2 Receipt of Reference Standards, Controls, and Test Substances

Reference standards, controls, and test substances will be packaged so as to minimize damage during transit and will be shipped according to proper regulatory procedures. Coded test substances will be packaged and shipped so as to conceal their identities. Each participating laboratory and the SMT will be notified by Substance Inventory and Distribution Management when any reference standards, controls, and test substances are shipped.

Upon receipt, substances should be stored in appropriate storage conditions as per recommendations provided by Substance Inventory and Distribution Management. Each participating laboratory should notify the SMT Project Coordinator upon receipt of the reference substances. Coded test substances, along with a sealed health and safety information package will be shipped to the designated Safety Officer. The Safety Officer should retain the safety information package and pass the coded test substances to the Study Director. The safety information package will contain necessary information about the substance hazards and provide instructions for emergency actions. A disclosure key for identifying the test substances by code will also be included in the package. If the health and safety package must be opened during the course of the validation study (see **Section 5.5**), the Safety Officer should immediately notify the SMT Project Coordinator.

5.1.3 Test Substance Information for the Study Director

Before shipping coded test substances, the SMT Project Coordinator will send the Study Director data sheets containing a minimum of essential information, including color, physical state, weight or volume of sample, specific density for liquid reference substances, and storage instructions to the Study Director.

5.2 Control Materials

The solvent control for both agonist and antagonist assays is 1.0% dimethyl sulfoxide (DMSO) in cell culture medium.

5.2.1 Positive Control (PC)

5.2.1.1 *Agonist Assay (PC)*

Methoxychlor (CASRN: 72-43-5) (3.13 μg/mL) is used as the agonist positive control for all comprehensive concentration-response tests for agonism.

5.2.1.2 *Antagonist Assay (PC)*

Flavone (CASRN: 525-82-6) (25 μg/mL) is used as the antagonist positive control for all comprehensive concentration-response tests for antagonism.

To demonstrate antagonism, a fixed concentration of estradiol (CASRN: 50-28-2) (2.5×10^{-5} μg/mL) is included as a control in all range finding and comprehensive concentration-response tests for antagonism.

5.2.2 Reference Standards

5.2.2.1 *Agonist Assay*

Estradiol (CASRN: 50-28-2) is used as the reference standard for agonist testing, run at 3 different concentrations for range finding and as an 10-point 2-fold serial dilution for comprehensive concentration-response testing.

5.2.2.2 *Antagonist Assay*

Estradiol (CASRN 50-28-2) (1.25×10^{-5} μg/mL) and raloxifene (CASRN 84449-90-1) run at 3 different concentrations for range finding and as a 9-point 2-fold serial dilution for comprehensive concentration-response testing is used as the reference standard for antagonist testing.

5.3 Inventory of Test Substances

The amount of test substance received, the amount used for specific tests, and the amount remaining should be documented by the participating laboratory.

5.4 Disposition of Test Substances

After the studies are completed, any remaining substance will be returned to Substance Inventory and Distribution Management or appropriately disposed of by the participating laboratory.

5.5 Handling of Test Substances

Appropriate safety procedures should be followed in handling the test substances. Personnel should be instructed to treat all test substances as ***very hazardous and potentially carcinogenic*** and to properly dispose of laboratory wastes as toxic wastes. The health and safety information package provided to the facility Safety Officer should be opened only during an emergency situation.

6.0 TEST SYSTEM

All testing procedures and data analyses should follow the Test Method Protocols (**Appendices B** and **C**) and Statement of Work provided by the SMT.

7.0 DATA COLLECTION

7.1 Nature of Data to be Collected

Both raw and summary data from experiments performed under this Statement of Work should be provided to the SMT via the SMT Project Coordinator.

7.2 Type of Media Used for Data Storage

All raw data should be collected and archived at the end of the study (under the direction of the Study Director). Backup files should be produced and maintained for data that are stored electronically.

7.3 Documentation

Raw data include, but are not limited to the following:

a) data recorded in the Study Workbook, which should consist of recordings of all activities related to preparing the LUMI-CELL® ER TA agonist and antagonist

reference standards, controls and test substances, and performing the agonist and antagonist assays

b) computer printouts of luminometer data

c) equipment logs

d) equipment calibration records

e) test substance logs

f) cryogenic freezer inventory logs

g) cell culture media preparation logs

8.0 VALIDATION STUDY PHASE DRAFT AND FINAL REPORTS

As noted in **Section 4.1.2.2**, a draft report should be submitted to the SMT Project Coordinator at the completion of each study phase (i.e., Phases I, IIa, IIb, III, and IV). Once the draft reports are accepted, a final report for each study phase should be prepared, signed by the Study Director and accompanied by a signed Quality Assurance Statement, and provided to the SMT Project Coordinator following acceptance of the corresponding draft report. See **Appendix A** for recommended phase-specific report contents and **Appendix D** for recommended report formats and styles.

9.0 RECORDS AND ARCHIVES

At the end of the validation study, the original raw and derived assay data, as well as copies of other raw data not exclusive to this validation study (instrument logs, calibration records, facility logs, etc.), should be stored and archived for at least five years. At the end of this five year-storage and archiving period, these stored/archived materials should be submitted to NICEATM for storage and archiving.

10.0 SUPPORTING DOCUMENTS

Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T, Hay R, Merten O, Price A, Schectman L, Stacey G, Stokes W. 2005. Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice. ATLA 33:261-287.

Federal Register (FR) Notice (Vol. 71, No. 51, pp. 13597-13598, March 16, 2006): Notice of Availability of a Revised List of Recommended Reference Substances for Validation of *In Vitro*

Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays: Request for Comments and Submission of *In Vivo* and *In Vitro* Data. Available: http://iccvam.niehs.nih.gov/docs/FR/frnotice.htm [accessed 24 March 2006]

ICCVAM. 2002. Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays - Expert Panel Final Report. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/docs.htm [accessed 24 March 2006]

ICCVAM. 2003. ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Pub. No. 03-4503. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine.htm [accessed 14 February 2006]

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD principles on Good Laboratory Practice. [as revised in 1997]. ENV/MC/CHEM[98]17. Paris: OECD

APPENDIX A

RECOMMENDED REPORT CONTENTS

STUDY STATUS REPORTS
LUMI-CELL® ER Validation Study – Phases I – IV

Report Date:

Substances Received:

Study status reports should include information on standards and controls received, with the information for those substances presented in tabular format as per **Table A-1**.

Table A-1 Substance Receipt Reporting Template for LUMI-CELL® ER Validation Study

XDS Identification Number	Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments

If no test substances were received during the time period described in the report, indicate "no test substances or controls received."

Range Finding Results:

Study status reports for range finding results should include:

- Information regarding any problems with test substance solubility in DMSO or 1% DMSO/aqueous cell culture media that prevented the conduct of experiments at the limit dose (1000 µg/mL) specified in the LUMI-CELL® ER assay protocols in **Appendices B** and **C**
- The number of range finder experiments performed during the time period described in the study status report. If no range finder experiments were conducted during this time, indicate "no range finder experiments conducted"
- Excel® spreadsheets of range finder data as described in LUMI-CELL® ER assay protocols in **Appendices B** and **C**
- Graphs of range finder results as per **Figures A-1** and **A-2** using instructions in the provided NICEATM Prism® Users Guide
- The recommended starting concentration for the comprehensive concentration-response experiments for each test substance and the rationale for its use

Comprehensive Concentration-Response Testing Results:

Study status reports for comprehensive concentration-response testing results should include:

- The number of comprehensive experiments performed during the time period described in the study status report. If no comprehensive experiments were conducted during this time, indicate "no comprehensive experiments conducted".
- Excel® spreadsheets of data as described in LUMI-CELL® ER assay protocols in **Appendices B** and **C**.
- Graphs of results as per **Figures A-3** and **A-4** using instructions in the provided NICEATM Prism® Users Guide.

Problems Encountered:

List any problems encountered during range finder, cytotoxicity, and/or comprehensive testing, and their resolution.

Other Information: (All copies of printouts, documents, and spreadsheets will be noted as exact duplicates of the data):

- Copies of raw data generated with the spectrophotometric plate reader
- Copies of completed Microsoft® Excel spreadsheets and Prism® files used for data collection and determination of the EC_{50} or IC_{50} values for the reference standard.
- Copies of the protocols
- Deviations to the protocols, SOPs, and/or Statement of Work

Projected Activities and Schedule:

Provide an estimate of the number and type of experiments (e.g., range finder or comprehensive experiments) to be conducted during the next biweekly study status reporting period. If no experiments will be performed, indicate that no experiments will be conducted.

APPENDIX A (cont.)

RECOMMENDED REPORT CONTENTS

DRAFT/FINAL REPORT NO. 1

LUMI-CELL® ER Validation Study – Phase I

TITLE PAGE

Study Title: Draft/Final Report 1: LUMI-CELL® ER Validation Study – Phase 1

Authors:

Testing Facility: Name and address

Experimental Start Date: The date on which the first phase specific data are collected.

Experimental End Date: The last date on which phase specific data are collected.

Archive Location: Name and address

Study Director: Name

Key Personnel: Laboratory technicians, QA Director, Safety Officer, Facility Manager

Scientific Advisor (if applicable): Name

QUALITY ASSURANCE STATEMENT (Final Reports Only)

The final reports for all phases of the validation study should be accompanied by a signed QA Statement that includes: 1) the phases and data inspected, 2) the dates of inspection, and 3) the dates findings were reported to the Study Director and laboratory management. The QA Statement should identify whether the methods and results described in the final report accurately reflect the raw data produced during the validation study.

TABLE OF CONTENTS

The Table of Contents should be formatted as specified by the provided "Style Guide for LUMI-CELL® ER Validation Study" (**Appendix D**).

EXECUTIVE SUMMARY

The executive summary should state the specific objectives of Phase I and review the experimental procedures and results that support the achievement of the objectives.

METHODS

A description of the protocol elements used for generation and analysis of data should be provided. This should also include information on standards and controls received, and be presented in tabular format as per **Table A-1**.

RESULTS

This section of Phase I should include a table containing the results from all experiments performed during Phase I as per **Table A-2**. This section should also include graphical representations of the data collected during the compilation of the historical database using instructions from the provided NICEATM Prism Users Guide as follows:

- Agonist Quality Controls
    - a graph depicting the combined results for the methoxychlor control
    - a graph depicting the combined results for the DMSO control
    - a graph depicting the combined results for the fold induction of the E2 reference standard
    - a graph depicting the combined EC_{50} values of the E2 reference standard
- Antagonist Quality Controls
    - a graph depicting the combined results for the flavone control
    - a graph depicting the combined results for the DMSO control
    - a graph depicting the combined results for the fold reduction of the Ral/E2 reference standard
    - a graph depicting the combined IC_{50} values of the Ral/E2 reference standard

DISCUSSION

Results, including a description of any problems that were encountered and how they were resolved, should be presented and discussed.

SIGNATURE PAGE

Study Director: Name, signature and date

Table A-2 Example Summary of Experiments Template

Experiments: Phase I						
Experiment I.D.	**Substance Code**	**Date**	**Plate Induction[1]**	**EC_{50} (µg/mL)[2]**	**Experiment Used for Data Analysis or Repeated**	**Reason Why Experiment Not Used**
AG1	E2	09/16/05	not calculated	not calculated	Repeated	Induction not ≥ to 3 fold
AG2	E2	09/16/05	not calculated	not calculated	Repeated	Positive control greater than historical mean plus 2.5 times the SD.
AG3	E2	09/16/05	not calculated	not calculated	Repeated	Plate was dropped
AG4	E2	09/23/05	8.4	2.95E-11	Used	N/A
AG5	E2	09/23/05	12.6	1.98E-11	Used	N/A
AG6	E2	09/29/05	7.4	1.95E-11	Used	N/A
AG7	E2	09/30/05	8.6	2.05E-11	Used	N/A
AG8	E2	10/06/05	6.5	2.35E-11	Used	N/A
AG9	E2	10/12/05	8.9	2.58E-11	Used	N/A
AG1-Repeat1	E2	10/12/05	9.9	2.90E-11	Used	N/A

[1] Column heading is "Plate Induction" for agonist testing and "Plate Reduction" for antagonist testing
[2] Column heading is "EC_{50}" for agonist testing and "IC_{50}" for antagonist testing

APPENDIX A (cont.)

RECOMMENDED REPORT CONTENTS

DRAFT/FINAL REPORTS NO. 2-5
LUMI-CELL® ER Validation Study – Phases II - IV

TITLE PAGE

Study Title:

Draft/Final Report 2: LUMI-CELL® ER Validation Study – Phase IIa

Draft/Final Report 3: LUMI-CELL® ER Validation Study – Phase IIb

Draft/Final Report 4: LUMI-CELL® ER Validation Study – Phase III

Draft/Final Report 5: LUMI-CELL® ER Validation Study – Phase IV

Authors:

Testing Facility: Name and address

Experimental Start Date: The date on which the first phase specific data are collected.

Experimental End Date: The last date on which phase specific data are collected.

Archive Location: Name and address

Study Director: Name

Key Personnel: Laboratory technicians, QA Director, Safety Officer, Facility Manager

Scientific Advisor (if applicable): Name

QUALITY ASSURANCE STATEMENT (Final Reports Only)

The final reports for all phases of the validation study should be accompanied by a signed QA Statement that includes: 1) the phases and data inspected, 2) the dates of inspection, and 3) the dates findings were reported to the Study Director and laboratory management. The QA Statement should identify whether the methods and results described in the final report accurately reflect the raw data produced during the validation study.

TABLE OF CONTENTS

The Table of Contents should be formatted as specified by the provided "Style Guide for LUMI-CELL® ER Validation Study" (**Appendix D**).

EXECUTIVE SUMMARY

The summary should state the specific objectives of Phases II to IV and review the experimental procedures and results that support the achievement of the objectives.

METHODS

A description of the protocol elements used for generation and analysis of data should be provided. This section should include information on coded test substances received as per **Table A-1**.

RESULTS

Range Finding:

The results section relevant to the range finding experiments conducted in Phases II to IV should include the following:

- Information regarding any issues with test substance solubility in DMSO or 1% DMSO/aqueous cell culture media that prevented the conduct of experiments at the limit dose (1.0×10^3 µg/mL) specified in the LUMI-CELL® ER assay protocols in **Appendices B** and **C**
- A table indicating the concentrations tested and the cell viability results for each concentration tested as per **Table A-3**
- A table containing all phase specific range finding experiments performed during the Phase as per **Table A-4**
- Graphical representation of range finding results for each test substance experiment as per **Figures A-1** and **A-2** using instructions from the provided NICEATM Prism® Users Guide
- The recommended starting concentration for comprehensive concentration-response experiment for each test substance and the rationale for its use

Table A-3 Example Table for Range Finding Concentrations Tested and Cell Viability

Substance Code	Concentrations Tested (µg/mL)	Cell Viability Results
V0001	$1.00 \times 10^{+2}$	
	$1.00 \times 10^{+1}$	
	$1.00 \times 10^{+0}$	
	1.00×10^{-1}	
	1.00×10^{-2}	
	1.00×10^{-3}	
V0002	$1.00 \times 10^{+2}$	
	$1.00 \times 10^{+1}$	
	$1.00 \times 10^{+0}$	
	1.00×10^{-1}	
	1.00×10^{-2}	
	1.00×10^{-3}	

Table A-4 Example Summary of Experiments Template: Range Finder Testing

Experiments: Phase IIa Range Finder Testing						
Experiment I.D.	**Substance Code**	**Date**	**Plate Induction[1]**	**EC_{50} (µg/mL)[2]**	**Experiment Used for Data Analysis or Repeated?**	**Rationale for Unacceptability**
RF 1	V0001	09/16/05	9.1	2.94E-11	Used	Acceptable
RF 2	V0002	09/16/05	8.9	2.92E-11	Used	Acceptable
RF 3	V0003	09/16/05	2	not calculated	Repeated	Induction too low
RF 4	V0004	09/23/05	9.3	2.98E-11	Used	Acceptable
RF3-Repeat	V0003	10/12/05	9.9	2.90E-11	Used	Acceptable

[1] Column heading is "Plate Induction" for agonist testing and "Plate Reduction" for antagonist testing
[2] Column heading is "EC_{50}" for agonist testing and "IC_{50}" for antagonist testing

Figure A-1 Example Agonist Range Finder Results Graph

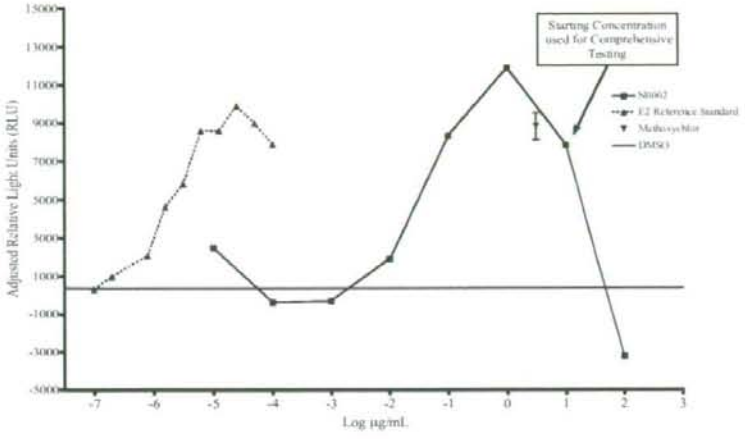
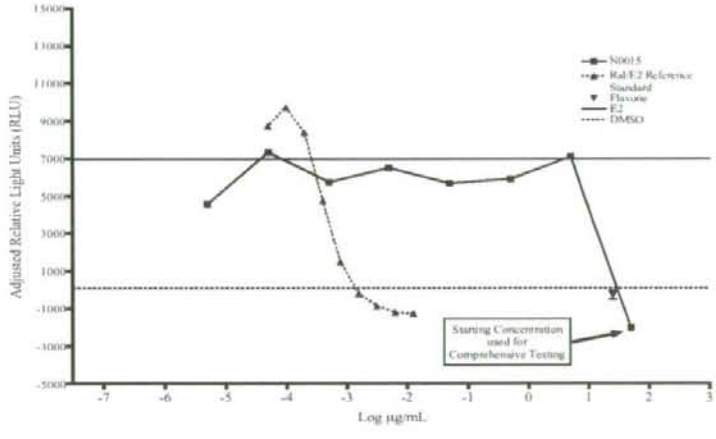


Figure A-2 Example Antagonist Range Finder Results Graph



Comprehensive Concentration Response Testing:

The results section relevant to the comprehensive concentration-response experiments conducted in Phases II-IV should include the following:

- A table indicating the concentrations tested for each substance tested during the phase and the cell viability results for each concentration tested as per **Table A-5**
- A table containing the phase specific experiments performed during the phase as per **Table A-6**

- Graphical representation of the combined results for each substance tested in the comprehensive concentration-response experiment as per **Figures A-3** and **A-4** using instructions from the provided NICEATM Prism® Users Guide

Table A-5 Example Concentrations Tested and Cell Viability Table

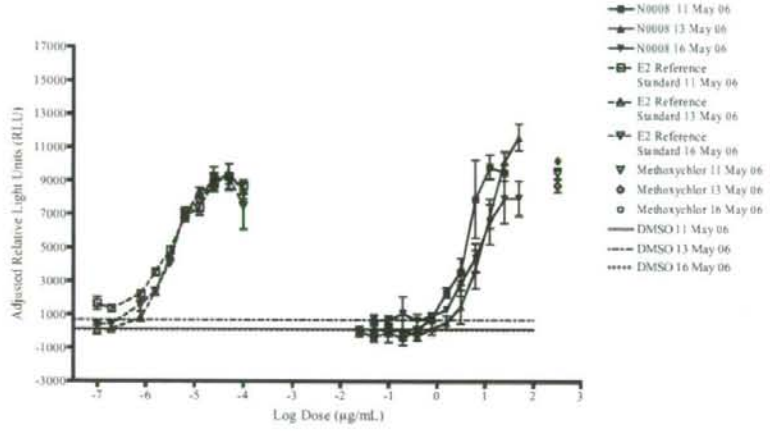
Substance Code	Concentrations Tested (µg/mL)	Cell Viability Results
V0001	1.00×10^{-2}	
	5.00×10^{-3}	
	2.50×10^{-3}	
	1.25×10^{-3}	
	6.25×10^{-4}	
	3.13×10^{-4}	
	1.56×10^{-4}	
	7.81×10^{-5}	
	3.91×10^{-5}	
	1.95×10^{-5}	
	9.77×10^{-6}	
V0002	5.00×10^{-3}	
	2.50×10^{-3}	
	1.25×10^{-3}	
	6.25×10^{-4}	
	3.13×10^{-4}	
	1.56×10^{-4}	
	7.81×10^{-5}	
	3.91×10^{-5}	
	1.95×10^{-5}	
	9.77×10^{-6}	
	4.89×10^{-6}	

Table A-6 Example Summary of Experiments Template: Comprehensive Testing

Experiments: Phase II-IV Comprehensive Testing						
Experiment I.D.	**Substance Code**	**Date**	**Plate Induction[1]**	**EC50 (µg/mL)[2]**	**Experiment Used for Data Analysis or Repeated?**	**Rationale for Unacceptability**
CT 1	V0001	09/16/05	2	not calculated	Repeated	Induction too low.
CT 2	V0002	09/16/05	8.9	2.92E-11	Used	Acceptable
CT 3	V0003	09/16/05	9.1	2.94E-11	Used	Acceptable
CT 4	V0004	09/23/05	9.3	2.98E-11	Used	Acceptable
CT1-Repeat	V0001	10/12/05	9.9	2.90E-11	Used	Acceptable

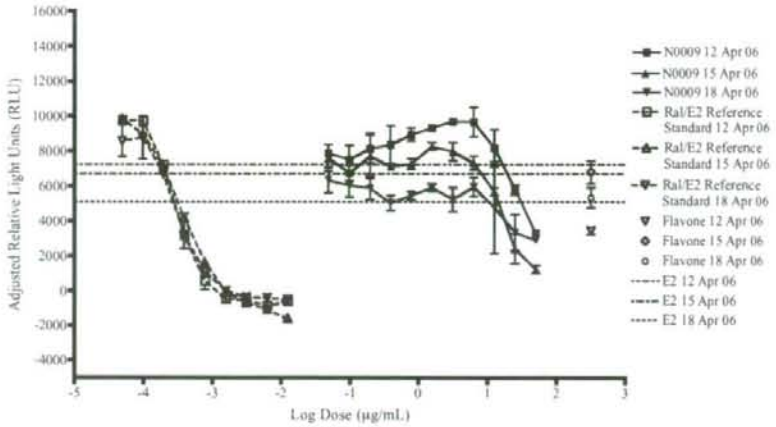
[1] Column heading is "Plate Induction" for agonist testing and "Plate Reduction" for antagonist testing
[2] Column heading is "EC_{50}" for agonist testing and "IC_{50}" for antagonist testing

Figure A-3 Agonist Comprehensive Testing for N0008[1]



[1] Line represents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

Figure A-4 Antagonist Comprehensive Testing for N0009[1]



[1] Line represents the mean of three raloxifene/E2 replicates minus three times the standard deviation of the raloxifene/E2 mean

Quality Controls:

This section should include graphical representations of quality control data used for acceptance or rejection of experiments conducted during each phase using Excel® as follows:

- Agonist Quality Controls
    - a graph depicting the combined results for the methoxychlor control
    - a graph depicting the combined results for the DMSO control
    - a graph depicting the combined results for the fold induction of the E2 reference standard
    - a graph depicting the combined EC_{50} values of the E2 reference standard
- Antagonist Quality Controls
    - a graph depicting the combined results for the flavone control
    - a graph depicting the combined results for the DMSO control
    - a graph depicting the combined results for the fold reduction of the Ral/E2 reference standard
    - a graph depicting the combined IC_{50} values of the Ral/E2 reference standard