July, 2013, Tokyo

日本での皮膚感作性代替法開発状況について

Update on *in vitro* skin sensitization assay in Japan

Hajime Kojima,

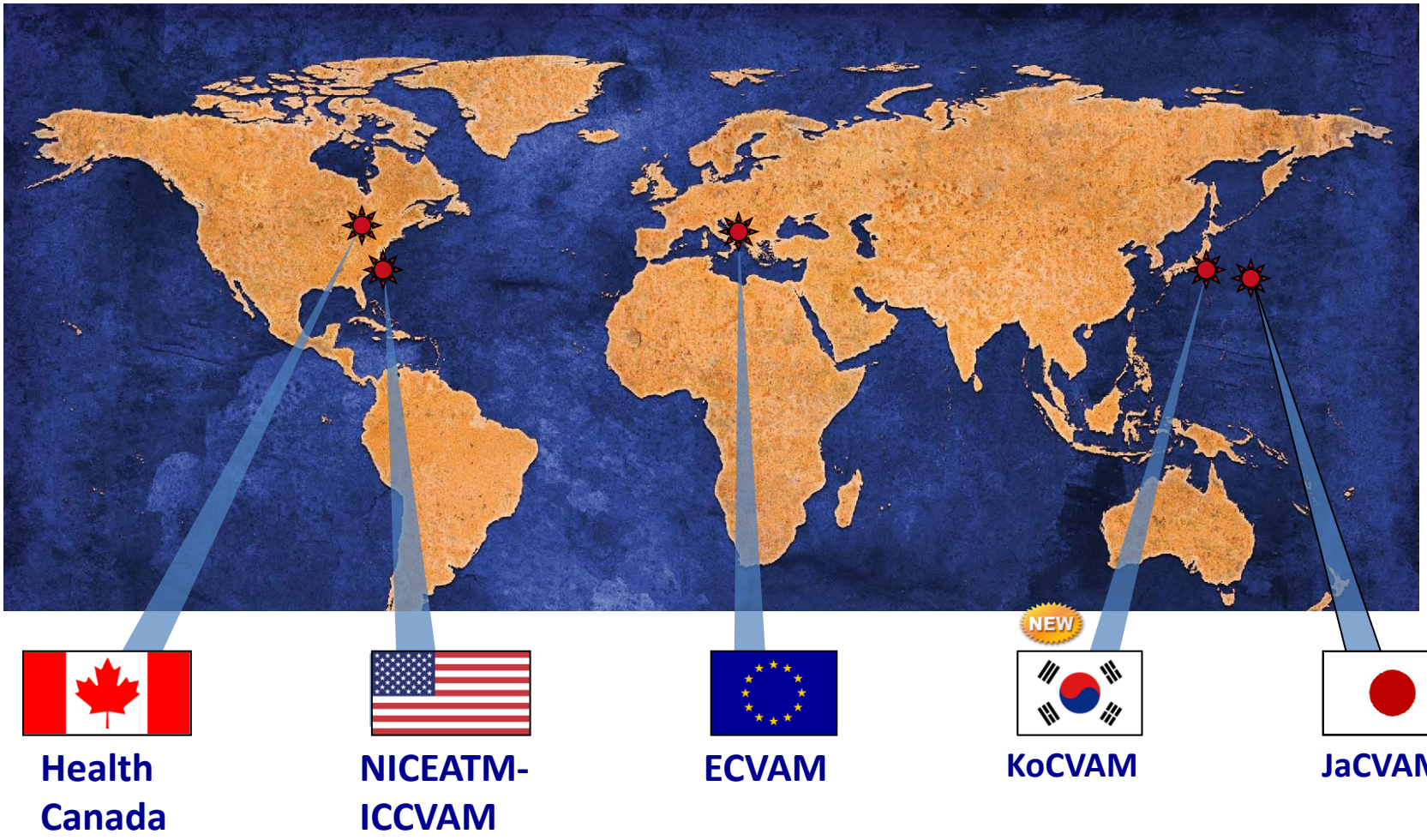
JaCVAM, NIHS

Contents

1. ICATM cooperation
2. IL-8 Luc assay
3. AOP and IATA

ICATM Flam work

ICATM is a **voluntary** international cooperation of national organizations: Canada, the European Union, Japan, South Korea, and the United States.



Acute Contact Dermatitis Test Methods 1

Method	Current status	Lead Organization	International acceptance
Murine Local lymph Node Assay (LLNA)	Completed		OECD TG 429 (2002), ISO (2002)
Updated LLNA	Completed		Updated OECD TG 429 (2010), ISO (2010)
Reduced LLNA (rLLNA)	Completed		Updated OECD TG 429 (2010)
LLNA:DA	Completed		OECD TG442A (2010)
LLNA:BrdU-ELISA	Completed		OECD TG 442B (2010)
Harminized performance standard for the LLNA	Completed		Updated OECD TG 429 (2010)

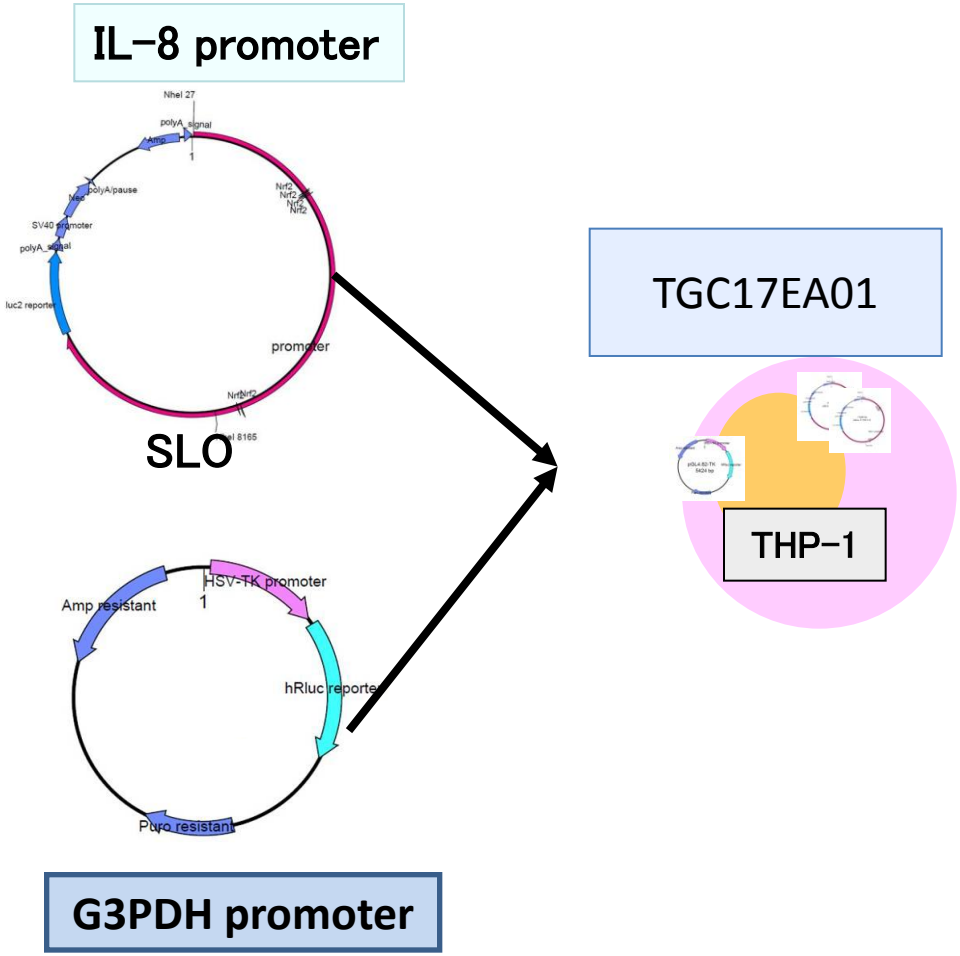
Acute Contact Dermatitis Test Methods 2

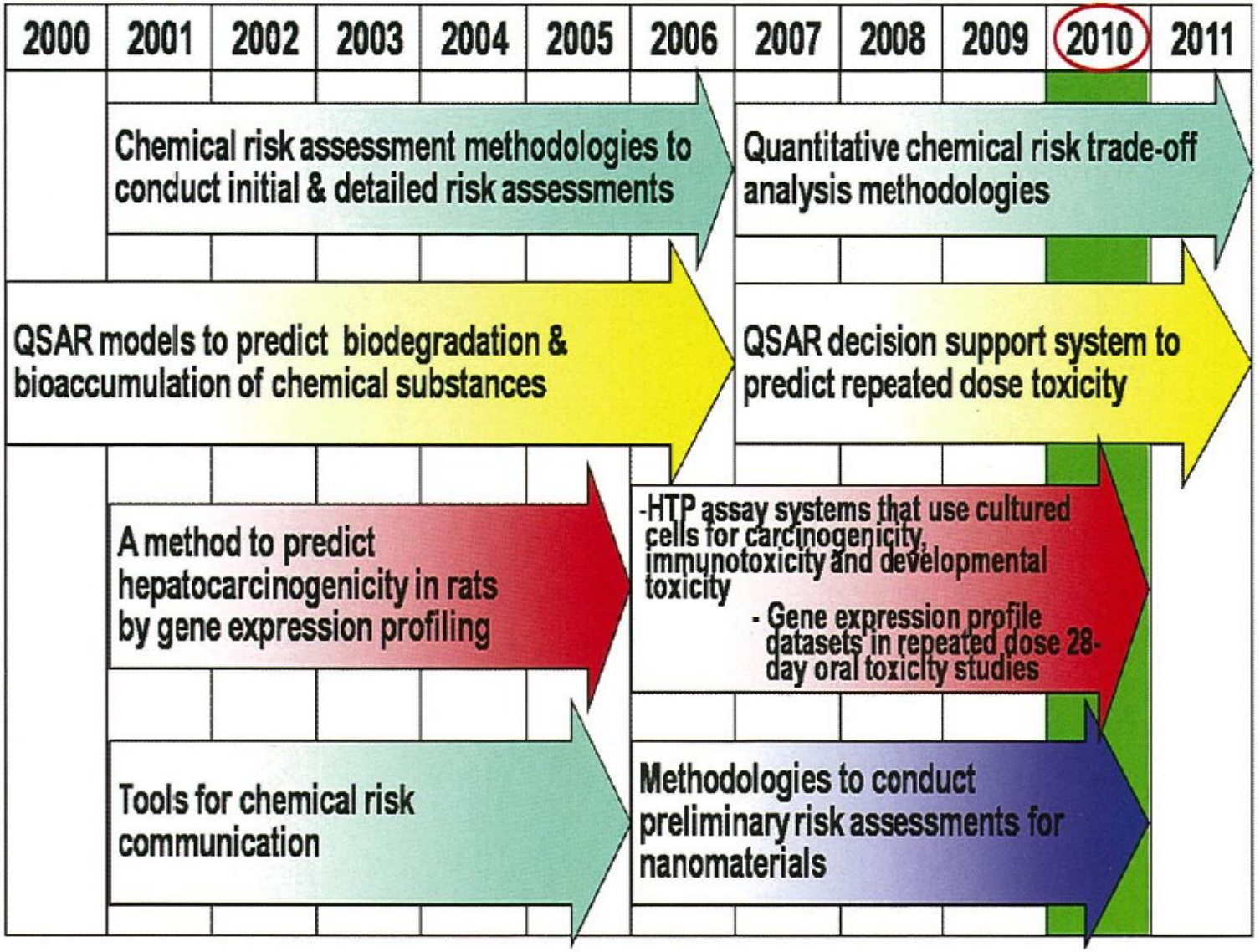
Method	Current status	Lead Organization	International acceptance
Nonradioactive LLNA protocol (LLNA: BrdU-Flow Cytometry)	- ICCVAM international peer review, 2009 - Recommendations pending a review of lead laboratory data and inter-laboratory study. KoCVAM validation study planned	NICEATM-ICCVAM, KoCVAM	
In vitro skin sensitization assays (h-CLAT; DPRA; MUSST)	Multi-laboratory validation ends in August 2012 (h-CLAT and MUSST). DPRA peer review is ongoing.	EURL ECVAM; JaCVAM and NICEATM-ICCVAM VMT liaison members	Proposal for an Adverse Outcome Pathway for skin sensitization has been approved by the OECD. SPSFs approved for the DPRA, Keratinosens and h-CLAT.
In vitro skin sensitization assay KeratinoSens	External Validation Study, peer review ongoing	EURL ECVAM	SPSF approved
In vitro skin sensitization assay IL-8 Luc assay	METI-sponsored validation study ongoing	JaCVAM; EURL ECVAM, NICEATM-ICCVAM, KoCVAM and Health Canada VMT liaisons	

JaCVAM correlated International validation studies for skin sensitization assay

1. LLNA:DA
2. LLNA:BrdU-ELISA
3. h-CLAT assay (with ECVAM)
4. IL-8 reporter gene assay (with ICATM)

IL-8 reporter gene assay (Il-8 Luc assay)




2000
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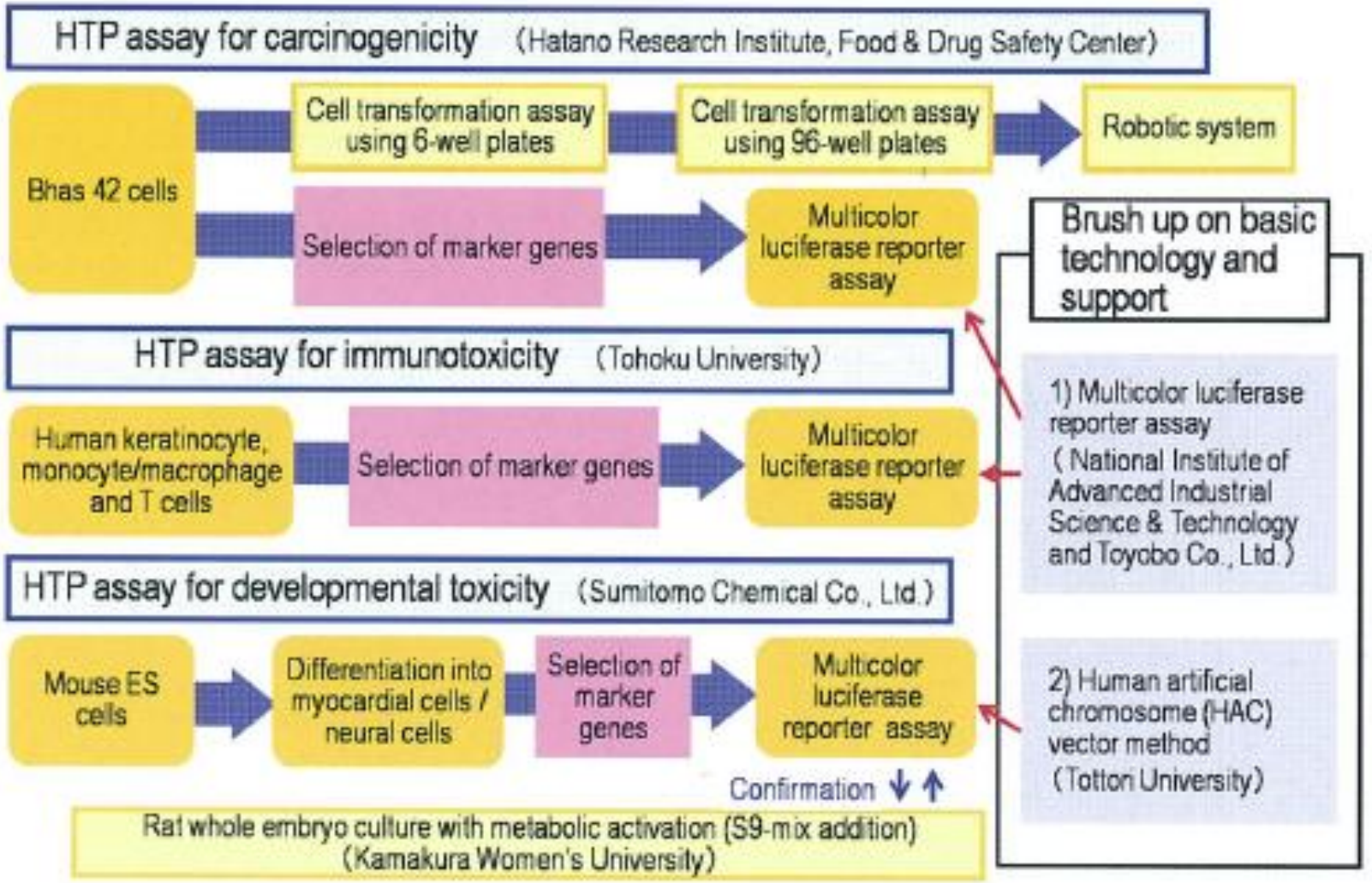
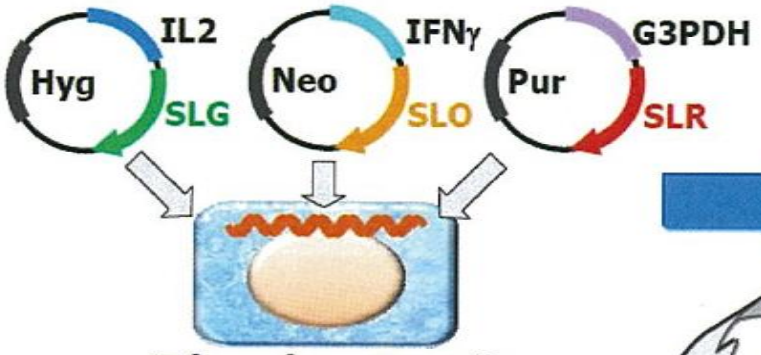
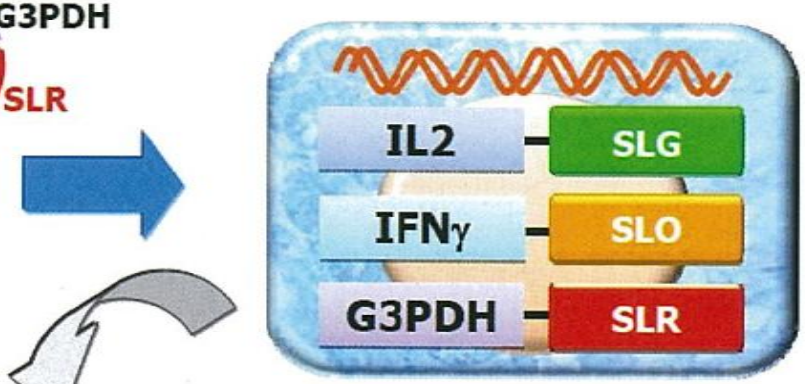
Chemical risk assessment methodologies to conduct initial & detailed risk assessments
Quantitative chemical risk trade-off analysis methodologies
QSAR models to predict biodegradation & bioaccumulation of chemical substances
QSAR decision support system to predict repeated dose toxicity
A method to predict hepatocarcinogenicity in rats by gene expression profiling
-HTP assay systems that use cultured cells for carcinogenicity, immunotoxicity and developmental toxicity
- Gene expression profile datasets in repeated dose 28-day oral toxicity studies
Tools for chemical risk communication
Methodologies to conduct preliminary risk assessments for nanomaterials

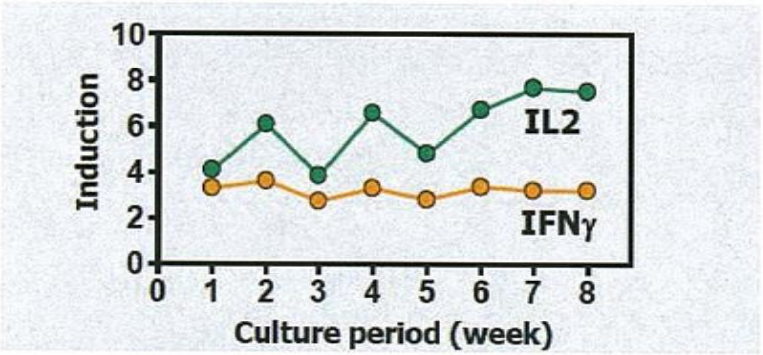

HTP assay for carcinogenicity (Hatano Research Institute, Food & Drug Safety Center)
Bhas 42 cells
Cell transformation assay using 6-well plates
Cell transformation assay using 96-well plates
Robotic system
Selection of marker genes
Multicolor luciferase reporter assay
HTP assay for immunotoxicity (Tohoku University)
Human keratinocyte, monocyte/macrophage and T cells
Selection of marker genes
Multicolor luciferase reporter assay
HTP assay for developmental toxicity (Sumitomo Chemical Co., Ltd.)
Mouse ES cells
Differentiation into myocardial cells / neural cells
Selection of marker genes
Multicolor luciferase reporter assay
Confirmation
Rat whole embryo culture with metabolic activation (S9-mix addition) (Kamakura Women's University)
Brush up on basic technology and support
1) Multicolor luciferase reporter assay (National Institute of Advanced Industrial Science & Technology and Toyobo Co., Ltd.)
2) Human artificial chromosome (HAC) vector method (Tottori University)

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.



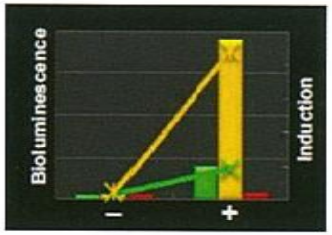




Cells display stable luciferase expressions and respond to chemicals during prolonged culture.

Chemical risk analysis in a 96well plate format HTP assay

Reaction with Tripluc® assay Reagents



Main members for IL-8 Luc assay Validation Management Team

Name	Role and expertise	Affiliation
Trial Coordinator **Noriho Tanaka**	VMT Chairperson,	**HRI and OTIP, Japan**
Lead Lab **Yutaka Kimura*** **Setsuya Aiba****	*VMT Co-chair **Developer of this assay Test method, expertise underlying science	**Tohoku Univ., Japan**
Hajime Kojima	Management of quality control	**JaCVAM, NIHS, Japan (JaCVAM representative)**
Takashi Omori	Data analysis, biostatistics dossier	**Doshisha Univ., Japan**
Liaison members		
ECVAM liaison **Emanuela Corcini**	Test system expertise, multi-study validation expertise, immunotoxicity expertise	**Mila Univ., Italy**
ICCVAM liaison **Warren Casey**	Test system expertise, multi-study validation expertise	**NICEATM, USA**
KoCVAM liaison **Ai-Young Lee**	Test system expertise, multi-study validation expertise	**KoCVAM, Korea**

Stages of IL-8 Luc assay validation study under Modular approach

Module 2: Within-lab Reproducibility (5 coded)

Module 3: Transferability

Phase 0 (*finished*) 10 non-coded

Module 4: Between-Lab Reproducibility

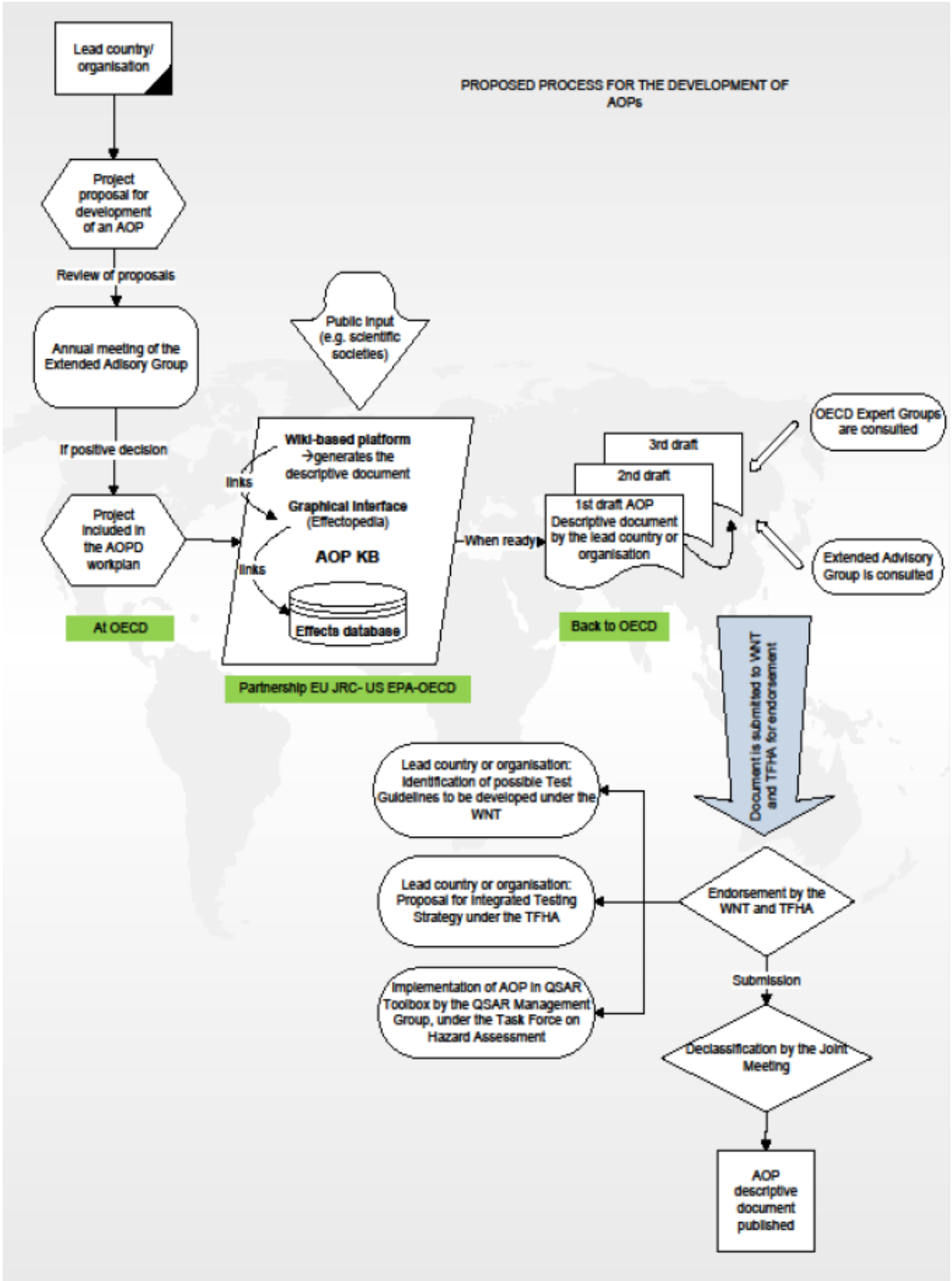
Phase I 20 coded

Present time

Module 5: Predictive capacity

Phase II ?? coded

AOP and IATA


PROPOSED PROCESS FOR THE DEVELOPMENT OF AOPs
Lead country/ organisation
Project proposal for development of an AOP
Review of proposals
Annual meeting of the Extended Adisory Group
If positive decision
Project included in the AOPD workplan
At OECD
Public input (e.g. scientific societies)
Wiki-based platform →generates the descriptive document
links
Graphical interface (Effectopedia)
AOP KB
links
Effects database
Partnership EU JRC- US EPA-OECD
When ready
3rd draft
2nd draft
1st draft AOP Descriptive document by the lead country or organisation
Back to OECD
OECD Expert Groups are consulted
Extended Advisory Group is consulted
Document is submitted to WNT and TFHA for endorsement
Lead country or organisation: Identification of possible Test Guidelines to be developed under the WNT
Lead country or organisation: Proposal for integrated Testing Strategy under the TFHA
Implementation of AOP in QSAR Toolbox by the QSAR Management Group, under the Task Force on Hazard Assessment
Endorsement by the WNT and TFHA
Submission
Declassification by the Joint Meeting
AOP descriptive document published

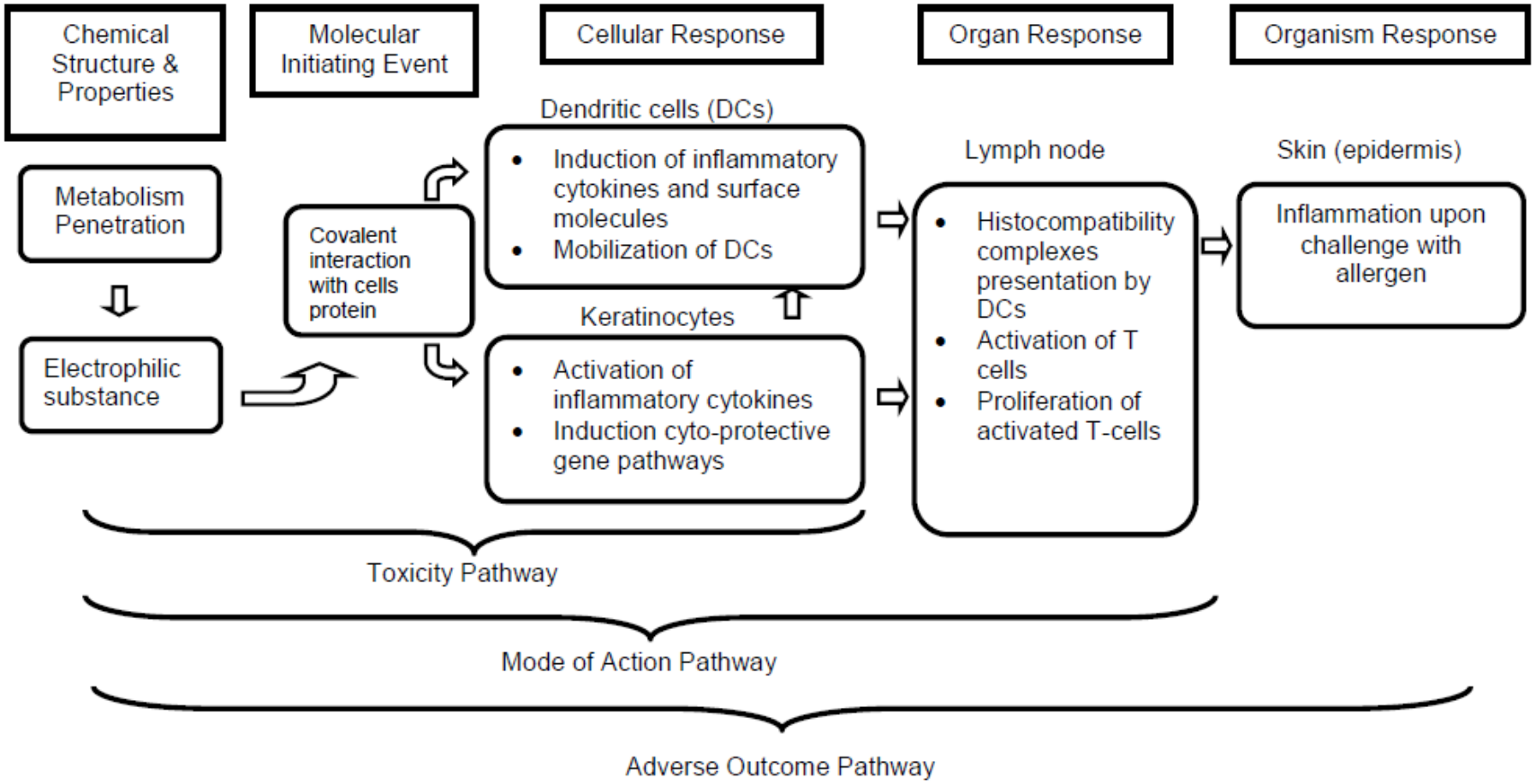
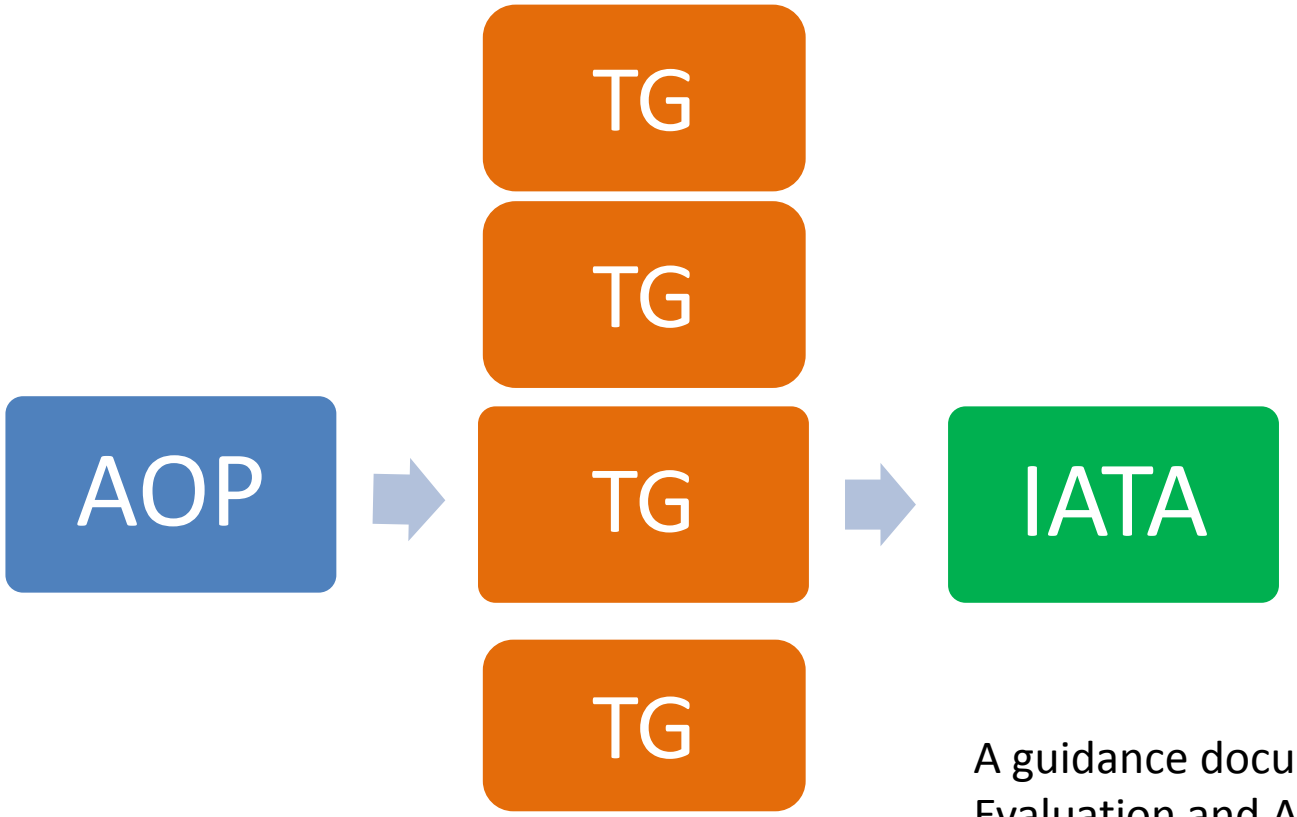


Figure 3. **Flow diagram of the pathways associated with skin sensitisation.**

OECD strategy for Regulatory acceptance

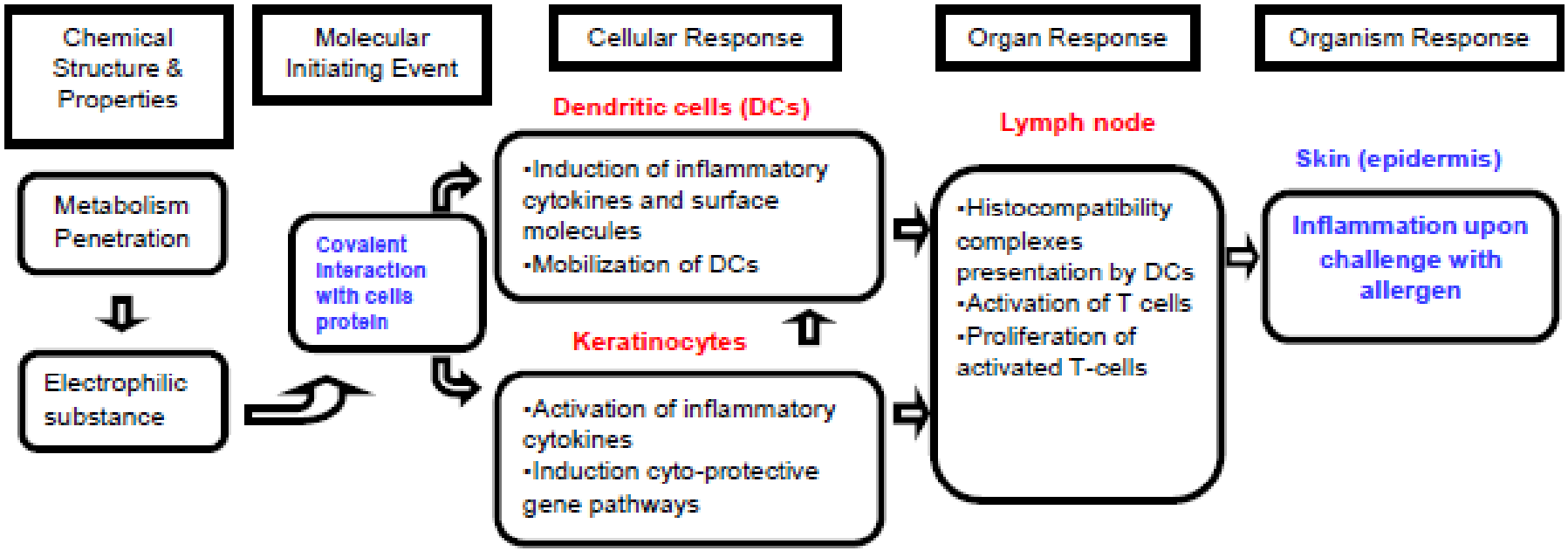


A guidance document on the Evaluation and Application of Integrated Approaches to Testing and Assessment （IATA)

AOP: Adverse Outcome Pathway

TG: Test Guideline

Adverse Outcome Pathways on skin sensitization assay



DPRA

DCs

H-CLAT,
MUSST?,
IL-8 Luc assay?

Keratinocytes

KeratinoSense

Local Lymph Node assay (LLNA)

Maximization or Buehler test using guinea pig

Figure 3: Timeline for implementation of EU legislation and skin sensitisation information requirements.

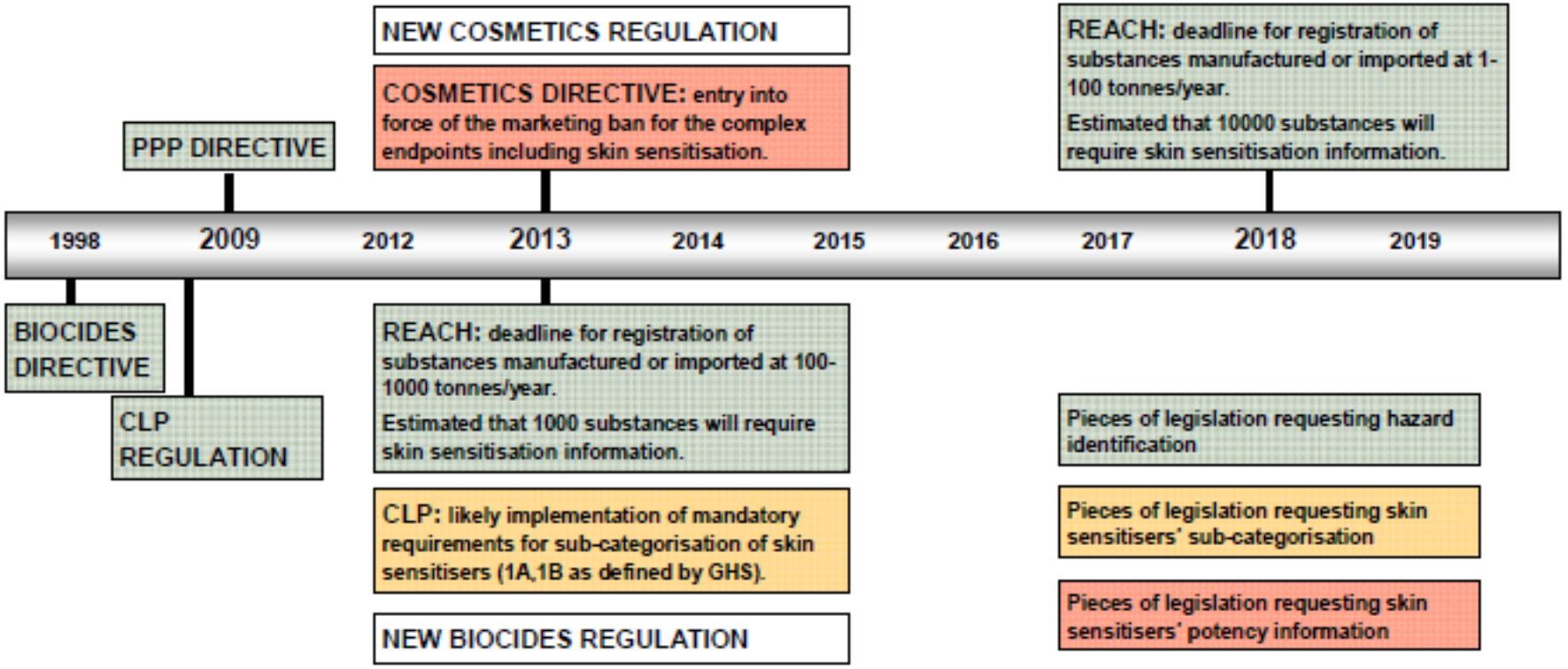
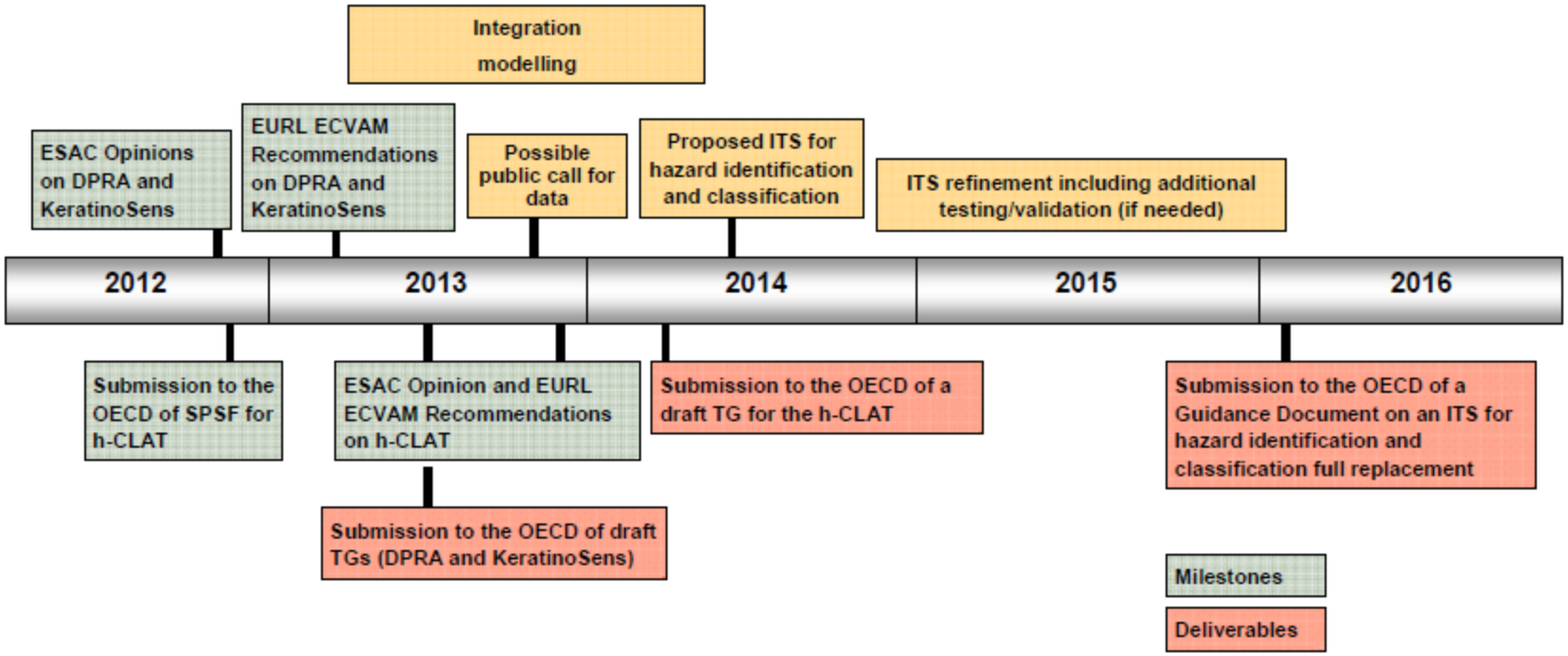


Figure 1: EURL ECVAM roadmap for achieving skin sensitisation hazard identification and classification based on alternative methods.



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)

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Thank you for your attention

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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- JaCVAM Activities

インターネット | 保護モード: 有効