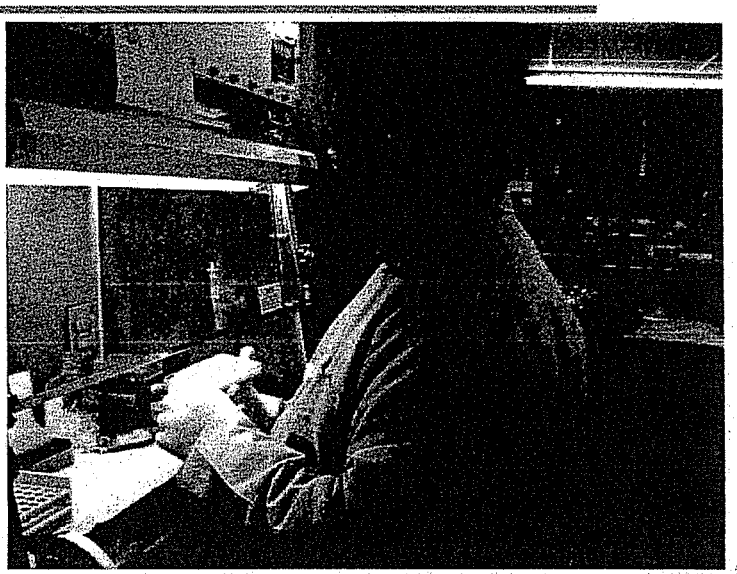
VI. Dr. Gordon in XDS Tissue culture lab



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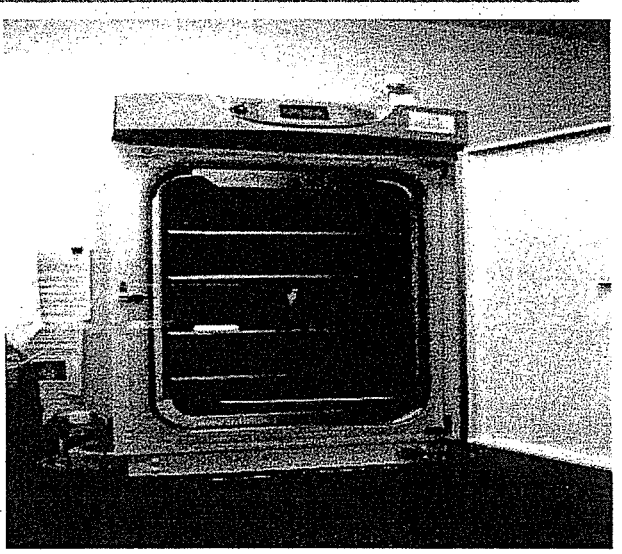
ICCVAM
NICEATM

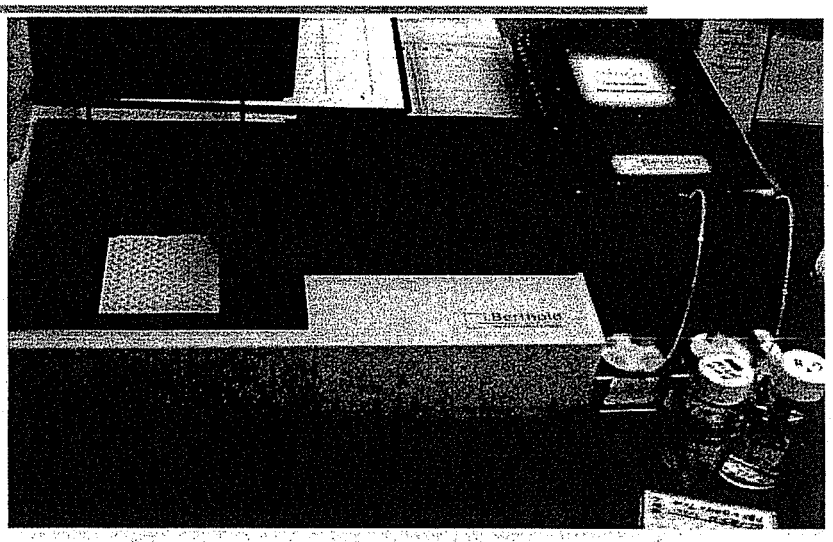
VI. CO^2 Incubator



36

ICCVAM
NICEATM

VI. Berthold Luminometer



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ICCVAM
NICEATM

Draft Minutes
International ED Study Management Team (SMT) Meeting
Thursday, 13 April 2006
(7:30 a.m. – 10:00 AM, US, EDT,
1:30 p.m. – 4:00 p.m. Italy, 8:30 PM – 10:00 p.m. Japan)

Teleconference

ECVAM:

Dr. Patricia Pazos Pereyra

JaCVAM:

Dr. Hajime Kojima

NICEATM:

Dr. William Stokes, NIEHS
Dr. Raymond Tice, NIEHS

Dr. Jeff Charles, ILS, Inc.
Mr. Frank Deal, ILS, Inc.
Ms. Patricia Ceger, ILS, Inc.

Call to Order and Introductions

Dr. Stokes called the meeting to order at 7:30 a.m. He welcomed everyone to the meeting and asked for introductions by members of ECVAM and JaCVAM followed by NICEATM staff. After introductions, Dr. Stokes briefly described the responsibilities of the NICEATM staff:

- Frank Deal and Patricia Ceger serve as project coordinators for the validation study and will coordinate communications between participating laboratories and the SMT.
- Dr. Jeff Charles, the Principle Investigator for the NICEATM support contract, will provide scientific advice and oversee support from ILS, Inc.
- Drs. Ray Tice and Bill Stokes will have direct oversight for the ED validation project within the United States.

After introductions, the following agenda items were discussed

The Collaboration of NICEATM and ECVAM During the *In Vitro* Cytotoxicity Test Methods Validation Study

The validation of two *in vitro* neutral red uptake cytotoxicity test methods was the first collaboration between NICEATM and ECVAM in the arena of international test method validation. One major lesson learned was that the study management team (SMT) must carefully oversee laboratories to ensure that they do not make unauthorized changes to the protocol and that all proposed protocol changes must be reviewed and approved by the SMT.

Review of Roles and Responsibilities

Dr. Stokes discussed the proposed responsibilities of the SMT, the local SMT, and the lead and participating laboratories. These are as follows:

- SMT
  - Overall coordination/management
  - Approval of study design, protocols, time lines, participating laboratories, etc.
  - Test substance selection, acquisition, coding and distribution
  - Data evaluation/interpretation
  - Information exchange
  - Approval of all reports from the study
- National Validation Centers
  - Manage contractual/financial considerations for the respective participating laboratory sponsored by each Center
- Lead Laboratory (Activities if so directed by the SMT)
  - Training/Instructions
  - Coordination of SOPs
  - Troubleshooting
- Participating Laboratories
  - Study conduct
  - Data collection
  - Data submission
  - Study reports

To adhere to the timeline, Dr. Stokes proposes that all data and reports be sent directly to NICEATM with copies, as considered appropriate, sent also to the sponsoring Center. NICEATM would be responsible for processing the data and the reports and for keeping all members of the SMT informed of progress on a biweekly basis.

Potential Conflicts of Interest

To avoid potential bias and conflict of interest, it was agreed that a laboratory that has developed a test method should not be involved in the validation of a competing test method. Thus,

- In the United States, XDS should not test the CERI HeLa-9903 method
- In Japan, CERI should not test the XDS LUMI-CELL assay

Review of HeLa-9903 (CERI) Test Method and Validation Status

Dr. Kojima discussed the CERI test method and indicated that CERI felt that validation testing for their test method was complete and that they (CERI) had submitted the validation data to the OECD for their review. The extent to which the CERI test method will be included in this proposed validation effort will depend on the results of their review. Dr. Stokes pointed out that OECD is currently only reviewing an agonist version of the assay and that for these test method to be useful for regulatory applications, an antagonist version of the assay is needed also.

Review of LUMI-CELL Test Method and Validation Status

- In June 2001, XDS received a Small Business Innovation Research grant from the U.S. National Institute of Environmental Health Sciences to develop the LUMI-CELL assay, an estrogen receptor transcriptional activation assay using the BG-1Luc4E2 cell line, a line derived from the BG-1 immortalized human ovarian cancer cell line that has been stably transfected with an estrogen receptor response element coupled to a luciferase reported gene.
- In April 2004, NICEATM received the XDS test method nomination for validation studies.

- The XDS nomination was approved for further pre-validation protocol standardization, and in October 2005, XDS begins protocol standardization and testing of eight coded substances for agonism and eight coded substances for antagonism, following the guidelines established by ICCVAM in 2003.
- The prevalidation effort is schedule for completion in June, 2006.

Funding Issues

- ECVAM is responsible for placing a call for tender to identify a EU laboratory to test LUMI-CELL and potentially the CERI HeLa-9903 method (this could be the same laboratory).
- NICEATM is responsible for identifying and arranging for a U.S. laboratory to test LUMI-CELL and, if this laboratory is XDS, for another laboratory to test the CERI HeLa-9903 method.
- JaCVAM is responsible for identifying and arranging for a Japanese laboratory to test LUMI-CELL (the laboratory cannot be CERI) and, if additional validation is needed of the CERI HeLa-9903 method, for a laboratory to test that assay.

Action Items:

- JaCVAM will identify and arrange for funding for a Japanese laboratory to validate LUMI-CELL and potentially to further validate the CERI test method.
- ECVAM will identify and arrange for funding for an E.U. laboratory to validate LUMI-CELL and potentially to further validate the CERI test method.
- NICEATM will identify and arrange for funding for a U.S. laboratory to validate LUMI-CELL and potentially another laboratory to further validate the CERI test method.
- NICEATM will send information to ECVAM and JaCVAM on the laboratories in the E.U. and Japan that have worked with XDS in the past.
- NICEATM will send a revised copy of the XDS Statement of Work containing updated validation study timelines to ECVAM and JaCVAM.

- Decisions regarding whether any further validation studies are necessary for the CERI test method will be deferred until after the OECD has made its decision about the validation status of this test method.

A Proposed Phased Approach to Validation

A phased approach to validation was reviewed. Concern was expressed that in this phased approach, negative substances would not be tested during Phase I. Dr. Tice pointed out that:

- Prior to Phase I, the test methods will have completed pre-validation evaluation to determine whether they appropriately identify negatives.
- Once Phase I is completed and actual testing of the substances on the ICCVAM Recommended Reference Substances list begins, ~25% of the substances tested will be negatives.
- The solvent control, which is used throughout the validation, should not have any ED activity and can be used to demonstrate the proper baseline ER activity for the test method.

Action Items:

- NICEATM will propose test substances to be used in each of the validation phases. Note: The identity of these substances must not be distributed outside the SMT in order to ensure that the participating labs do not learn the identity of the substances in each phase.
- The SMT will review and approve the proposed substances for each validation phase prior to the start of the study.

It was asked whether there would be an ongoing evaluation of cytotoxicity during the validation process. XDS is currently evaluating cytotoxicity by visual observation and by the Promega CellTiter Glo assay, which detects levels of ATP. CERI only evaluates cytotoxicity during antagonist testing, using both visual observations and a reporter cell line with a constitutively active reporter gene. Cell death in the reporter cell line is measured as a decrease in luminescence. The SMT will need to consider the data collected by XDS and

CERI in deciding to what extent and how cytotoxicity data will need to be collected during the validation study.

Action Item:

- The SMT will evaluate information from CERI and XDS to make a decision regarding the need for cytotoxicity evaluation and the method(s) to be used.

The Revised ICCVAM ED Recommended Reference Substances List

Dr. Tice reviewed the development of the ICCVAM ED Recommended Reference Substances list of 78 substances. This list contains an ER minimum list of 53 substances that must be used during ER test method validation, and an AR minimum list of 44 substances that must be used during AR test method validation. Each list contains:

- the widest possible range of ED responses (from strong response to negative)
- a wide range of chemical classes
- a wide range of applicable physico-chemical properties
- ~25% substances that are expected to be ER or AR negative

This list has recently been revised by replacing two substances that were considered to be excessively costly and four substances that are not commercially available. NICEATM released a *Federal Register* notice (Vol. 71, No. 51, pp. 13597-8, 03/16/06; http://iccvam.niehs.nih.gov/methods/endocrine.htis) requesting comments on revisions to the reference substances list, and submission of *in vivo* and *in vitro* ED data for substances on the list. Data submitted will be used to generate a database of ED responses to substances on the reference list. Comments are due May 1, 2006.

In regards to the revised ED reference substances list, Dr. Tice reminded Dr. Pazos of the discussion that had taken place during the ECVAM Endocrine Disruptor Task Force (EDTF) teleconference on 30 November, 2005, in which the EDTF agreed to search for a source of adequate quantities of ICI 182,780 (ICI), a proposed reference substance for all ER antagonist assays. She responded that there was a source in England that can provide sufficient compound for validation purposes. Dr. Tice responded by saying that the primary

concern was not directed at the availability of the ICI compound for the validation study but rather its ready availability for all future ER antagonist testing. It was agreed that this issue would require further investigation.

Action Items:

- To the extent they have comments or data to provided, CERI, ECVAM, and JaCVAM will provide comments on revisions to the ICCVAM reference substances list and *in vivo* and *in vitro* ED data to NICEATM by May 1st.
- ECVAM will further consider the ICI 182,780 issue.

International Study Validation Timeline

Dr. Stokes reviewed the proposed timeline for international study validation, emphasizing the need for constant monitoring of data by the SMT:

- During Phase I, test method protocol and SOP transferability will be evaluated. Participating laboratories will be provided with the test method protocol and SOPs and asked to attempt to run the protocol with no additional training. As needed and requested by the SMT, the lead laboratory will answer test method questions and work to solve problems. If there is a need for additional training, it will be provided.
- Repeat testing in the final phase will only be considered if runs between laboratories are significantly different
- Peer Review for the international validation study should be completed before the end of 2007 or early 2008.
- Depending on the results obtained in various phases, the timeline may need to be adjusted to extend one or more phases.

Next meeting:

NICEATM will query the SMT for dates for a May meeting. All agreed that the 0730-0930 EDT timing was acceptable to accommodate the different time zones.

The meeting was adjourned at 9:45 a.m. (EST)

Draft Minutes
International ED Study Management Team (SMT) Meeting
Monday, 15 May 2006
(7:30 a.m. – 10:00 AM, US, EDT,
1:30 p.m. – 4:00 p.m. Italy, 8:30 PM – 10:00 p.m. Japan)

Teleconference

ECVAM:

Dr. Patricia Pazos Pereyra

JaCVAM:

Dr. Hajime Kojima

NICEATM:

Dr. Raymond Tice, NIEHS
Dr. Jeff Charles, ILS, Inc.
Mr. Frank Deal, ILS, Inc.
Ms. Patricia Ceger, ILS, Inc.

Call to Order and Introductions

Dr. Tice called the meeting to order at 7:30 a.m. He welcomed everyone to the meeting and asked for introductions by members of ECVAM and JaCVAM followed by NICEATM staff.

After introductions, the following agenda items were discussed.

Review of LUMI-CELL® Test Method and Validation Status

Mr. Deal reported that the prevalidation effort currently being conducted at XDS is on schedule for completion at the end of June 2006.

Discussion of OECD Pre-Screen Evaluation of CERI ER TA Test Method Submission

Dr. Tice stated that the current focus of the SMT should be restricted to initiating and conducting validation studies based on the XDS LUMI-CELL® ER TA agonist and antagonist protocols. Decisions by the SMT regarding the initiation and conduct of validation studies for CERI's hER-Hela-9903 ER TA assay will be deferred until after the OECD

preliminary validation assessment panel has made its recommendation as to whether or not this test method will undergo an independent peer review. However, Dr. Tice pointed out that the CERI test method is currently being considered for detecting agonists only and that the test method may need to be validated for antagonist activity regardless of what OECD recommends.

Discussion of ICCVAM ED Recommended Reference Substances List to be used for Phased Validation

Dr. Kojima agreed that the number (12) and choice of proposed substances was appropriate for testing in Phases I and II but questioned the number of substances proposed for Phases III and IV. Dr. Tice reviewed the development of the ICCVAM ED Recommended Reference Substances list of 78 substances, which included input from Japanese and EU scientists in the selection process and the criteria used in substance selection. Briefly, this included:

- covering the widest possible range of ED responses (from strong response to negative)
- covering a wide range of chemical classes
- including a wide range of applicable physico-chemical properties
- including ~25% substances that are expected to be ER negative

Dr. Tice emphasized that the 53 substances proposed for testing in all participating laboratories (Phases II and III) are from the ER minimum list that ICCVAM has recommended as essential for the validation of an ER TA test method and that the remaining 25 substances are proposed for testing by one of the participating laboratories or divided between two or more of the participating laboratories (Phase IV). Dr. Tice also emphasized that CERI used at least 56 substances in an effort to validate their proprietary hER-HeLa-9903 ER TA assay. Dr. Kojima acknowledged the recommendation for testing substances on the ER minimum list but specifically questioned the need for testing all of the substances in all participating laboratories as this was done for only 10 of the 56 substances used by CERI in their validation studies.

Dr. Pazos also agreed with the number and choice of proposed substances for testing in Phases I and II but questioned the number of substances proposed for Phases III and IV. Dr. Pazos stated that current ECVAM practices are requiring no more than the testing of 25-30 substances for the validation of *in vitro* endocrine disruptor assays.

Dr. Tice stated that the United States would consider taking responsibility for testing the 25 reference substances included in Phase IV, since those substances were scheduled to be tested in one laboratory only. He then asked Drs. Kojima and Pazos to send their list of recommended substances and accompanying rationale for Phase III. Dr. Pazos replied that she would send the list to the ECVAM ED Task Force for their comment.

Dr. Tice indicated that the purchase, storage, and shipping of test substances would be the responsibility of the U.S. via the NIEHS Chemical Inventory Group.

Action Items:
Drs. Kojima and Pazos to send the SMT their list of recommended substances, including rationales, for testing in Phase III.

Identification of Laboratories to Conduct LUMI-CELL® ER TA Validation Studies

Dr. Tice stated that, currently, XDS is the intended laboratory for the conduct of validation studies for the LUMI-CELL® assay in the U.S.

Dr. Pazos stated that ECVAM colleagues responsible for the contractual and legal considerations of the tender offer for the conduct of the LUMI-CELL® validation study in the EU had a number of questions regarding certain proprietary aspects of the assay (patent issues, trade secrets, etc.). She also requested information on the specific cell line/plasmid construct used in the LUMI-CELL® assay.

Dr. Pazos asked if GLP compliance is a requirement for participating laboratories. Dr. Tice answered that, yes, GLP compliance is a requirement for all participating laboratories.

Dr. Kojima asked for clarification regarding restrictions to the placement of the LUMI-CELL® validation study in Japan. Dr. Tice stated that to avoid conflict of interest and a number of legal issues, the LUMI-CELL® validation study could not be placed in a laboratory that has a proprietary competing commercial ER TA assay. A specific reference was made to the current situation with CERI and their competing hER-Hela-9903 ER TA assay as an example of a laboratory that could *not* be used to conduct the LUMI-CELL® validation study in Japan. Dr. Kojima stated that he would continue to review the qualifications of potential participating laboratories in Japan. He also stated that he did not think that Hyoshi, Inc., a laboratory that is currently licensed to use XDS's proprietary CALUX assay, is GLP compliant and therefore could not be considered as a potential participating laboratory. Dr. Kojima did say that he was interacting with Dr. Tanaka at Hatano Research Institute about participating in the validation study and that Dr. Tanaka has requested copies of the XDS agonist and antagonist protocols to review.

Dr. Tice indicated that participating laboratories will be sending reports and data to the NICEATM project coordinators (Mr. Deal and Ms. Ceger) for evaluation and statistical analysis, and for their use in preparing the draft test method background review document (BRD). Dr. Joe Haseman, the ILS consulting statistician, will provide consultation services for statistical analyses. In addition, ECVAM and JaCVAM are invited to have their own statisticians review the data and the analyses.

Action Items:

- Dr. Pazos to send NICEATM list of questions regarding contractual and legal considerations for the ECVAM tender offer.
- NICEATM to send Dr. Pazos information on the construct of the cell line/plasmid used in the LUMI-CELL® assay.
- Dr. Kojima to identify and recommend a qualified Japanese laboratory to conduct LUMI-CELL® validation studies.
- NICEATM to send current LUMI-CELL® ER TA agonist and antagonist draft protocols to Drs. Kojima and Pazos.

International Study Validation Timeline

Dr. Tice briefly reviewed proposed study timelines as outline in Attachment 2 of the agenda and emphasized that the intended study start date of July 2006 and completion of Phase II in March 2007.

A date for the next meeting was not established (one would likely be held in June).

The meeting was adjourned at 8:30 a.m. (EDT)

Action Items from 2 August 2006 EDWG Meeting:

For the NICEATM LUMI-CELL® protocol standardization study draft report

- NICEATM to provide the revised PP presentation on the restricted website.
- EDWG to provide any other comments on the draft report by end of business, 18 August 2006.
- NICEATM needs to remove the error bars in figures for any data based on two replicates.
- The figures presenting the CellTiterGlo®data needs to include a line at the 80% level.
- The Range Finder figures should indicate (e.g., by an *) the highest concentration selected for testing.
- NICEATM to calculate "Absolute" IC_{50} values and to provide positive response ranges for substances positive for antagonism.
- NICEATM to provide maximum fold increases and positive response ranges for substances positive for agonism.
- NICEATM to re-evaluate flavone as the weak positive control in the LUMI-CELL® antagonist protocol.
- NICEATM to conduct a linear regression analysis comparing the XDS visual observation and CellTiterGlo® methods of assessing cell viability to establish the extent of concordance.
- NICEATM to send EDWG the approach used by the NIH Chemical Genomics Center Group) to concomitantly evaluate agonism and antagonism on the same plate.

For the International Validation Study

- The EDWG agreed, with the concerns raised, that LUMI-CELL® could move forward into an international validation study
- NICEATM to develop a comprehensive standard operating procedure (SOP) for conducting an assessment of cell viability based on visual observations.
    - SOP to include photographic images of representative cell morphology and cell density for each visual observation cell viability classification.

Action Items from 27 July 2006 teleconference meeting

- ECVAM will notify NICEATM of publication of the Tender Offer (expected first week of August, with award expected by late September to mid October). Like JaCVAM, ECVAM will use a laboratory that is not GLP compliant but will monitor their work using their Quality Assurance Unit.
- NICEATM will modify the validation study timelines to reflect ECVAM and JaCVAM study initiation dates.
- NICEATM will add Drs. Kojima and Kanno to the EDWG as JaCVAM liaisons.
- The International Study Management Team will provide comments to NICEATM on draft LUMI-CELL® Protocol Standardization Study report. Comments to particularly address methods for evaluation of cell viability and analysis of antagonist data.
- NICEATM will arrange for an International Study Management Team teleconference for late August or early September to finalize any remaining validation study issues.
- NICEATM will distribute to the participating labs the reference standards, positive controls, and coded reference substances prior to initiation of experiments, by phases.
- Based on Dr. Kanno's statement that CERI's HeLa ER TA assay (for testing agonist and antagonist) will be reviewed through OECD, NICEATM will place on hold efforts to locate a U.S. lab to participate in a validation of this assay.

Draft Minutes
International ED Study Management Team (SMT) Meeting
Monday, 02 October 2006
(7:30 a.m. – 10:00 AM, US, EDT,
1:30 p.m. – 4:00 p.m. Italy, 8:30 PM – 10:00 p.m. Japan)

Teleconference

ECVAM:

Dr. Miriam Jacobs

JaCVAM:

Dr. Hajime Kojima, NIHS

NICEATM:

Dr. Raymond Tice, NIEHS
Dr. Dave Allen, ILS, Inc.
Mr. Frank Deal, ILS, Inc.
Ms. Patricia Ceger, ILS, Inc.

Call to Order and Introductions

Approval of Minutes

Minutes of 27 July International Study Management Team teleconference were approved

After approval of minutes, the following agenda items were discussed:

Status of LUMI-CELL® ER TA Agonist and Antagonist Protocol Standardization StudyTest

Mr. Deal reported that data from the protocol standardization study conducted at XDS was being further analyzed, and a revised study report and revised protocols were being drafted.

Action Item:

NICEATM to send ECVAM and JaCVAM revised study report and LUMI-CELL® ER protocols for review and consideration.

Status of the Contractual Arrangements with Laboratories that will be Conducting the LUMI-CELL® ER TA Validation Studies

Dr. Jacobs stated that the ECVAM Call for Tender (for the conduct of LUMI-CELL® validation study) was published 29 Sep. ECVAM to award study following 21- day response period. Expected study start date is Nov. 2006.

Action Items:

- ECVAM will notify NICEATM as soon as the Call for Tender is published
- Dr. Kojima to send NICEATM names of Hiyoshi Corp. (the Japanese laboratory participating in the validation study) contacts responsible for receipt of reference standards and controls needed to initiate Phase I of LUMI-CELL® validation study.
- Following the selection of the European laboratory by ECVAM, Dr. Jacobs to also send NICEATM names of laboratory contacts responsible for receipt of reference standards and controls needed to initiate Phase I of LUMI-CELL® validation study.
- NICEATM to work with NTP Chemistry Resources Group to arrange for the shipping of reference standards and controls to participating laboratories.

Tentative Timelines

Dr. Tice reviewed proposed validation study timelines and recommended that 1) a Study Management Team teleconference with appropriate personal from XDS, Hiyoshi Corp., and the selected European laboratory should be scheduled to review protocols and discuss international study coordination before initiating Phase I, 2) a Study Management Team meeting should be scheduled in North Carolina to discuss progress of LUMI-CELL® validation study in conjunction with the 2007 Society of Toxicology meeting being held in Charlotte, North Carolina.

Action Items:

- NICEATM to arrange for an International Study Management Team teleconference with appropriate personal from XDS, Hiyoshi Corp., and the selected European laboratory for protocol review and to discuss international study coordination before initiating Phase I.

- Study Management Team to arrange a "face to face" meeting in North Carolina to discuss progress of LUMI-CELL® validation study in conjunction with the 2007 Society of Toxicology meeting being held in Charlotte, North Carolina. If feasible, the SMT meeting will be held at NICEATM immediately following the SOT meeting (i.e., Friday, March 30, 2006).

Discussion of JaCVAM Request for Validation of CERI ER TA Antagonist Assay Validation

Dr. Kojima requested that ICCVAM and ECVAM consider participation in a study to validate the CERI ER TA Antagonist Assay. Dr. Tice reviewed the ICCVAM process for recommending and prioritizing new test methods for validation. Dr. Jacobs indicated that formal documents submitted to OECD as part of the peer review of the CERI ER TA Agonist Assay can not be considered public until completion of the peer review (spring 2007). Therefore, these documents could not be used as part of the formal recommendation process for the validation of the CERI ER TA Antagonist Assay until completion of the peer review.

Action Items:

- Dr. Kojima to work with CERI to obtain data and documents other than those submitted for formal OECD peer review, to begin the process for recommending U.S. participation an international validation study for the CERI ER TA Antagonist Assay.
- ECVAM to consider participating in international validation studies for CERI ER TA Antagonist Assay (although they could not commit until 2007).

Next Meeting

The next International Study Management Team teleconference will be scheduled for late October or early November to resolve remaining validation study issues.

The meeting was adjourned at 8:45 a.m. (EDT)

化学物質リスク評価　大野班　班会議　2006.08.24

HeLa-9903細胞を用いた レポーター遺伝子アッセイの バリデーションの進捗について

財団法人化学物質評価研究機構
武吉 正博

1

1. HeLa-9903細胞を用いたレポーター遺伝子アッセイ OECDにおけるレポーター遺伝子アッセイ位置付けと背景

OECD Test Guideline Programme

Special Activity

EDTA
(Endocrine Disruptors Testing and Assessment)

- VMG-mammalian (est.1999)
- VMG-eco (est.2001)
- VMG-non-animal (est.2002)

2003年3月 第1回VMG-NA会議にて
- Pre-Validationが終了し、Multi-Lab Validationを進める旨、報告。
- 日本がLeadすることが決まった。

[OECD EDTA Conceptual Framework]

Level 1
Sorting and prioritizing based upon existing information

Level 2
In vitro assays providing mechanistic data
- -ER, AR, TR receptor binding assay
- -Transcriptional activation
- -Aromatase and steroidogenesis in vitro
- -Aryl hydrocarbon receptor recognition/binding
- -QSARs
- -High Through Put Screening
- -Thyroid function
- -Fish hepatocyte VTG assay
- -Others (as appropriate)

Level 3
In vivo assays providing data about single endocrine mechanisms and effects

Level 4
In vivo assays providing data about multiple endocrine mechanisms and effects

Level 5
In vivo assays providing data on effects from endocrine & other mechanisms

2