

July, 2015, Tokyo

皮膚基礎研究クラスターフォーラム

動物実験代替法の国内外の最新動向



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JaCVAM, NIHS

内容

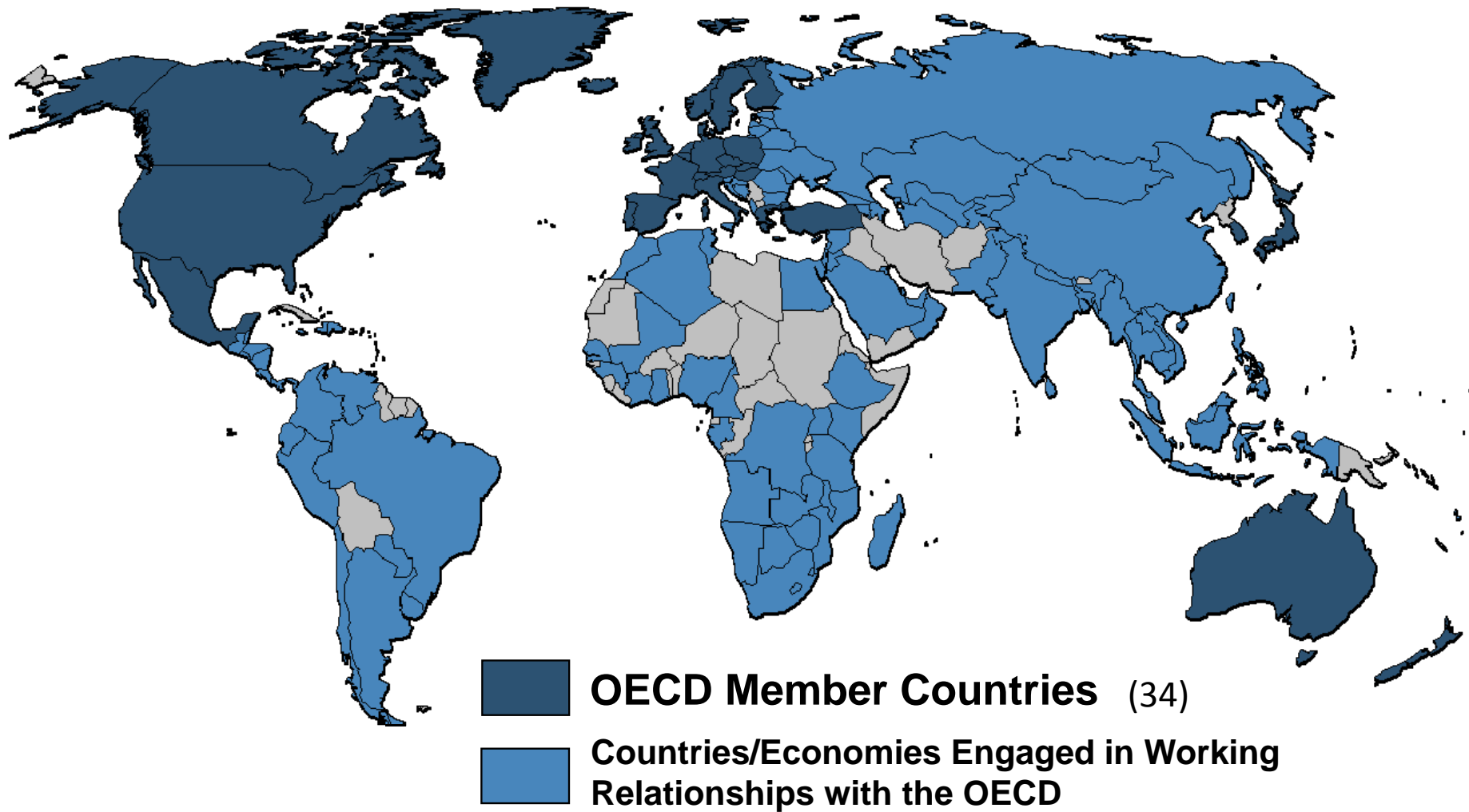
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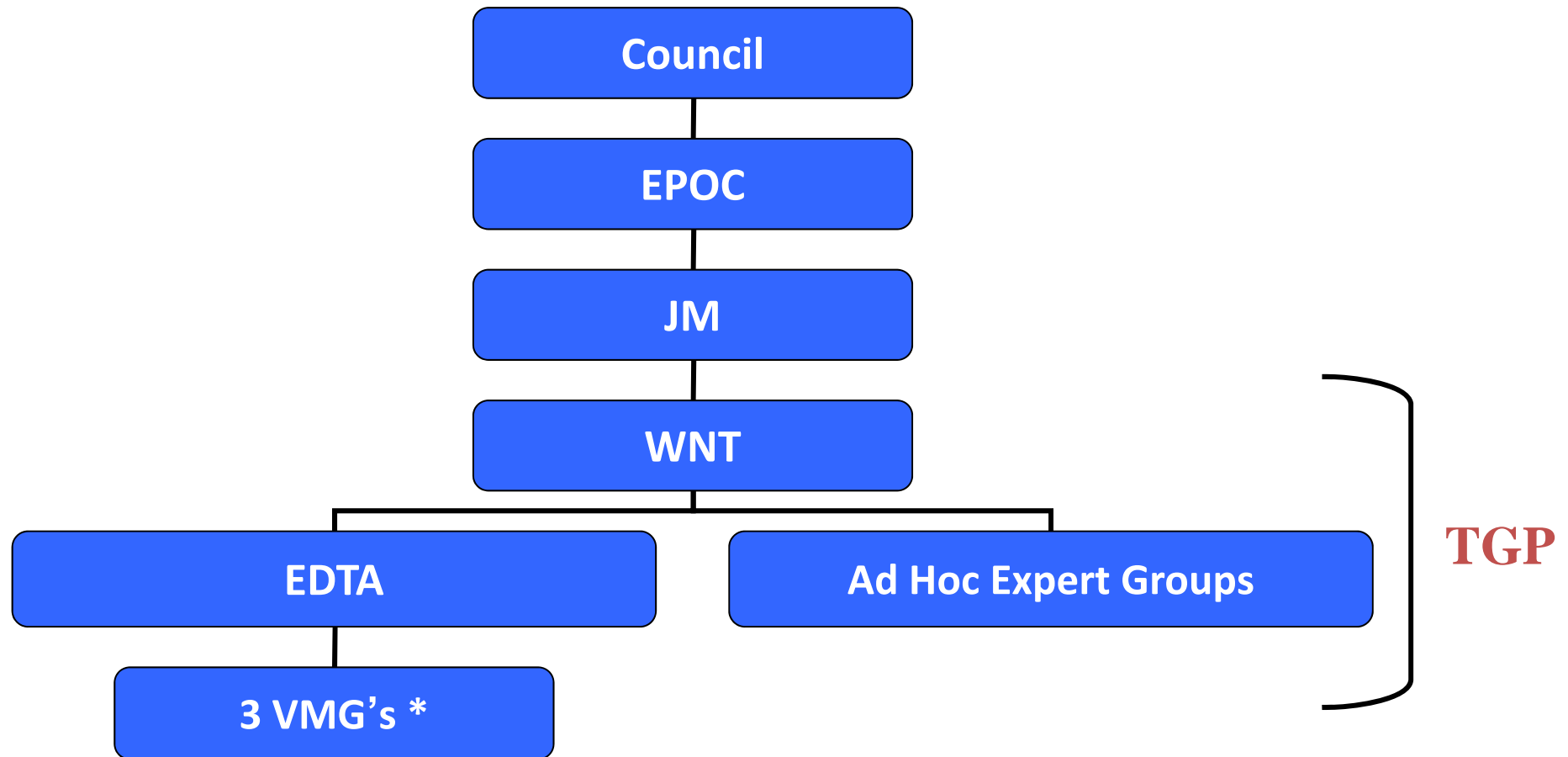
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A global outreach

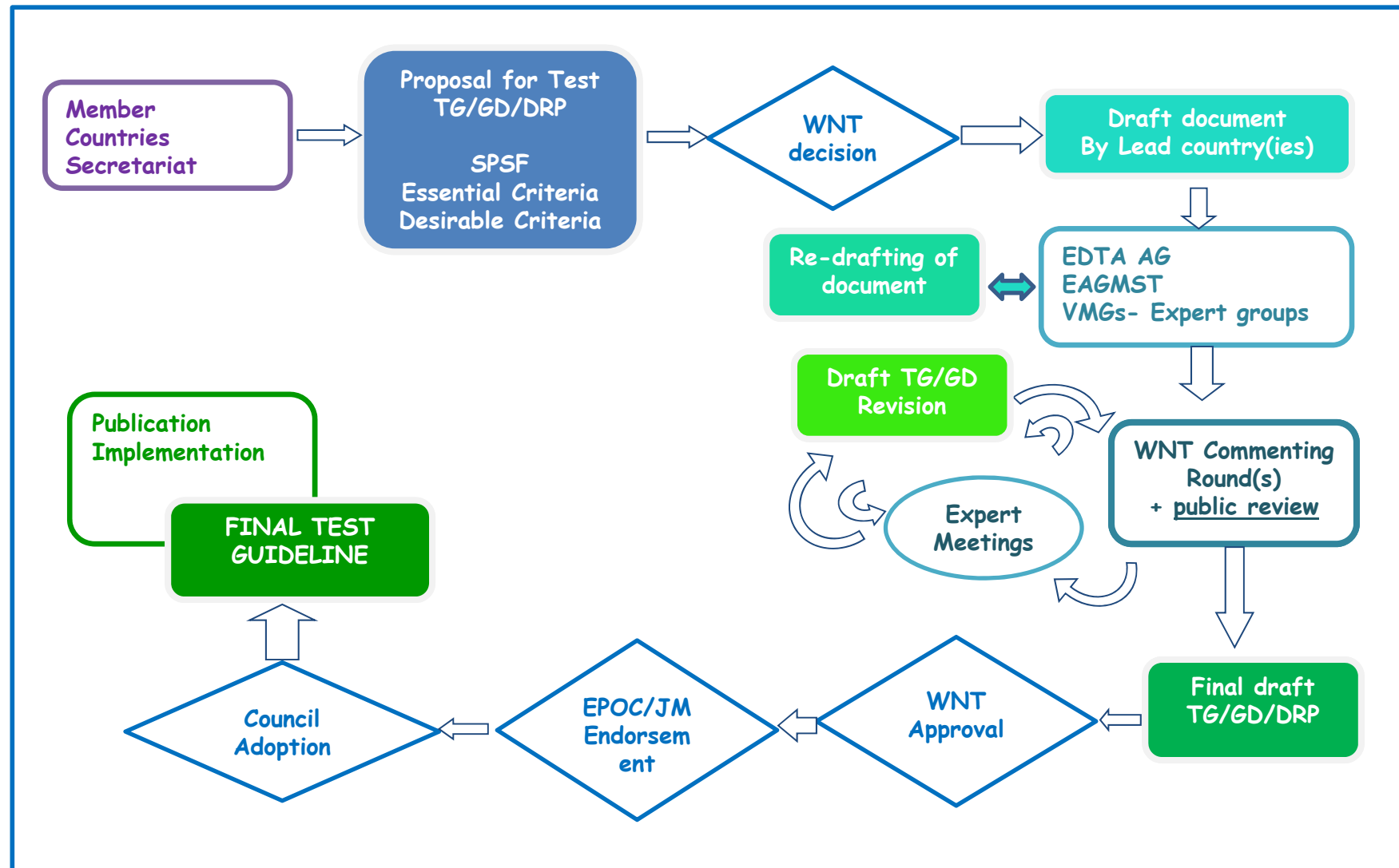


Decision Layers at the OECD



***Mammalian, Ecotoxicity and Non-Animal**

Process for the development of documents



Functioning of the Programme

- Work plan includes projects lead by member countries, updated and declassified annually.
- SPSF template for project proposal, available to NCs, concerns projects on:

New Test Guideline

Guidance document

Revised Test Guideline

Detailed Review Paper

Deletion of an existing Test Guideline

Other, specify:

- Regulatory need
 - Animal welfare
 - Cost effectiveness
- } main motivations
for projects

Important documents elaborated for the Programme

- Series Testing and Assessment (>200 publications):
 - Guidance Document **1** for the Development of OECD **Guidelines** for Testing of Chemicals (last updated 2009)
 - Guidance Document **34** on the **Validation and International Acceptance** of New or Updated Test Methods for Hazard Assessment (2005)
 - Guidance Document **150** on Standardised Test Guidelines for Evaluating Chemicals for **Endocrine Disruption** (2012)
 - +Numerous Guidance Documents and testing strategies for several hazard endpoints

2014年に成立した試験法

Method	Lead Country	International acceptance
<i>In vivo</i> Mammalian Alkaline Comet Assay	Japan	TG489(2014)
<i>In vitro</i> Micronucleus Test	Canada	Updated TG487 (2014)
Mammalian Bone Marrow Chromosomal Aberration Test	Canada	Updated TG475 (2014)
Mammalian Erythrocyte Micronucleus Test	Canada	Updated TG474 (2014)
<i>In vitro</i> Mammalian Chromosome Aberration Test	Canada	Updated TG473 (2014)
<i>In vitro</i> Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method : epiCS	German	Updated TG431 (2014)
<i>In vitro</i> Skin Sensitisation: ARE-Nrf2 Luciferase Test Method	EU	TG442D(2014)
<i>In Chemico</i> Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)	EU	TG442C(2014)

2015年に成立する予定の試験法

Method

TG 490: Short-Time Exposure for the detection of chemicals causing Serious Eye Damage and chemicals Not Requiring Classification for Serious Eye Damage or Eye Irritation

TG 491: Reconstructed Human Corneal Epidermis for the Detection of Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage

TG 492: *In vitro* Thymidine Kinase mutation Test

TG 493: Estrogen Receptor Binding Assay

GD:*In vitro* Carcinogenicity: Cell Transformation Assay Bhas 42 Assay

GD:*In vitro* Carcinogenicity: Syrian Hamster Embryo (SHE) Cell Transformation Assay

2015年に成立する予定の試験法

Method
Updated TG 404: Acute Dermal Irritation/Corrosion
Updated TG 421: Reproduction/Development Toxicity Screening Test
Updated TG 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
Updated TG 430: <i>In vitro</i> Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)
Updated TG 431: <i>In vitro</i> skin corrosion (Human Skin Model Test)
Updated TG 435: <i>In vitro</i> Membrane Barrier Test Method for Skin Corrosion
Updated TG 439: <i>In vitro</i> Skin Irritation - Reconstructed Human Epidermis Test Method
Updated TG 455: The Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals
Updated TG 476: <i>In vitro</i> Mammalian Cell Gene Mutation Test
Updated TG 478: Rodent Dominant Lethal Test
Updated TG 483: Mammalian Spermatogonial Chromosome Aberration Test

2015年に成立する予定の日本提案のOECD TG案

- Cell Transformation Assay Bhas 42 Assay
- Eye Irritation Test: Short Time Exposure (STE) Assay
- Endocrine Disruptor Screening: Stable Transfected Transcriptional Activation (STTA) Antagonist Assay

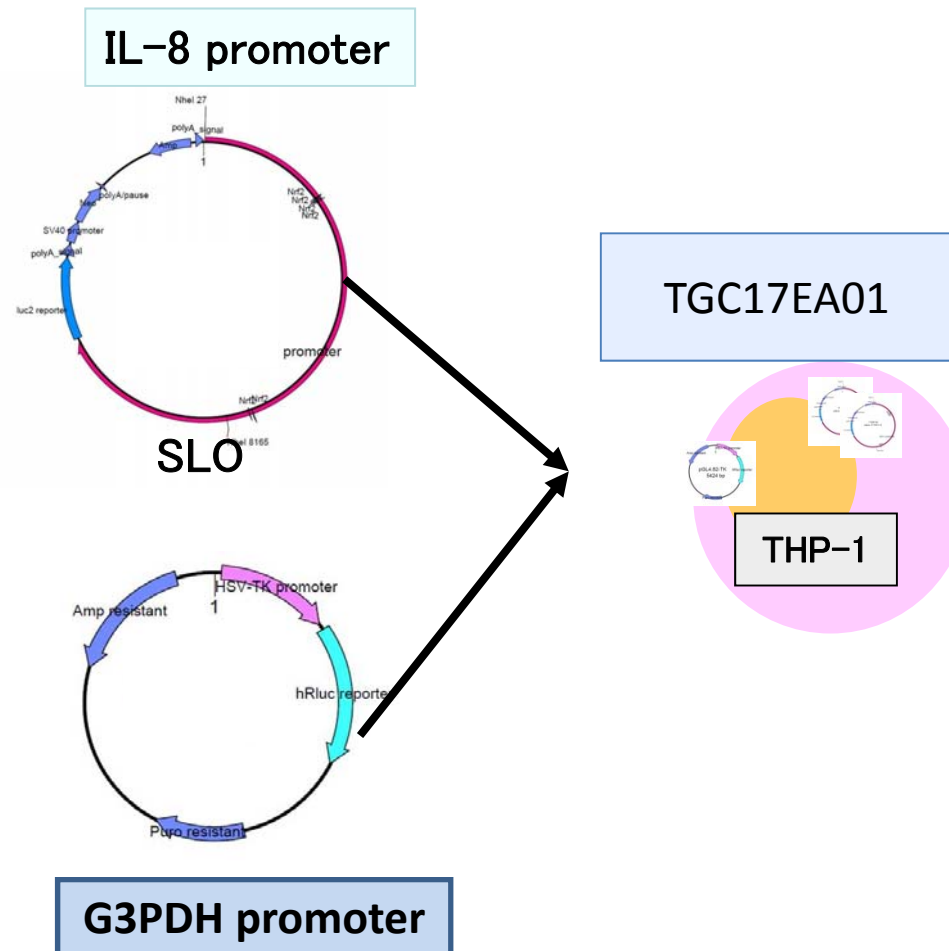
2015年に作業計画に載った試験法

United States	GD IATA on Eye irritation/corrosion	Approved
France	RhCE test method using SkinEthic for Eye	Approved
Italy	In vitro Macromolecular Test Method for identifying Chemicals inducing serious eye damage	Approved
Netherlands	Extension of the ICE (TG 438)	Approved
Netherlands	Revision of GD 160 on BCOP and ICE: collection of tissue for histological evaluation	Approved
France	MUSST for in vitro skin sensitisation	Approved
Japan	IL-8 Luc assay for in vitro skin sensitisation	Conditionally approved
United States	Pig-A genetox TG	Approved
European Commission	New GD on genotoxicity testing of nanomaterials	Approved
United Kingdom	IATA document on non-genotoxic carcinogens	Approved
Denmark	Feasibility study for minor enhancements of TG414	Approved
United Kingdom	Development of a reference/characterising chemical set for testing <i>in vitro</i> metabolism systems in EAS assays	Approved
Korea	Androgen Receptor Transactivation Assay	Approved
United Kingdom/Netherlands	Cross-species concordance and difference for in vitro extrapolation	Approved
United States	GD on AOPs for E, A, T pathways	Approved
United Kingdom	Case studies on Integrated Risk Assessment for illustrating cross-species linkages in the Conceptual Framework	Approved
Sweden	DRP on retinoic acid	Approved

OECD作業計画にある日本から提案されたテストガイドライン案

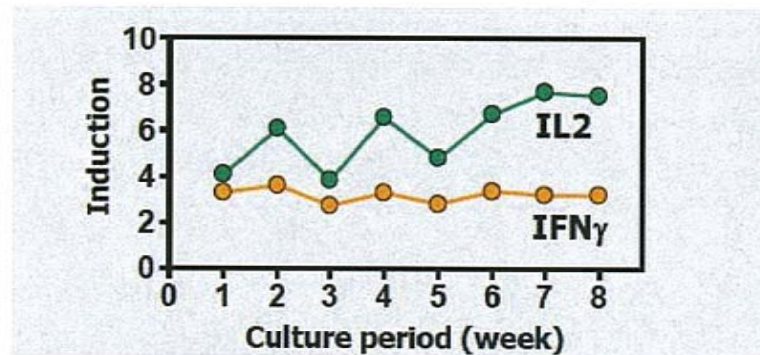
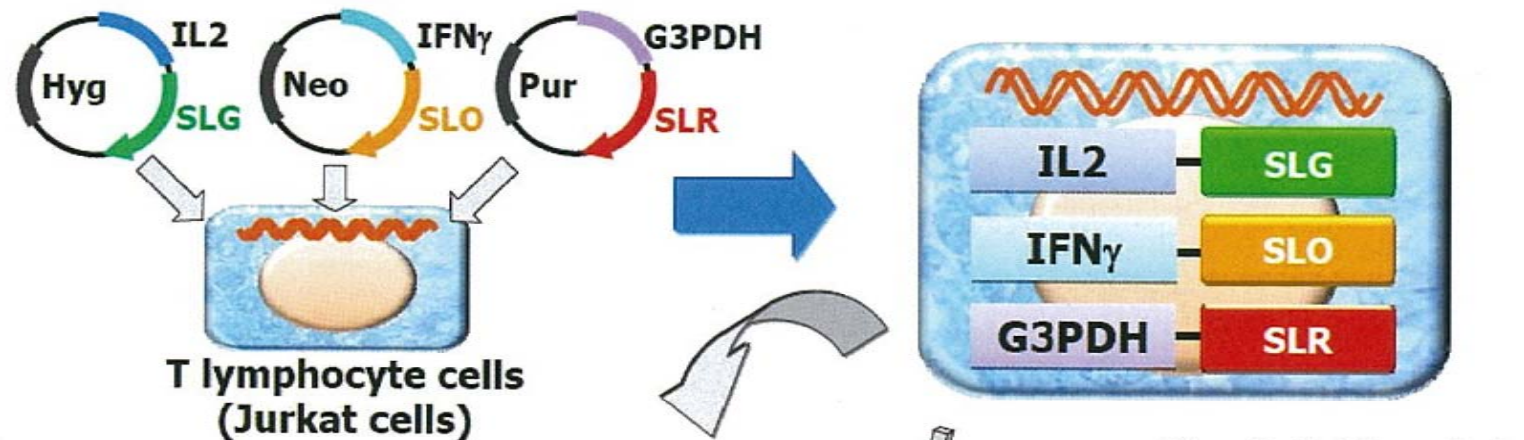
- Skin sensitization assay h-CLAT assay
- IL-8 reporter gene assay for skin sensitization testing (今年度に計画として承認された)
- Androgen disruptor screening Stable transfected transcriptional activation (STTA) assay (AR-Ecoscreen)

IL-8 Luc assay



Example of toxicity test for immunology using a multireporter assay

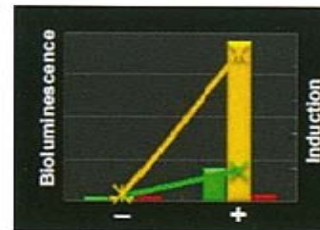
Generation of T cells stably express SLG, SLO and SLR enzymes under two marker gene promoters and internal control gene promoter.



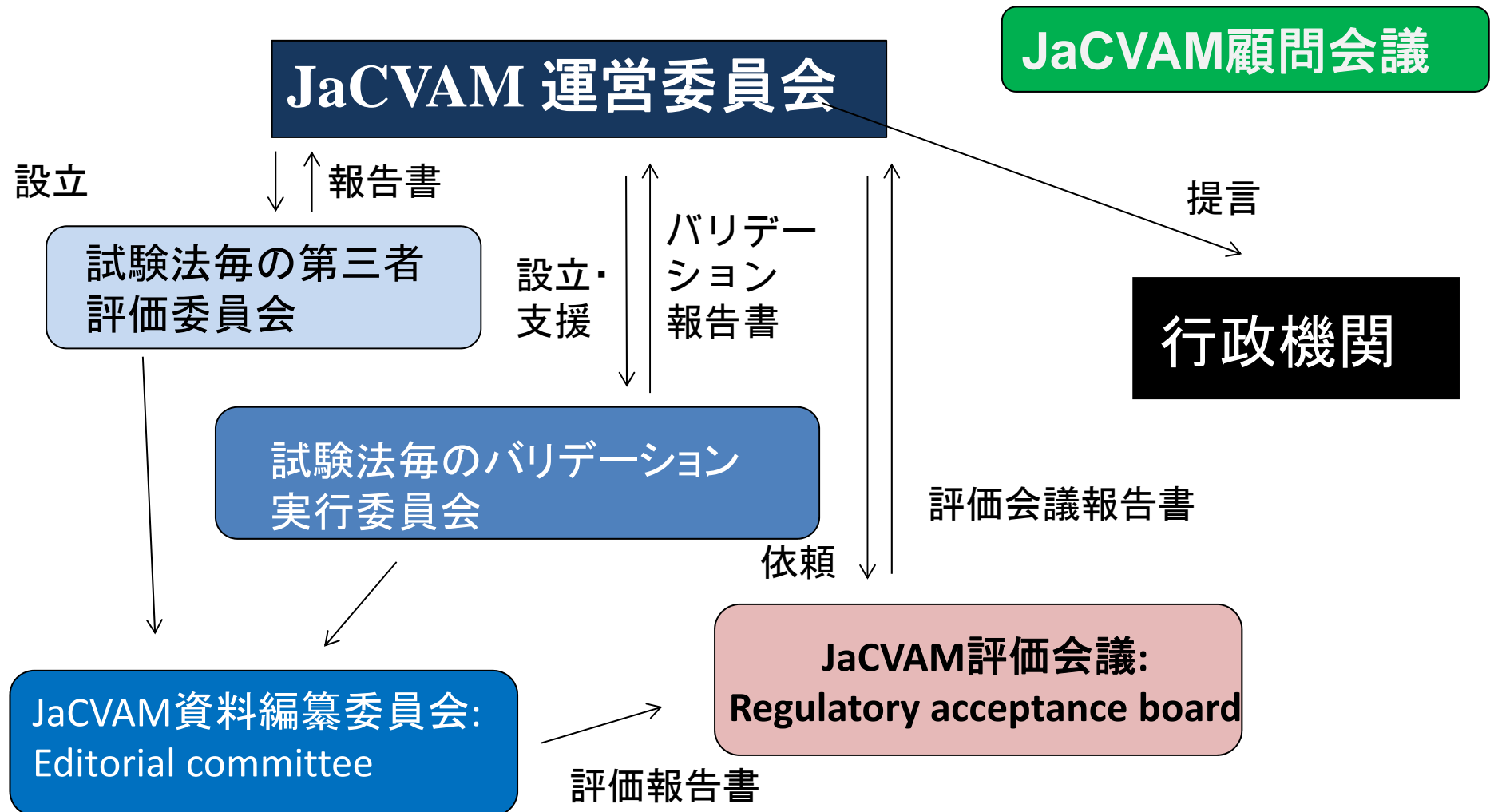
Cells display stable luciferase expressions and respond to chemicals during prolonged culture.

Chemical risk analysis
in a 96well plate
format HTP assay

Reaction with Tripluc[®] assay Reagents



JaCVAM 組織 2014



新規試験法提案書

平成 25 年 1 月 20 日

No. 2012-04

皮膚感作性試験代替法 Local Lymph Node Assay (LLNA): BrdU-ELISA の判定基準の変更に関する提案

平成 24 年 10 月 1 日に東京、国立医薬品食品衛生研究所にて開催された新規試験法評価会議（通称：JaCVAM 評価会議）において以下の提案がなされた。

提案内容：皮膚外用剤として用いる医薬品、医療機器、化粧品、皮膚適用の医薬部外品、農薬等に含まれる物質又はそれらの製品の皮膚感作性を予測する皮膚感作性試験代替法 Local Lymph Node Assay (LLNA): BrdU-ELISA は、RI を使用せずとも従来試験法と同等の結果が得られることから、行政上利用することは可能である。

この提案書は、米国 Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) LLNA: BrdU-ELISA Evaluation Report (2010)、LLNA: BrdU-ELISA の JaCVAM 評価報告 (2011) および OECD Test Guideline (TG) 442B をもとに、皮膚感作性試験代替法評価委員会によりまとめられた文書を用いて JaCVAM 評価会議が評価および検討した結果、その有用性が確認されたことから作成された。

以上の理由により、行政当局の安全性評価方法として「皮膚感作性試験代替法 LLNA: BrdU-ELISA の判定基準の変更」に関する提案をするものである。

吉田武美 

JaCVAM 評価会議 議長

西川秋佳 

JaCVAM 運営委員会 委員長

JaCVAM statement on the Local Lymph Node Assay (LLNA): BrdU-ELISA for skin sensitization assay

At the meeting concerning the above method, held on 1 October 2012 at the National Institute of Health Sciences (NIHS), Tokyo, Japan, the members of the Japanese Center for the Validation of Alternative Methods (JaCVAM) Regulatory Acceptance Board unanimously endorsed the following statement:

The LLNA: BrdU-ELISA can be used to identify substances as potential skin sensitizers or nonsensitizers as well as LLNA for regulatory use, without Radio-isotope.

Following the review of the results of the ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods, USA) Evaluation Report, JaCVAM peer review panel reports, and OECD (Organisation for Economic Co-operation and Development) Test Guideline revised No. 442B, it is concluded that the LLNA: BrdU-ELISA for skin sensitization assay is clearly beneficial.

The JaCVAM Regulatory Acceptance Board has been regularly kept informed of the progress of the study, and this endorsement is based on an assessment of various documents, including, in particular, the evaluation report prepared by the JaCVAM ad hoc peer review panel for skin sensitization assay.



Takemi Yoshida
Chairperson
JaCVAM Regulatory Acceptance Board



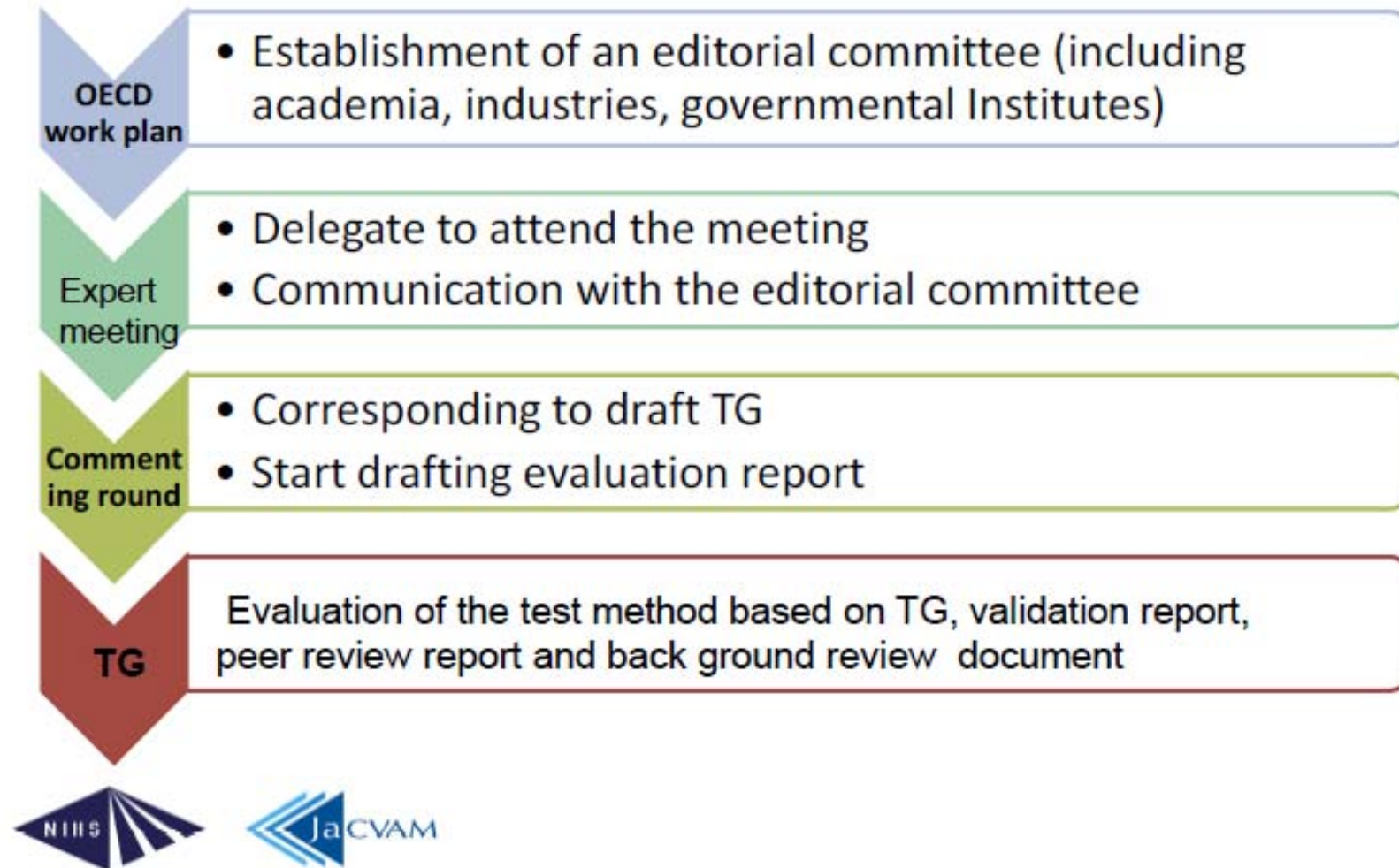
Akiyoshi Nishikawa
Chairperson
JaCVAM Steering Committee

20 January, 2013

2014年度にJaCVAM評価会議が認証した試験法 (赤字)

No.	Test Method
1	<i>In vitro</i> Skin Corrosion Testing: Vitrolife-Skin, EpiDerm
2	The Bovine Corneal Opacity and Permeability (BCOP) Test Method
3	The Isolated Chicken Eye (ICE) Test Method
4	Fluorescein leakage (FL) Test Methods for Identifying Ocular Corrosives and Severe Irritants
5	Skin Sensitization Assay, LLNA : DA
6	The Revised Acute Eye Irritation / Corrosion
7	Skin Sensitization Assay, LLNA : BrdU-ELISA
8	Skin Sensitization Assay, rLLNA
9	<i>In Chemico</i> Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)
10	<i>In vitro</i> Skin irritation Testing: Episkin, EpiDerm, SkinEthcs, LabCyte EPI-MODEL
11	<i>In vitro</i> Skin Absorption Assay
12	Utilization of Cytotoxicity Test for Acute Oral Toxicity Testing
13	BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists

OECD TG work plan and activity of JaCVAM editorial committee



活動中のJaCVAM資料編纂委員会

委員会	取り組んでいる試験法		
眼刺激性試験	サイトセンサーマイ クロフィジオメーター	STE法(短時間 曝露法)	角膜モデル EpiOcular
皮膚感作性試験	ARE-Nrf2 Luciferase Test Method	h-CLAT	
形質転換試験	SHEアッセイ	Bhasアッセイ	
内分泌かく乱	ER-STTA	AR-EcoScreen	
光毒性試験	ROSアッセイ		
急性毒性試験	3T3 NRU		
薬物代謝試験	TG案		

黒字: 成立

赤字: 評価会議

緑字: 資料編纂中

許認可に関与した代替法に関する通知

医薬部外品の製造販売承認申請等に添付する資料については、平成18年7月19日付医薬食品局審査管理課事務連絡「医薬部外品の製造販売承認申請及び化粧品基準改正要請に添付する資料に関する質疑応答集(Q&A)について」において、動物実験代替試験法等の利用に関してOECD等により採用された代替試験法あるいは適切なバリデーションでそれらと同等と評価された方法に従った試験成績であれば、当該品目の申請資料として差し支えない旨を示しているところです。

化粧品・医薬部外品ガイダンス 代替法普及のため厚生労働省が発出したガイダンス

赤字：本年度、緑字：まもなく

No.	試験法
1	皮膚感作性試験代替法としてのLLNAを化粧品・医薬部外品の安全性評価に活用するためのガイダンス
2	光毒性試験代替法としての <i>in vitro</i> 3T3 NRU光毒性試験を化粧品・医薬部外品の安全性評価に活用するためのガイダンス
3	皮膚感作性試験代替法としてのLLNA:DA を化粧品・医薬部外品の安全性評価に活用するためのガイダンス
4	皮膚感作性試験代替法としてのLLNA:BrdU-ELISA を化粧品・医薬部外品の安全性評価に活用するためのガイダンス
5	眼刺激性試験代替法としての牛摘出角膜の混濁および透過性試験法(BCOP)を化粧品・医薬部外品の安全性評価に資するためのガイダンス
6	眼刺激性試験を化粧品・医薬部外品の安全性評価に活用するための留意事項について
7	眼刺激性試験代替法としての鶏摘出眼球試験法(ICE)を化粧品・医薬部外品の安全性評価に資するためのガイダンス

個別試験の動向

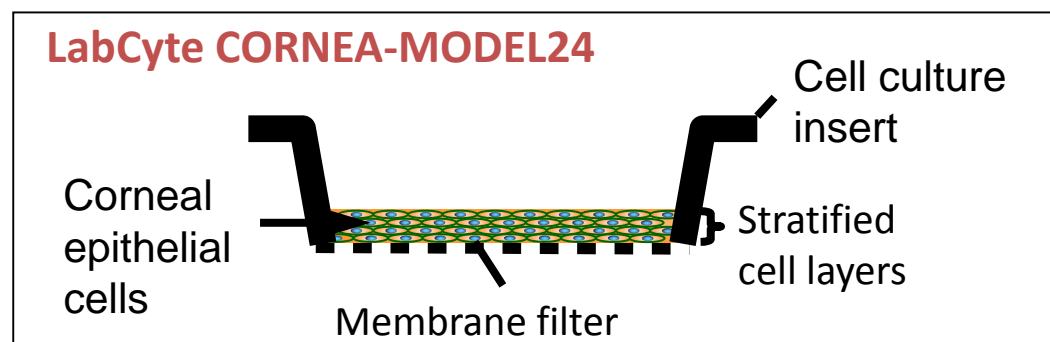
眼刺激性試験代替法の国際動向

Method	Current status	International acceptance
Bovine Corneal Opacity and Permeability (BCOP) Test Method	Completed	Updated TG 437 (2013)
Isolated Chicken Eye (ICE) Test Method	Completed	Updated TG 438 (2013)
Use of Histopathology as an Additional Endpoint in Ocular Safety Testing	Completed	GD 160 (2011)
Revised TG 405	Completed	Updated TG 405 (2012)
Fluorescein Leakage (FL) Test Method	Completed	TG 460 (2012)
Cytotoxicity Test: Short Time Exposure (STE) Test		TG490(2015)
Human Reconstructed Tissue Models for Eye Irritation EpiOcular EIT™		TG491(2015)

眼刺激性試験代替法に関する日本の動向

Method	Current status	Lead Organization
Cytotoxicity Test: SIRC CVS	JaCVAM-sponsored validation study ongoing	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT
Vitrigel-EIT method	MAFF-sponsored validation study ongoing	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, and Health Canada VMT
Human Reconstructed Tissue Models for Eye Irritation LabCyte CORNEA-MODEL24	Pre-validation study	

培養角膜モデルLabCyte CORNEA-MODEL24を用いた眼刺激性試験代替法



Liquid test chemicals

Liquid test chemicals are applied to the RhCE surface for **1 minute**. After the application, chemicals are removed and tissues are washed, and then the RhCE are post-cultivated for **24 hours**.

Solid test chemicals

Solid test chemicals are applied to the RhCE surface for **24 hours**. After the application, chemicals are removed and tissues are washed. **Post-cultivation is not set.**

皮膚感作性試験代替法の国際動向1

Method	Current status	International acceptance
Murine Local lymph Node Assay (LLNA)	Completed	Updated TG 429 (2010), ISO (2010)
Reduced LLNA (rLLNA)	Completed	Updated TG 429 (2010)
LLNA:DA	Completed	TG442A (2010)
LLNA:BrdU-ELISA	Completed	TG 442B (2010)
<i>In Chemico</i> Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)	Completed	TG442C(2014)
<i>In vitro</i> Skin Sensitisation: ARE-Nrf2 Luciferase Test Method	Completed	TG442D(2014)

皮膚感作性試験代替法の国際動向2

Method	Current status	Lead Organization	International acceptance
Nonradioactive LLNA protocol (LLNA: BrdU-Flow Cytometry)	<ul style="list-style-type: none"> - ICCVAM international peer review, 2009 ➤ KoCVAM validation study ongoing 	Korea	
<i>In vitro</i> Skin Sensitization Assays : h-CLAT	Multi-laboratory validation ends in 2013. h-CLAT peer review is over.	EURL ECVAM and JaCVAM	OECD Work Plan
<i>In vitro</i> Skin Sensitization Assay: IL-8 Luc Assay	METI-sponsored validation study ongoing	JaCVAM	OECD Work Plan
MUSST	Cosmetic Europe-sponsored validation study ongoing	France	OECD Work Plan

皮膚感作性試験の有害性転帰事象(AOP)

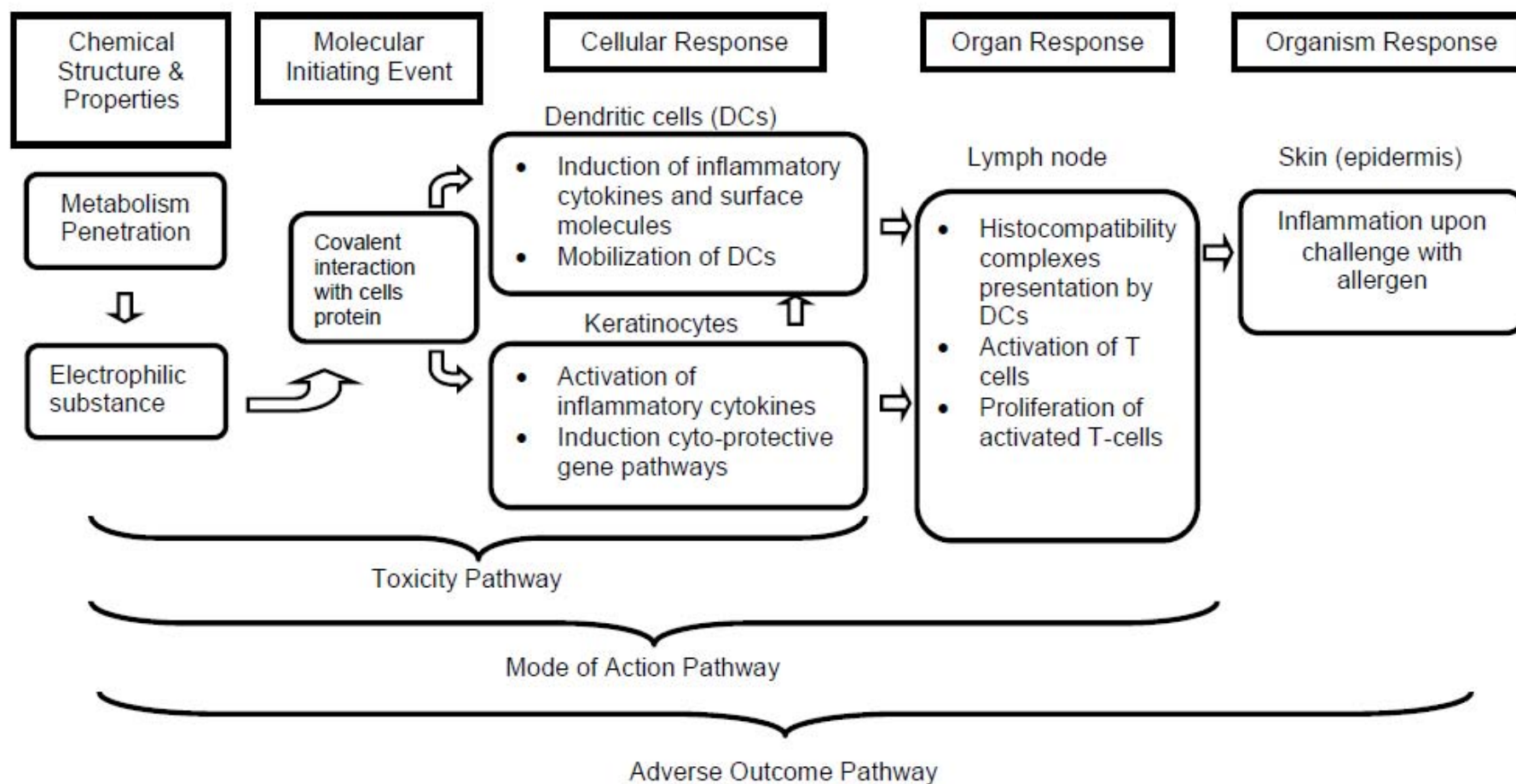


Figure 3. Flow diagram of the pathways associated with skin sensitisation.

**GUIDANCE DOCUMENT ON THE REPORTING OF STRUCTURED APPROACHES TO DATA
INTEGRATION AND INDIVIDUAL INFORMATION SOURCES USED WITHIN IATA FOR SKIN
SENSITISATION**

Table1: IATA elements and information sources

IATA Elements	Information sources informing the IATA elements
Exposure consideration	<ul style="list-style-type: none"> Dose per unit area of skin ($\mu\text{g}/\text{cm}^2$) Frequency of exposure Formulation effects <i>In vitro</i> to <i>in vivo</i> extrapolation
Chemical descriptors	<p>Chemical structure</p> <p>Physico-chemical properties</p> <ul style="list-style-type: none"> Molecular Weight pKa Log Kow Evaporation rate/Vapour pressure Water solubility
Dermal bioavailability <ul style="list-style-type: none"> Skin penetration Skin metabolism 	<p>Non-testing methods</p> <ul style="list-style-type: none"> Characterisation of skin absorption(e.g. physiologically based-pharmacokinetic (PBPK) models) <p>Testing methods</p> <ul style="list-style-type: none"> TG 428 (Skin absorption: <i>in vitro</i> method) TG 427 (Skin absorption: <i>in vivo</i> method) TG 428 modified to include time course (Pendlington et al. 2008; Davies et al. 2011) <p>Non testing methods:</p> <ul style="list-style-type: none"> <i>In silico</i> (e.g. structure-metabolism rules encoded in the expert system TIMES-SS, Meteor , Nexus (not this is limited to liver metabolism rather than skin metabolism), simulators for skin metabolism and autoxidation within the OECD Toolbox

	Testing methods <ul style="list-style-type: none"> Incubation with S9 or microsomes from skin or surrogate systems (e.g. liver) Peroxidase-peroxide system
AOP key event 1: Covalent interaction with cellular proteins	
	Non-testing methods <ul style="list-style-type: none"> Protein binding/reactivity alerts (e.g. OECD Toolbox, Derek Nexus, Toxtree, TIMES-SS)¹ Testing methods <ul style="list-style-type: none"> TG442C (Direct Peptide Reactivity Assay) Adduct formation or relative reactivity rate, with or without metabolic activation, e.g. Cor1C420 assay (Natsch and Gfeller, 2008) PPRA (Gerberick et al., 2009) Kinetic DPRA (Roberts and Natsch et al., 2009) Glutathione depletion assay (Aptula et al. 2006; Schultz et al., 2005) TG 428 modified to include free/bound measurements (Pickles et al, submitted) Allergen-protein interaction assay (APIA, Dietz, 2013) Amino acid Derivative Reactivity Assay (ADRA, Yamamoto et al., 2015)
AOP key event 2: events in Keratinocytes	
Activation of biochemical pathways Pathways-associated gene expression Pathways-associated protein expression Release of pro-inflammatory mediators	Testing methods <ul style="list-style-type: none"> TG 442D (ARE-Nrf2 Luciferase Test Method-KeratoSensTM) LuSens (Ramirez et al., 2014) AREc32 cell line assay (Natsch and Emter, 2008). Sens-is (Cottrez et al., 2015) HaCaT gene signature (van der Veen et al., 2013) SenCeeTox (McKim et al., 2012) Epidermal Sensitization Assay (EpiSensA, Saito et al., 2013) Proteomic signature in keratinocytes (Thierse et al., 2011) RhE-IL-18 (Gibbs et al. 2013)
AOP key Event 3: Events in Dendritic cell	
	Testing methods

Expression of co-stimulatory and adhesion molecules in dendritic / monocytic cells Pathways-associated protein expression in dendritic / monocytic cells Pathways-associated gene expression in dendritic / monocytic cells	<ul style="list-style-type: none"> h-CLAT (Ashikaga et al., 2010, OECD draft TG available) U-SENSTM (Piroird et al., 2015) modified MUSST (Bauch et al., 2012) PBMDC (Reuter et al., 2011) MUTZ SensiDerm (Thierse et al., 2011) IL-8 Luc assay (Takahashi et al., 2011) GARD (Johansson et al., 2013) VitoSens (Hooyberghs et al., 2008)
AOP key event 4: Events in Lymphocytes	
	Testing methods <ul style="list-style-type: none"> Human T cell priming/proliferation assay (hTCPA) (Moulon et al., 1993; Krasteva et al., 1996; Dietz et al., 2010; Martin et al., 2010, Richter et al., 2013; Popple et al., 2015) (Existing) animal data <ul style="list-style-type: none"> TG 429 (LLNA) TG 442A (LLNA: DA) TG 442B (LLNA: BrdU-ELISA)
AOP Adverse Outcome	
	(Existing) human data <ul style="list-style-type: none"> Human Repeat Insult Patch Test (HRIPT) Clinical data Data from occupational exposure Epidemiological data (Existing) animal data <ul style="list-style-type: none"> TG 406 (Guinea-pig Maximisation Test; Buehler Test)
Others	<ul style="list-style-type: none"> Skin corrosion (e.g. OECD TG 430,431,435, 404) Skin irritation (e.g. OECD TG439, 404) Genotoxicity (e.g. OECD TG 471)

¹ Note Derek Nexus and TIMES-SS are expert systems that aim to provide a prediction of likely skin sensitisation hazard and potency drawing on knowledge captured in SARs and in the case of TIMES-SS additionally underpinned by QSARs. As such their scope is broader than simply providing insight of potential electrophilic reaction centres indicative of protein binding potential which itself defines the MIE.

今後の懸念

- h-CLATをいつTGに承認させることができるか。
- IATAは行政的な受入れに必要となるか。
- 今年から来年に掛けて、どの試験法の申請を行うか。
- ガイダンスとなった形質転換試験をどのように扱っていくか。



Japanese Center for the Validation of Alternative Methods

Office : New Testing Method Assessment, Division of Pharmacology,
National Biological Safety Research Center (NBSRC),
National Institute of Health Sciences (NIHS)

日本語

English



Search



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About JaCVAM



Update on JaCVAM



Academic activities



Submission of Alternative
Methods to JaCVAM



International Cooperation

御静聴ありがとうございました

Policy and Mission: JaCVAM's policy and mission is to promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and establish guidelines for new alternative experimental methods through international collaboration.

the 3Rs in animal experiments—Reduction (of animal use)

Refinement (to lessen pain or distress and to enhance animal well-being)

Replacement (of an animal test with one that uses non-animal systems or phylo-genetically lower species)
(OECD GD34)

News

⊕【NEW】news texts dummy texts news texts dummy texts
news texts dummy texts(2009.7.16)

⊕news texts dummy texts news texts (2009.7.3)

⊕news texts dummy texts news texts dummy texts news
texts dummy texts (2009.7.3)

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