Ministère de la Santé et des Services aux personnes

Signé le _____ jour de _____ 2009.

_____

Elke Anklam, Ph.D.
Directeur

Au nom de l'Institut de santé et de protection des consommateurs
Centre commun de recherche, Commission européenne de l'Union européenne

Signé le _____ jour de _____ 2009.

_____

David H. Blakey, D.Phil.
Directeur

Au nom du Bureau de la science de la santé environnementale et de la recherche
Programme de la sécurité des milieux
Direction générale de la santé environnementale et de la sécurité des consommateurs
Santé Canada

U.S. Department of State
Office of Language Services
Translating Division

04-2009-0138-2
French/English

COMPARISON

Date: April 20, 2009

Reply to Attn of: A/OPR/LS/T Joseph P. Mazza

Subject: Recomparison of the "Memorandum of Cooperation between the Japanese Center for the Validation of Alternative Methods, National Institute of Health Sciences, Ministry of Health, Labour, and Welfare of Japan; and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services of the United States of America; and the European Centre for the Validation of Alternative Methods, Institute of Health and Consumer Protection Joint Research Center, European Commission of the European Union, and the Environmental Health Science and Research Bureau Safe Environments Programme, Healthy Environments and Consumer Safety Branch, Health Canada Regarding the International Cooperation on Alternative Test Methods (ICATM)" as per documents sent by NIH to LS on 4/20/09

To: NIH– Dr. John R. Bucher

The English and French texts of the above-mentioned Memorandum of Cooperation have been recompared by a responsible language officer of this Division and found to have the same meaning in all substantive respects.

A/OPR/LS: GPG
cc: L/T

添付資料５

Coordination Meeting Notes

International Cooperation on Alternative Test Methods (ICATM)

7th World Congress on Alternatives to Animal Testing, Rome, Italy

Tuesday, September 1, 2009

6:30 p.m. – 7:30 p.m.

ICATM Organizational Participants:

ECVAM	Dr. Joachim Kreysa Dr. Claudius Griesinger
Health Canada	Dr. David Blakey
JaCVAM	Dr. Hajime Kojima Dr. Yasuo Ohno
NICEATM-ICCVAM	Dr. William Stokes Dr. Marilyn Wind Dr. David Allen, ILS, Inc.

1. Inclusion of New Validation Centers in ICATM

- At the 7WC, there were announcements of three new national validation centers that have been or will be established in the near future (Brazil, Korea, Finland). This raises the possibility that one or more of these organizations may apply for observer or full member status in ICATM, and therefore ICATM should discuss what criteria might be used as the basis for accepting new organizations.
- The MoC states that …"non-member governmental organizations that perform validation activities and seek limited involvement with ICATM (e.g. observing meetings, sharing information) may do so when feasible upon application to ICATM and unanimous consent of the ICATM Validation Organizations". Such organizations may also seek full membership in ICATM with unanimous consent of the ICATM Validation Organization and execution and adoption of the MoC.
- Participants agreed that applications will be considered on a case-by-case basis, but that general criteria should be developed that can be used as guidance
- Participants agreed that the following general criteria were applicable:
  - Consistent with the MoC, such organizations should be government entities
  - Organizations should be involved in the conduct of validation studies, evaluation and review of proposed alternative test methods, and/or the development of formal recommendations for regulatory acceptance consideration
  - Individual EU member countries should not have separate membership within ICATM, as ECVAM coordinates government validation activities for the EU.

2. ICATM Procedures

- Participants expressed a need for a password-protected website with which to share draft documents for review
  - Dr. Griesinger stated that he would investigate the feasibility of establishing a web-based database that would allow for direct posting of documents by any of the participants.
- NICEATM-ICCVAM will post the latest version of draft proposed ICATM procedures as soon as the site is active

3. Future ICATM Coordination Meetings

- **Future ICATM Coordination meetings were discussed, with agreement on the following schedule:**
- Meet for a full day prior to the next ESAC meeting at ECVAM, currently planned for January or February, 2010. However, this meeting is contingent upon formal establishment of the new ESAC.
- If the ESAC meeting is delayed, then ICATM will plan to meet in conjunction with the 2010 SOT in Salt Lake City, UT, USA (March 7-11).
- A full day meeting is scheduled for June 16, 2010 (9:00 am – 5:00 pm) in conjunction with the next SACATM meeting (June 17-18) in Research Triangle Park, NC

4. Other Business/Activities

- Participants agreed that pdf versions of their presentations from the WC7 ICATM session should be posted on their respective websites
- JaCVAM intends to submit the LabCyte human skin model for skin irritation testing to OECD for peer review, since this was already discussed at the June OECD Expert Consultation meeting, and its validation status is based on the ECVAM performance standards agreed on at this meeting.
- ESAC nominees are due Sept 30. Interested participants must "self nominate" themselves (i.e., NICEATM-ICCVAM, JaCVAM, or Health Canada cannot simply submit individuals that they endorse)
  - The number of non-EU members will be based on the comparative expertise of EU vs. non-EU nominees (i.e., the membership of ESAC will be driven by expertise, not regional designation).

ICCR-3 report

Alternative Test Methods Update

September 8-10, 2009

Tokyo

VAM（Validation of Alternative Methods）

Group

International Cooperation on Alternative Test Methods

Representative Members

Dr. Marilyn Wind, for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Dr. William S. Stokes, for the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Dr. Elke Anklam, for the European Centre for the Validation of Alternative Methods (ECVAM)

Dr. Joachim Kreysa, for ECVAM

Dr. David Blakey, for the Environmental Health Science and Research Bureau within Health Canada

Dr. Hajime Kojima, for the Japanese Center for the Validation of Alternative Methods (JaCVAM)

ICATM

On April 27, 2009, representatives from four international agencies, including the director of the NTP, signed a Memorandum of Cooperation (MOC) establishing an **International Cooperation on Alternative Test Methods (ICATM)**. The agreement promotes enhanced international cooperation and coordination on the scientific validation of non- and reduced-animal toxicity testing methods. If the toxicity testing methods are shown to be reproducible based on strong scientific information, and able to accurately identify product related health hazards, the tests are more readily accepted by regulatory agencies.

According to Dr. William Stokes, D.V.M., Director of NICEATM and Executive Director of ICCVAM, "This international cooperation will benefit both people and animals. The cooperation will serve an important role in translating research advances into more effective public health prevention tools. It will speed the adoption of new test methods based on advances in science and technology that will provide more accurate predictions of safety or hazard. Animal welfare will also be improved by the national and international acceptance of alternative test methods that reduce, refine, and replace the use of animals."

The MOC was signed by:

- Dr. Linda Birnbaum, for the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- Dr. Elke Anklam, for the European Centre for the Validation of Alternative Methods (ECVAM)
- Dr. David Blakey, for the Environmental Health Science and Research Bureau within Health Canada
- Dr. Masahiro Nishijima, for the Japanese Center for the Validation of Alternative Methods (JaCVAM)

Four country representatives signed the international agreement to reduce animal use in toxicity testing. Shown left to right are Linda Birnbaum, David Blakey, Elke Anklam, and Hajime Kojima representing Masahiro Nishijima of Japan. Standing are Marilyn Wind, Michelle Limoli of FDA, and William Stokes. (Photo courtesy of NIH)
Birnbaum signed as the U.S. representative on behalf of the NTP Interagency

Update from JaCVAM on cosmetic issues

JaCVAM has a framework for peer review and regulatory acceptance of alternative methods. JaCVAM has a Steering Committee, which has supported a Validation Management Team and has established oversight committee for each validation study. The oversight committee prepares the background review document (BRD), which is evaluated by an ad hoc Peer Review Panel (Panel). The Panel publishes a report, which goes to the Regulatory Acceptance Board (Board).

Last year, JaCVAM submitted statements on two test methods: 1) the Vitrolife-Skin™, a 3-dimensional cultured skin model for skin corrosivity testing, and 2) the LLNA (Local Lymph Node Assay) -DA for skin sensitization testing. JaCVAM receives a report from the Board and then prepares a statement for Japan's regulatory agencies.

1. Skin irritation

JaCVAM has proposed a new OECD test guideline (TG) for an *in vitro* skin irritation assay (LabCyte EPI-MODEL24). The validation study using this assay was completed in June 2009. Peer reviews are ongoing by the Panel for the *in vitro* skin irritation assays LabCyte EPI-MODEL24, EpiDerm and SkinEthics. Acceptance of another assay by the Board (EPISKIN) is pending.

With regard to a battery system to predict phototoxicity (the Yeast Growth Inhibition Phototoxicity Assay and Red Blood Cell Photohemolysis Assay), the Board recommends that further work should be performed before a definitive statement on its scientific validity can be made.

2. Skin sensitization

JaCVAM has proposed new TGs for a non-radioisotope versions of the LLNA. One of them, the LLNA–DA, was accepted by the Board last year. The other assay, the LLNA: BrdU-ELISA, is pending acceptance by the Board.

A peer review of the reduced LLNA (rLLNA) is in progress by the Panel.

A validation study of h-CLAT (human cell line activation test) will begin in collaboration with ECVAM this fall.

3. Eye irritation

The BCOP (Bovine Corneal Opacity and Permeability) and ICE (Isolated Chicken Eye) test methods were accepted by the Board, Peer reviews of other eye irritation test methods, cytotoxicity tests using SIRC cells or a 3-dimensional dermal model (MATREX), are on-going by the Panel.

4. Acute toxicity

The peer review of *in vitro* cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests starts in July.

5. Genotoxicity

JaCVAM has proposed a TG for a comet assay for genotoxicity testing, and the validation study of *in vivo*/*in vitro* assays is on-going.

6. Carcinogenicity:

The validation study of the Bhas cell transformation assay developed by Hatano Institute, Food and Drug Safety Center.is on-going.

NICEATM-ICCVAM Update on cosmetic issues

NICEATM-ICCVAM identified four key challenges in the Five-Year Plan:

1. **Identify priorities and conduct and facilitate alternative test method activities**
2. **Incorporate new science and technology**
3. **Foster regulatory acceptance and use of alternative methods**
4. **Develop partnerships**

The four highest priority areas are below:

- Dermal Toxicity
- Ocular Toxicity
- Acute Toxicity
- Biologics/Vaccines

With the exception of biologics/vaccines, all of these priority areas are related to the regulation of cosmetics.

Dermal toxicity:

1. Skin irritation

1) Draft OECD Test Guideline on *In Vitro* Skin Irritation Assays

ICCVAM provided comments on two rounds of draft documents; delegates participated in OECD Expert meetings, Berlin, October, 2008 and Washington, June 15-17, 2009.

2) ICCVAM submitted proposals to update OECD Test Guidelines for *In Vitro* Skin Corrosivity Assays to include performance standards:

TG 430: Transcutaneous Electrical Resistance Test (TER)

TG 431: Reconstructed Human Epidermis (RhE) Test Methods

Draft revised TG 430 and TG431 are currently out for review and comment by member countries.

2. Skin sensitization

ICCVAM submitted a proposal to update TG 429 with the reduced LLNA, performance standards, and a revised protocol. A draft revised TG 429 is currently out for review and comment by member countries.

An OECD Expert Consultation meeting is planned at CPSC (Bethesda, MD) for October 20-22, 2009.

The LLNA public peer review panel meeting was held on April 28-29, 2009, NIH Natcher Conference Center, Bethesda, MD. In this meeting, updated data on three non-radioactive versions of the LLNA and an expanded LLNA applicability domain was evaluated.

The final Panel report was published on June 1 and is available on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm). ICCVAM will publish final recommendations in fall 2009.

In conjunction with JaCVAM, draft OECD Test Guidelines have been submitted for 2 non-radioactive methods (LLNA: BrdU-ELISA and LLNA: DA).

3. Ocular toxicity:

1) BCOP and ICE

ICCVAM and NICEATM developed drafts of OECD Test Guidelines for BCOP and ICE in coordination with ECVAM and JaCVAM

Timeline to OECD Adoption:

Submitted to OECD in August, 2008

OECD Expert Consultation Meeting was held at EPA, Washington D.C., on December 4-5, 2008.

Draft Test Guidelines were approved at National Coordinators Meeting, March 31-April 2, 2009. Formal adoption by the OECD Council is expected in September 2009.

- TG 437: BCOP
- TG 438: ICE

2) Other methods

An independent international peer review meeting on alternative ocular safety testing methods and strategies was held on May 19-21, 2009, CPSC Headquarters, Bethesda, MD (view the Panel report at http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm). Ten alternative methods and strategies were evaluated in this meeting, including:

- Routine use of analgesics and topical anesthetics
- Low Volume Eye Test (Note: also evaluated in July by ESAC)
- *In vitro* methods and testing strategies (Note: CM also evaluated in July by ESAC)

4. Acute Toxicity:

In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests

All U.S. agencies endorsed the ICCVAM recommendations with the stated limitations and where applicable to their agency.

A draft OECD Guidance Document developed by NICEATM-ICCVAM was forwarded to OECD in July 2009 for consideration and adoption. The draft GD is currently out for review and comment by member countries.

5. Genotoxicity:

***1) In Vitro* Micronucleus Test: OECD draft Test Guideline 487**

The ICCVAM Genetic Toxicity Working Group (GTWG) provided comments on cytotoxicty procedures and study design

***2) In vivo* rodent Comet assay (**JaCVAM international validation study)

GTWG has provided comments on proposed study plan, protocol, and reference substances

3) **Cell transformation assays**

- GTWG nominated experts for ESAC Peer Review Panel
- JaCVAM validation study planned, GTWG comments

ECVAM Update on cosmetic issues

1. Skin irritation:

In 2007, the EPISKIN® method was validated by ECVAM (European Center for the Validation of Alternative Methods) as a potential stand-alone method, capable of reliably distinguishing irritant from non-irritant substances. The EpiSkin test method may thus be able to replace the Draize skin irritation test, a classic test introduced for safety testing for drugs and chemicals 60 years ago for many testing situations. In 2008, two skin irritation methods (SkinEthic RhE model and EpiDerm SIT model) were validated on the basis of the Performance Standards for applying human skin models to *in vitro* skin irritation testing.

In April 2009, the ECVAM Scientific Advisory Committee (ESAC) endorsed the satisfactory performance of three ECVAM-validated *in vitro* skin irritation test methods based on reconstructed human epidermis (RhE) also under the UN GHS (United Nations Globally Harmonized System of Classification and Labeling of Chemicals) system.

A Draft OECD Test Guideline for human skin model systems as irritation test methods was submitted in 2007. This draft guideline was discussed in OECD Expert meetings, Berlin, October, 2008 and Washington, June 15-17, 2009.

2. Skin sensitization:

1) LLNA

The ESAC endorsed the scientific validity of the harmonized ECVAM performance standards for the Local Lymph Node Assay (LLNA) at its 29th meeting held in November 2008. These harmonized Performance Standards for the LLNA represent a significant accomplishment of international collaboration in the field of alternative methods.

2) Other assays

The first meeting of the Validation Management Team for a planned study on skin sensitization will be held in September-October, 2009, Ispra.

The Direct Peptide Reactivity Assay (DPRA).

The Human Cell Line Activation Test (hCLAT)

The Myeloid U937 Skin Sensitisation Test (MUSST)

3. Ocular toxicity:

1) BCOP and ICE

The two validated organotypic *in vitro* assays [the Bovine Corneal Opacity and Permeability (BCOP) and the Isolated Chicken Eye (ICE) test methods] identify severe eye irritants using tissues from slaughterhouses which would otherwise be discarded. The tests will replace the use of animals to identify severe irritants, though some animal testing will still be required for mild irritants.

Eight other test methods are currently under evaluation by ECVAM.

2) Cytotoxicity-/ cell function- based assays

A review of scientific reports was conducted on the following *in vitro* tests, which had been subjected to a retrospective validation study:

1. Cytosensor microphysiometer (INVITOX 97 and 102);
2. Neutral red release (INVITTOX 54 en Predisafe);
3. Fluorescein leakage (INVITTOX 71, 82, 86 and120);
4. Red blood cell haemolysis test (INVITOX 37 and 99).

2-1)Of these, the Cytosensor Microphysiometer (INVITTOX 102) is considered to have been scientifically validated and to be suitable for use for regulatory purposes as a Top-Down

screen to distinguish strong irritants (EU R41, GHS Category 1, and EPA Category I) and a Bottom-Up screen for consideration for regulatory use to distinguish non-irritants (EU:NC; GSH: NC; EPA: cat IV) from all other classes as part of a tiered-testing strategy in a weight-of-evidence approach for the applicability domain given (water-soluble surfactants, multi-component water-soluble surfactant containing mixtures, and water-soluble chemicals/materials).
2-2) The Fluorescein Leakage test (INVITTOX protocol 71) is considered to have been scientifically validated and to be suitable for regulatory use as a Top-Down screen to distinguish strong irritants (EU: R41; GSH: cat 1; EPA: Category I) from all other classes, for chemicals only. Additional testing and further refinement, in particular with respect to variability and definition of the applicability domain, by expanding the dataset of tested chemicals and direct comparison with *in vivo* data is recommended, and further review conducted once this is done.
2-3) With regard to the remaining tests, ESAC recommends that further work should be performed before a definitive statement on their scientific validity can be made.

3) LVET

The ESAC does not recommend the use of the Low Volume Eye Test (LVET) method, a minor modification of the Draize eye test, for the prospective (future) generation of data of any use or applicability domain.

However, the ESAC recommends the use of existing Low Volume Eye Test (LVET) data within Weight of Evidence (WoE) evaluations for decision making on the necessity to conduct standard test method(s) for eye irritation for purposes of classification and labelling for the limited use domain of household detergents and cleaning products as well as their main ingredient class.

The ESAC furthermore recognises that several alternative methods for eye irritation have databases of acceptable size only when also considering LVET testing data as an additional possible validation reference. While some differences of LVET data with respect to traditional reference data for eye irritation (i.e. Draize eye data) should be kept in mind (i.e. the tendency to give lower hazard categories than the classical Draize eye test) these data may still be useful and, in the case of household detergents, cleaning products and surfactants used in such products, may contribute to a knowledge base against which alternative methods may be validated for this specific use domain.

4) Reconstructed Human Tissue Models (SkinEthic™HCE and EpiOcular™) for discrimination of non-irritants (NC) from irritants (GHS cat.1 &2).

The validation management team has conducted pre-study discussions. The experimental part of the validation study will start in November, 2009.

4. Acute Toxicity

ECVAM is a partner in the integrated project A-Cute-Tox (funded by DG RTD), which overall objective is to develop an *in-vitro* test strategy sufficiently robust and powerful to completely replace *in-vivo* testing for acute toxicity testing of chemicals.

As a follow-up study to the international validation study on the prediction of acute toxicity by cytotoxicity assays and taking into consideration the high prevalence of non-toxic substances in the New Chemicals Database (87% with LD50 > 2000 mg/kg), ECVAM has organized a prospective validation study in which 57 industrial chemicals (of which 60% are cosmetic ingredients) are tested to assess the predictive capacity of the validated 3T3/Neutral Red Uptake (NRU) cytotoxicity assay to discriminate between toxic/hazardous (LD50 < 2000 mg/kg) and non-toxic (LD50 > 2000 mg/kg) substances.

5. Genotoxicity:

1) Retrospective validation of in vitro Micronucleus test completed
2) Analysis to reduce top dose of cytotoxicity in in vitro genotoxicity tests for a better test strategy with less false positives
3) (pre-)validation of COMET Assay ongoing (coordinated by JaCVAM)
4) Genotox assays in 3D-skin model (coordinated by COLIPA)
5) Recommendation for reduction in genotox - *in vivo* testing (ECVAM Workshop report in press)

6. Carcinogenicity:

ECVAM validation study on cell transformation assays [SHE (pH 6.7and pH 7) and Balb/c 3T3 cells] completed.

Peer review of validation of the three cell transformation assays (CTA)to be initiated end of 2009.

7. Toxicokinetics

Prevalidation of two human hepatic metabolic competent test systems: the HepaRG test system and cryopreserved human hepatocytes.

8. Reproductive toxicity

Due to the high impact of reproductive toxicity studies on the number of animals used for the detection of reproductive and developmental toxicants, ECVAM has drafted and initiated an integrated project "ReProTect" with the aim to stimulate further development and optimization of *in vitro* models for toxicological safety testing in this area (http://www.reprotect.eu/). Several promising tests have been included in order to make them available for entering the validation process. ECVAM is providing scientific/technical support to the Project coordinator as well as relevant information on the development of testing strategies.

LUMI-CELL estrogen receptor transcriptional activation assay: agonist and antagonist protocols currently under validation (ECVAM is one of the testing laboratories)

ICCR(International Cooperation on Cosmetics Regulations)と ICATM (International Cooperation on Alternative Test Methods)への関与

国立医薬品食品衛生研究所
小島　肇

ICCR動向3-1
化粧品規制協力国際会議(ICCR) 第3回結果概要

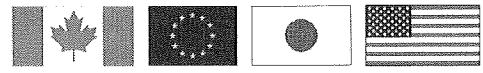
2009年9月9日～11日に化粧品規制協力国際会議(ICCR)が東京で開催された。ICCRは米国、日本、欧州連合及びカナダ化粧品規制当局からなる国際的グループである。ICCRの目的は、化粧品分野において、国際貿易への障壁を最小化しつつ、最高レベルの世界的な消費者保護を維持することである。

今回の会合では、以下の問題について議論した。
動物実験代替法
GMP
成分の安全性／認可物質リスト
ナノテクノロジー
化粧品表示
日焼け止め剤
規制当局と業界団体の技術ワーキンググループとの連携
ICCRの拡大

ICCR動向3-2
会合の結果の概略

動物実験代替法
- 規制当局はICATM の活動について報告を受け、進捗を確認した。
- 規制当局はICATMの活動への協力、調整、支援を継続する。

International Cooperation on Alternative Test Methods (ICATM)



ICATM

2009年4月27日　日米欧カナダの代表による調印式（日本からは国立衛研　所長代理として小島が出席）

ICATMの協力内容

3つの重要な領域における協力の枠組みを以下に示す:

1) バリデーション研究
2) 科学的妥当性についての第三者評価
3) 代替法における公式な試験法勧告の推奨

ICATM Current Alternative Test Method Validation and Regulatory Acceptance Status Report

September, 8, 2009

Dermal Corrosivity Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
CORROSITEX Skin Corrosivity Test	Adopted as OECD Test Guideline (TG) 435 (2006)	Completed	Yes, accepted by U.S. in 2000; as OECD TG 435 in 2006	OECD TG 435 (2006)
EpiSkin Skin Corrosivity Test	Adopted as OECD TG 431 (2004)	Completed	Adopted for regulatory use in EU in 2000	OECD TG 431 (2004)
EpiDerm Skin Corrosivity Test	OECD TG 431 (2004)	Completed	Adopted for regulatory use in EU in 2000	OECD TG 431 (2004)
SkinEthic Skin Corrosivity Test	OECD TG 431 (2004)	Completed	Adopted via OECD TG 431 (2004)	OECD TG 431 (2004)
Rat TER Skin Corrosivity Test	OECD TG 430 (2004)	Completed	Adopted for regulatory use in EU in 2000	OECD TG 430 (2004)
In vitro skin corrosivity assays -EpiDerm -EPISKIN -SkinEthic -Rat TER	Update of OECD test guidelines 430 and 431 in progress to incorporate performance standards	NICEATM-ICCVAM		

Dermal Irritation Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
In vitro reconstructed human epidermis test methods -EpiDerm -EPISKIN -SkinEthic	- Finalisation of draft OECD test guideline - Peer review of EpiDerm and SkinEthic in progress in Japan; EPISKIN pending acceptance by Japanese Regulatory Acceptance Board	ECVAM	Adopted for regulatory use in EU in 2009	
Investigation of *in vitro* dermal irritation assays for detecting false negative corrosives from in vitro corrosivity tests -EpiDerm -EPISKIN -SkinEthic	Study in progress	NICEATM-ICCVAM		
LabCyte EPI-MODEL24 *in vitro* test method	- JaCVAM-sponsored validation study completed June, 2009 - Peer review in progress	JaCVAM		

Phototoxicity Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
3T3 NRU Phototoxicity Test	OECD TG 432 (2004)	completed	OECD TG 432 (2004)	OECD TG 432 (2004)
3T3 NRU Phototoxicity Test: Application to UV Filter Chemicals	OECD TG 432 (2004)	completed	OECD TG 432 (2004)	OECD TG 432 (2004)
Tiered testing strategy to predict phototoxicity (3T3 NRU PT and reconstructed human epidermis models)	Study in progress	ECVAM		
Test method battery to predict phototoxicity (yeast growth inhibition phototoxicity assay and red blood cell photohemolysis assay)	Japanese Regulatory Acceptance Board recommended additional work be performed	JaCVAM		

Ocular Toxicity Test Methods -1-

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
Bovine Corneal Opacity and Permeability (BCOP) Test Method	Draft OECD TG approved, formal adoption pending	completed	U.S. acceptance in 2008	Draft OECD TG approved, formal adoption pending
Isolated Chicken Eye (ICE) Test Method	Draft OECD TG approved, formal adoption pending	completed	U.S. acceptance in 2008	Draft OECD TG approved, formal adoption pending
Cytotoxicity test: SIRC cells	JaCVAM peer review ongoing	JaCVAM		
Cytotoxicity test: three-dimensional dermal model (MATREX)	JaCVAM peer review ongoing	JaCVAM		
Routine use of anesthetics and analgesics, and humane endpoints in the Draize eye test	Development of ICCVAM final recommendations in progress	NICEATM-ICCVAM		
Low volume eye test	- Development of ICCVAM final recommendations in progress - ESAC recommendations issued	NICEATM-ICCVAM/ ECVAM		

Ocular Toxicity Test Methods -2-

Method	Current Status	Lead Action Organization	National acceptance	International Acceptance
In vitro methods for identification of moderate and mild irritants and substances not labeled as irritants: -BCOP -ICE -IRE -HET-CAM	Development of ICCVAM final recommendations in progress	NICEATM-ICCVAM		
In vitro approach for categorization of anti-microbial cleaning products	Development of ICCVAM final recommendations in progress	NICEATM-ICCVAM		
Four cell function-based *in vitro* assays -Cytosensor Microphysiometer® (CM) -Neutral red release -Fluorescein leakage -Red blood cell hemolysis	ESAC recommendations issued endorsing CM and fluorescein leakage tests and recommending further development on others	ECVAM		
Human reconstructed tissue models -EpiOcular -SkinEthic	ECVAM validation study started in 2009 (experimental part will start end of 2009)	ECVAM		

Acute Contact Dermatitis Test Methods -1-

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
Murine local lymph node assay (LLNA) for skin sensitization	OECD TG 429 (2002) ISO (2002)	complete	OECD TG 429 (2002) ISO (2002)	OECD TG 429 (2002) ISO (2002)
Nonradioactive murine local lymph node assay protocol (LLNA: DA)	- ICCVAM peer review completed, development of final ICCVAM recommendations in progress - Development of draft OECD test guideline in progress	JaCVAM (validation study); NICEATM-ICCVAM (international peer review)	Accepted by Japanese Regulatory Acceptance Board	
Reduced LLNA (rLLNA)	- Endorsed by ICCVAM, transmittal of ICCVAM recommendations to U.S. Federal agencies in progress - Update to TG 429 submitted to OECD - JaCVAM peer review in progress	NICEATM-ICCVAM/ ECVAM		

Acute Contact Dermatitis Test Methods -2-

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
Nonradioactive LLNA protocol (LLNA: BrdU-Flow Cytometry	- ICCVAM peer review completed, 2009 - Data audit and interlaboratory study planned, 2009	NICEATM-ICCVAM		
Nonradioactive LLNA protocol (LLNA: BrdU-ELISA)	- ICCVAM peer review completed, development of final recommendations in progress - Acceptance by Japanese Regulatory Acceptance Board pending - Development of draft OECD test guideline in progress	JaCVAM (validation study); NICEATM-ICCVAM (international peer review)		
Harmonized performance standards for the LLNA	- Endorsed by ESAC - Endorsed by ICCVAM, transmittal of ICCVAM final recommendations to U.S. Federal agencies in progress - Update to TG 429 submitted to OECD	NICEATM-ICCVAM; ECVAM; JaCVAM		
In vitro skin sensitization assays (h-CLAT; DPRA; MUSST)	Multi-laboratory validation study to start this fall	ECVAM; JaCVAM		

Acute Oral Systemic Toxicity Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
Up and Down Procedure (UDP)	OECD TG 425 (2001)	completed	OECD TG 425 (2001)	OECD TG 425 (2001)
Fixed Dose Procedure (FDP)	OECD TG 420 (2001)	completed	OECD TG 420 (2001)	OECD TG 420 (2001)
Acute Toxic Class Method (ATC)	OECD TG 423 (2001)	completed	OECD TG 423 (2001)	OECD TG 423 (2001)
In vitro cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests	- Draft OECD guidance document submitted - JaCVAM peer review in progress	NICEATM-ICCVAM/ECVAM	U.S., 2008	
In vitro cytotoxicity test (3T3 Neutral Red Uptake) for identifying substances with acute oral LD50 > 2000 mg/kg b.w.	ECVAM follow-up validation study completed	ECVAM		
In vitro hepatic biotransformation enzyme induction	Validation study planned	ECVAM		

Endocrine Disruptor Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
LUMI-CELL estrogen receptor transcriptional activation assay: agonist and antagonist protocols	- International validation study in progress (NICEATM, JaCVAM, ECVAM) - Independent peer review planned Spring 2010, including proposed ER-TA Performance Standards	NICEATM-ICCVAM		
CertiChem MCF-7 cell proliferation assay	Validation study in progress	NICEATM-ICCVAM		
Stably Transfected Transcriptional Activation (STTA) Assay for the detection of estrogenic activity of chemicals	International validation study on anti-estorogenic activity in progress (JaCVAM-Korea)	JaCVAM		Draft OECD TG approved, formal adoption pending

Genetic Toxicity Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
In vivo/In vitro comet assay	Validation study in progress	JaCVAM		
In vitro micronucleus test	Validation study completed	ECVAM		
In vitro micronucleus test	Finalisation of draft OECD test guideline	OECD/United Kingdom (Lead Country)		
Improvement of the In vitro test battery for genotoxicity	*Analysis of cytotoxicity top dose in in vitro genotoxicity tests on going*	ECVAM		
Genotoxicity assays in 3D skin models	Validation study ongoing	ECVAM		

Carcinogenicity Test Methods

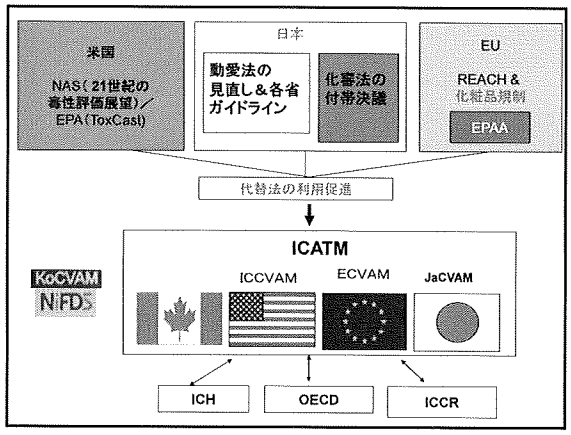
Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
Bhas cell transformation assay	Validation study in progress	JaCVAM		
SHE pH 6.7, SHE pH 7 and Balb/c 3T3 cell transformation assays	Validation study completed	ECVAM		
SHE pH 6.7, SHE pH 7 and Balb/c 3T3 cell transformation assays	Peer review to be initiated end of 2009	ECVAM		

Biologics Test Methods

Method	Current Status	Lead Action Organization	National Acceptance	International Acceptance
Use of Humane Endpoints in Animal Testing of Biological Products	Accepted in U.S.	NICEATM-ICCVAM	U.S., 9 CFR 117.4e	
Rabies Vaccine, Humane Endpoints	Accepted in U.S.	NICEATM-ICCVAM	U.S., 9 CFR 117.4e	
ELISA Test for Batch Potency Testing of Erysipelas Vaccines (refinement)	Accepted in U.S. and EU		U.S., per 27 CFR 610.10; are reviewed on a case-by-case basis; published in European Pharmacopeia	
Relevance of the Target Animal Safety Test for batch safety testing of vaccines for veterinary use	Accepted in U.S. and EU		U.S., per 9 CFR 113.4; published in European Pharmacopeia	
ELISA Test for Batch Potency Testing of Human Tetanus Vaccines refinement	Accepted in U.S. and EU		U.S., per 27 CFR 610.10; are reviewed on a case-by-case basis; published in European Pharmacopeia	
ToBI Test for Batch Potency Testing of Human Tetanus Vaccines refinement	Accepted in U.S. and EU		U.S., per 27 CFR 610.10; are reviewed on a case-by-case basis; published in European Pharmacopeia	

ICATMにおける試験法開発の方向性

- 国際協調が徐々に進んでいる。
- OECDテストガイドラインの設定が大きな目標である。
- 比較すべき動物実験結果はGHS基準である。
- 局所毒性から一般毒性にシフトする過程にある。
- 疑陰性をなくすべく、新規試験法の開発および既存試験法の見直しが行われている。



Ⅳ．分担研究報告（非臨床有効性部門）

厚生労働科学研究費補助金（医薬品・医療機器等レギュラトリーサイエンス総合研究事業）
平成21年度小括研究報告書

－非臨床有効性評価一般に関する研究－

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研究要旨

本研究では、国際的動向を踏まえた医薬品の新たな**品質・有効性評価に関する非臨床研究として１**．Q8（R1）製剤開発 補遺 ステップ４合意に関する**研究と２**．first-in-man試験に用いる治験薬の製造・品質管理に関する研究（アカデミアのトランスレーショナルリサーチにおける被験物質の品質管理基質に関する研究）を遂行した。

１．Q8（R1）製剤開発 補遺 ステップ４合意に関する研究：本年度は、特に変更管理の柔軟性を獲得するために注目を浴びているデザインスペースの設定のためのアプローチをQ8（R）およびQ&Aから抽出し、解析した。Q8（R）は、製品開発戦略が製薬企業や開発すべき製品によって異なることを前提としており、構築すべきデザインスペースの種類は、どの様な変更管理や操作の柔軟性を必要とするかで異なる。

２．first-in-man試験に用いる治験薬の製造・品質管理に関する研究：本年度は米国及び欧州におけるfirst-in-manなどの特に早期臨床試験における被験物質の品質管理に関する基準ついての調査を行い、国内の状況と比較した。日米欧３極での臨床試験における被験物質の品質管理に関する基準は、基本的な考え方は同じであるが、法律上・制度上の扱いが大きく異なっていた。共通している基本的な考え方は、被験物質の品質管理の目的が、被験者を保護し、臨床試験の信頼性と市販後の製品の有効性・安全性を保証することにある一方、開発段階であることから基準の弾力的な運用を認めていることである。

キーワード：製剤開発研究、デザインスペース、製造プロセス、品質管理、GMP

A．研究目的

１．製剤開発補遺Q8（R）は、親ガイドライン（Q8）では新規概念の詳細な説明までは至っていなかったことから、デザインスペース設定の手順等を具体的に示すことを目的として作成された。Q8（R）につき平成20年度研究ではQ8ステップ４の設立の背景およびわが国の品質保証システムに与える影響を考察した。本年度、Q8（R）に示された新しい概念の実現方法を考察すると共に、Q8（R）の翻訳案の作成を行った（厚生労働省に提出済み）が、本報告書では、同時期に活動したQ8-11ガイドラインの実施のための作業グループ（Q-EWG）が作成したQ&Aのうちデザインスペースに関するQ&A（以下Q&A）も含めて、現在ICHで議論されているデザインスペースの設定及び運用を紹介し、考察した。

２．トランスレーショナルリサーチ（TR）においては、ヒトに初めて使用される（first-in-man）被験

物質を用いた試験も実施されるが、特に治験以外で臨床試験を実施する場合には、その品質管理方法には、明確な基準等がないのが現状である。日本以外のICH地域である米国と欧州においては、治験と治験以外の臨床試験の区別はなく、アカデミアでの学術的な臨床試験と承認申請を目的とした臨床試験は同じ規制の下に行われている。米国、欧州ともそれぞれ臨床試験の被検薬製造のガイドライン（GMP）があり、日本も治験薬を対象とした治験薬GMPが作成されている。一方ICHの品質（Quality）ガイドラインは承認申請を目的とした医薬品を対象としており、臨床試験段階の品質管理に関しては、原薬のGMPガイドライン（Q7A）「19 臨床試験に使用する原薬」以外は、特に定められたものがない状況である。本年度は米国及び欧州におけるfirst-in-manなどの特に早期臨床試験における被験物質の品質管理ついての調査を行い、国内の状況と比較し、違いを明らかにすることを目的に研究を遂行した。

B．研究方法

1．Q8（R1）製剤開発 補遺 ステップ４合意に関する研究：下記のICHガイドラインおよびQ&Aを対象とした。

- PHARMACEUTICAL DEVELOPMENT (Q8R1) Current Step 4 version: http://www.ich.org/LOB/media/MEDIA4986.pdf
- Q8/Q9/Q10 - Questions & Answers: http://www.ich.org/LOB/media/MEDIA5783.pdf

2．first-in-man試験に用いる治験薬の製造・品質管理に関する研究：調査は全て公表されている資料（規制当局HP、ガイドライン、論文等）を用いた。

C．研究結果

1．Q8（R1）製剤開発 補遺 ステップ４合意に関する研究：Q8（R）は、デザインスペースを設定する際に留意すべき６個の項目を論じている。即ち、① 変数の選択、② 承認申請資料におけるデザインスペースの説明 ③ 単位操作のデザインスペース、④ デザインスペースとスケール及び操作との関係 ⑤ デザインスペースと立証された許容範囲、⑥ デザインスペースと不適合境界。各項目の解説は以下のとおりである。

①変数の選択

CQA（重要品質特性）はデザインスペースを適切に設定することにより保証できること、その設定に際しては、工程パラメータだけでなく物質特性も変数として用いることができることが明記されるとともに、デザインスペースの設定に際しては、検討した全パラメータを変数とする必要はないとなっている。Q&Aではさらにデザインスペースを構築するためには、全パラメータの多変量的な相互作用を検討する必要はなく、リスク評価と申請者が希望する工程操作のフレキシビリティーに応じて、適宜パラメータと物質特性を選択すればよいとしている。

②承認申請資料におけるデザインスペースの説明

Q8（R）ではデザインスペース設定のアプローチは一つには限定されていない。また、同一のデータセットを用いても複数のデザインスペースの設定があり得ることをQ8（R）は指摘している。

③単位操作のデザインスペース

申請者がどのような工程操作上のフレキシビリティーを求めるかによってデザインスペースの設定の戦略が変わる。

④デザインスペースとスケール及び操作との関係

どの製造スケールのデータでデザインスペースを設定するかは、実際にデザインスペースを設定する場合には極めて重要な問題であるが、本ガイドラインでは、申請者の戦略によって異なるとしている。小スケールの開発データに基づいて設定されたデザインスペースを大規模な製造に適応するためにはリスクに関する考察が必要になることを指摘している。また、複数のスケールに適用可能なデザインスペースを設定するためには、スケールとは独立したパラメータを用いてデザインスペースを設定することを推奨している。Q&AではEFPIA Mock P2（欧州製薬協が発表した製剤開発研究の実物大模型）におけるスケール非依存性のパラメータを用いたデザインスペースをスケールに依存しない例として紹介している。即ち、流動層乾燥工程にPAT（Process Analytical