ChemicalWatch
GLOBAL RISK & REGULATION NEWS

Skin Sensitization Webinar

28 January 2015, 4:00pm GMT

Today's webinar

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This webinar will:

- Discuss data requirements and in vivo classification for skin sensitisation in order to define what is required to replace the animal test;
- Provide an overview of the key mechanism of skin sensitization, based on the published adverse outcome pathway, and describe the in vitro and in chemico methods that can be used to assess skin sensitisation with a specific focus on the validated methods;
- Review current OECD activities in the field of skin sensitisation.

Speakers

- **Dr Susanne Kolle**, BASF

- **Dr Silvia Casati**, EURL ECVAM

Chair: **Dr Gilly Stoddart**, PETA International Science Consortium, Ltd

Chair: **Philip Lightowlers**, Chemical Watch

Questions

- Please submit questions during the webinar using your chat box

- Any unanswered questions can be raised on our Forum following the webinar: http://forum.chemicalwatch.com/

Upcoming Webinars

Webinar 5: Mammalian Acute Toxicity

March 5, 2015

11am ET, 4pm GMT

Pilar Prieto, EURL ECVAM

Lawrence Milchak, 3M

Webinar 6: Ecotoxicity (fish embryo test)

April 2015

11am ET, 4pm GMT

Marlies Halder, EURL ECVAM

Thomas Braunbeck, University of Heidelberg

Scott Belanger, Procter & Gamble

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www.piscltd.org.uk

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Skin Sensitisation

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EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)
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http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam

Information requirements under REACH

EC proposal to amend point 8.3 of Annex VII

ANNEX VII

STANDARD INFORMATION REQUIREMENTS FOR SUBSTANCES MANUFACTURED OR IMPORTED IN QUANTITIES OF 1 TONNE OR MORE[1]

8.3. Skin sensitisation	8.3. Step 2 does not need to be conducted if:
The assessment of this endpoint shall comprise the following consecutive steps:	– the available information indicates that the substance should be classified for skin sensitisation or corrosivity; or
(1) an assessment of the available human, animal and alternative data,	– the substance is a strong acid (pH < 2,0) or base (pH > 11,5); or
(2) *In vivo* testing.	– the substance is flammable in air at room temperature.
	The Murine Local Lymph Node Assay (LLNA) is the first-choice method for *in vivo* testing. Only in exceptional circumstances should another test be used. Justification for the use of another test shall be provided.

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REACH testing needs

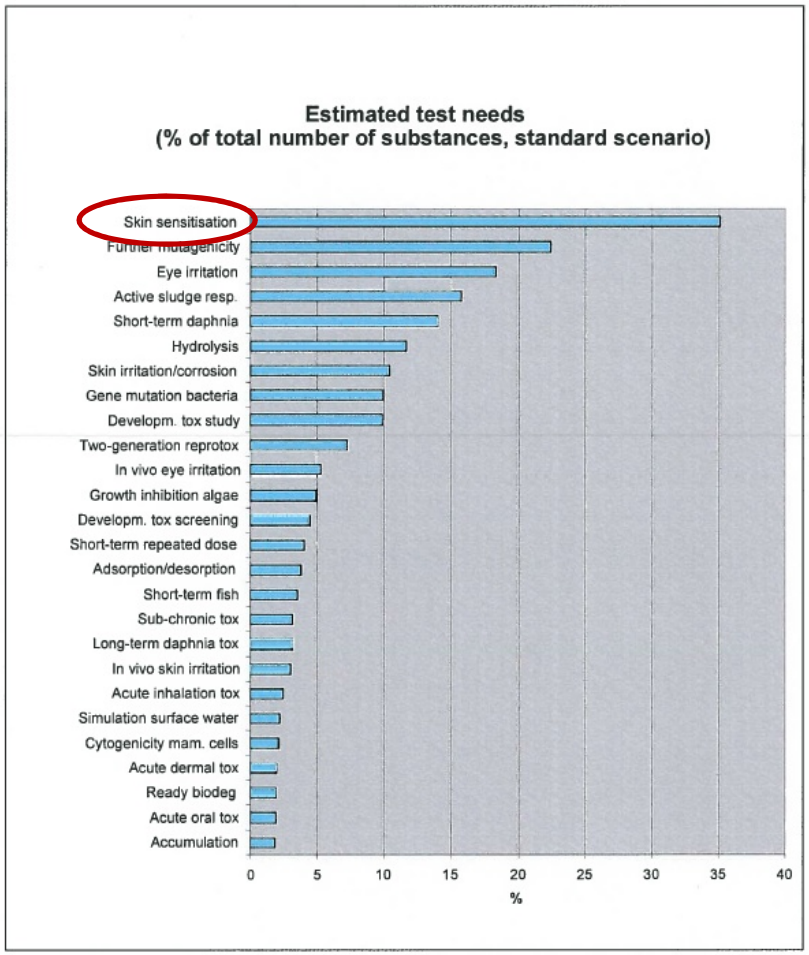

Figure 1 Estimated percentage of the total number of phase-in substances that will need to be tested for the different endpoints.

Between 25,000 and 50,000 substance registrations expected for the 2018 deadline

http://echa.europa.eu/documents/10162/684852/media_briefing_2014_musset_en.pdf

Van der Jagt, K., Munn, S., Torslov, J. & de Bruijn J.(2004). Alternative approaches can reduce the use of test animals under REACH. Addendum to the report "Assessment of additional testing needs under REACH. Effects of (Q)SARs, risk based testing and voluntary industry initiatives. EUR 21 405 EN.

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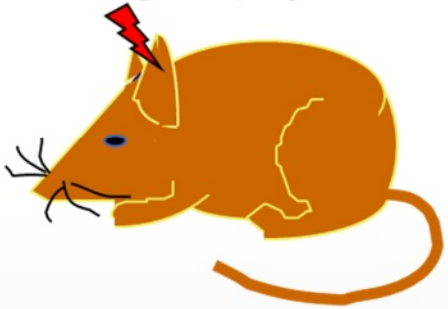
Animal tests

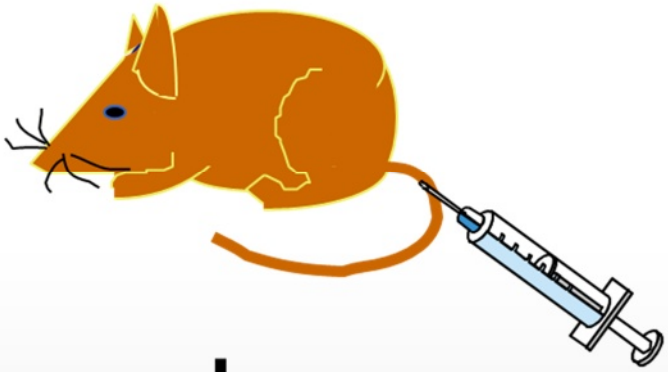
Test	Criteria for positive result
Guinea Pig Maximisation Test (GPMT) (OECD TG 406)	Positive response in ≥30% of the test animals
Buehler Test (OECD TG 406)	Positive response in ≥15% of the test animals
Mouse local lymph node assay (LLNA) (OECD TG 429)	Stimulation Index (SI) ≥3
LLNA: DA (OECD TG 442A)	SI ≥1.8
LLNA: BrdU-ELISA (OECD TG 442B)	SI ≥1.6

OECD TG 429: Local Lymph Node Assay

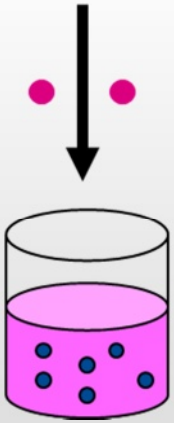
Epicutaneous induction: Application of the test material on days 1, 2 and 3 (3 dose groups plus vehicle and positive control groups)



Injection of ^{3}H-thymidine on day 6



Removal of lymph nodes 5 hours later; make a cell suspension



Determination ^{3}H-thymidine incorporation via liquid scintillation counting

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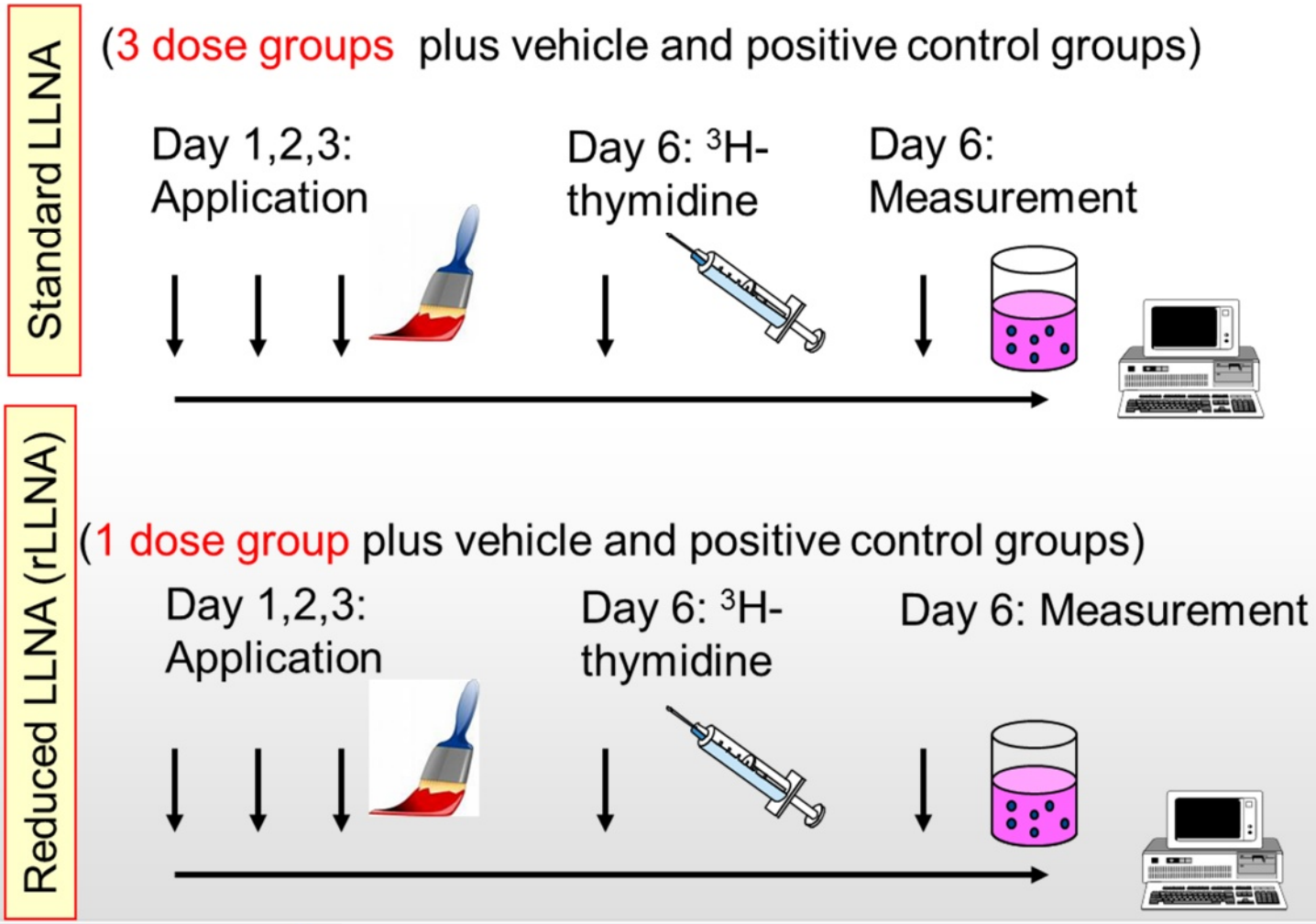
OECD TG 429: Reduced Local Lymph Node Assay



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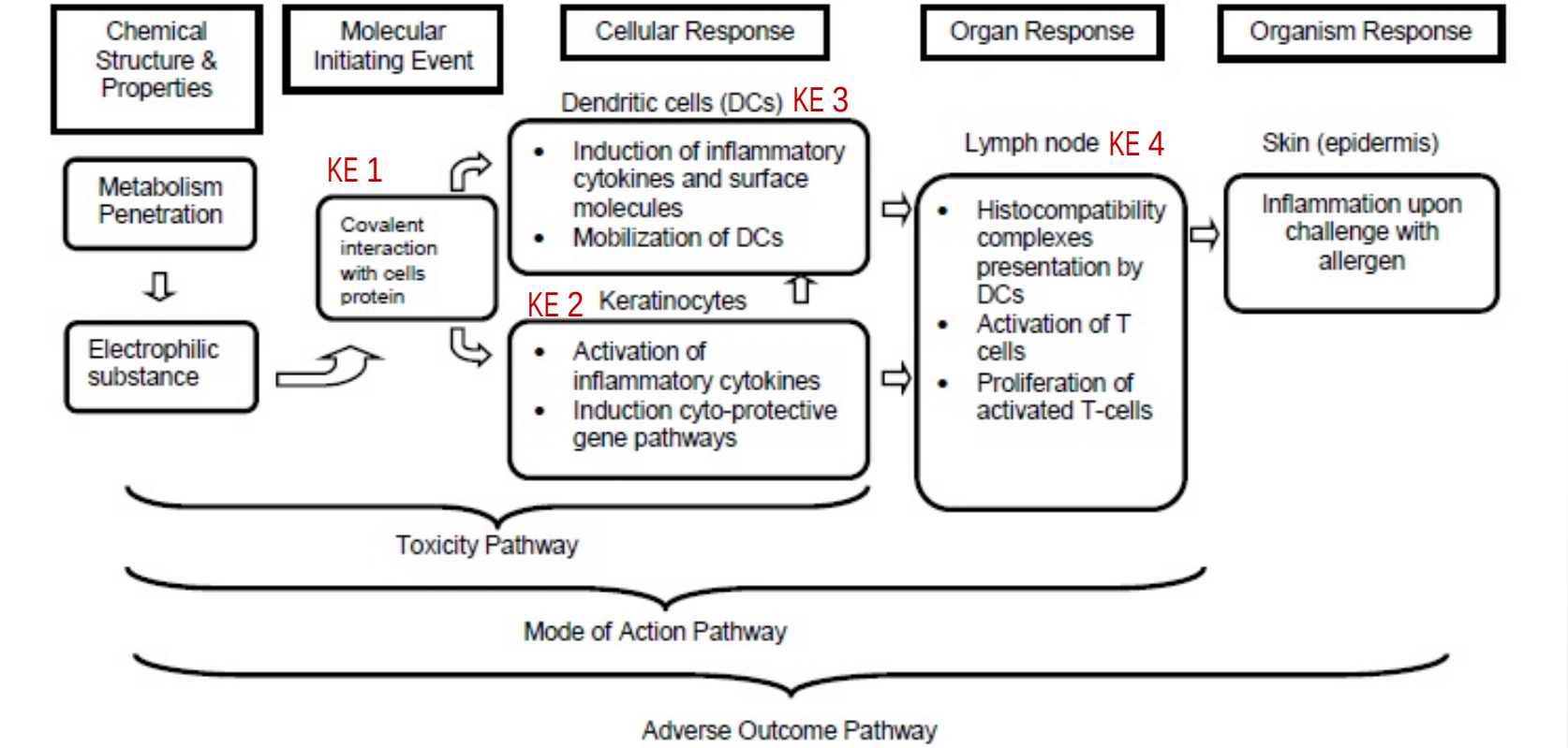
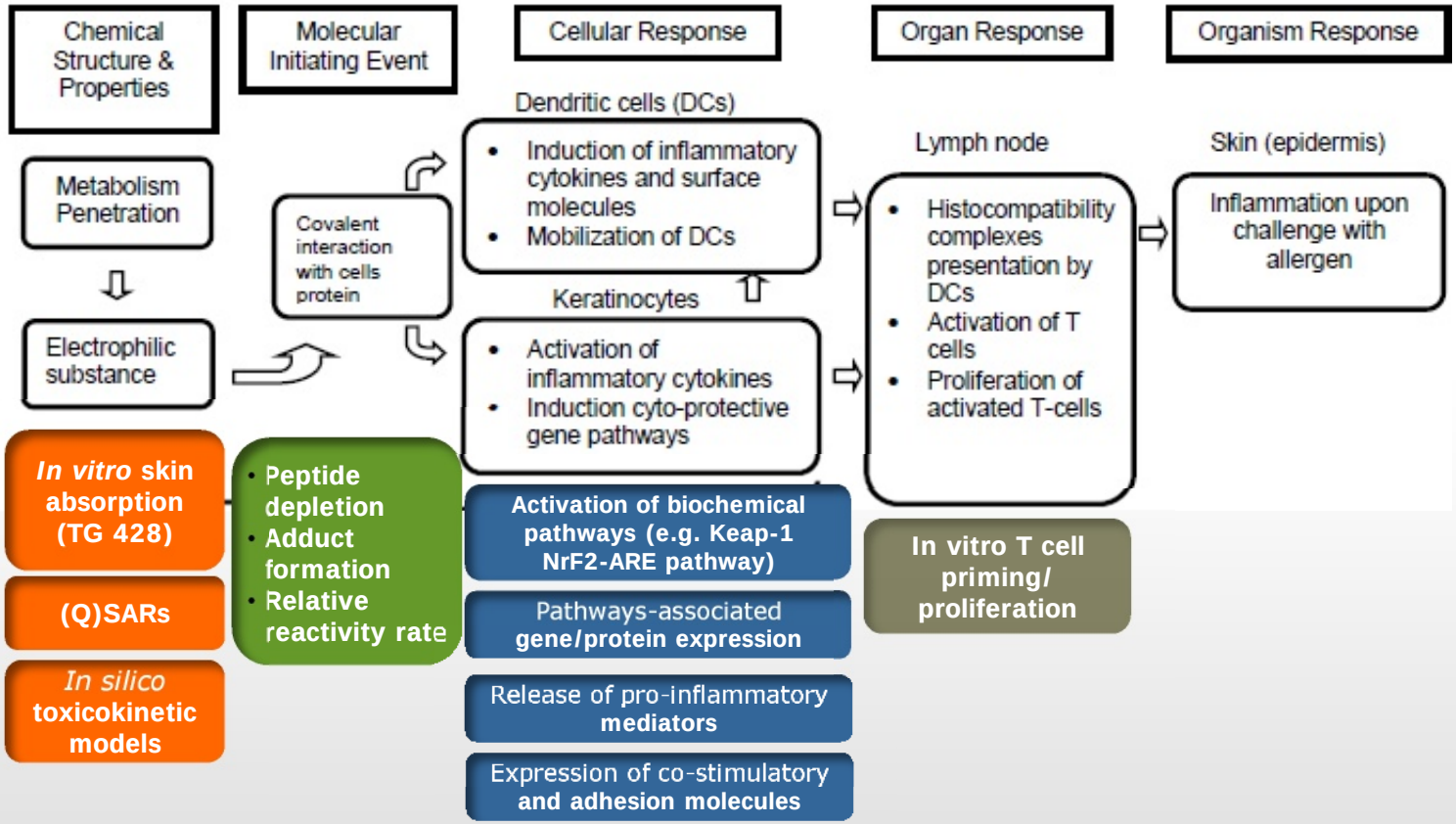
The **A**dverse **O**utcome **P**athway for skin sensitisation

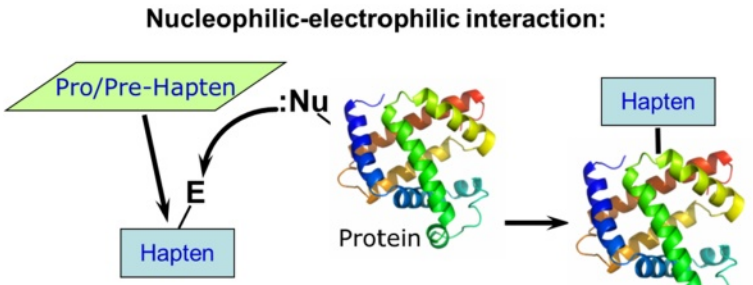


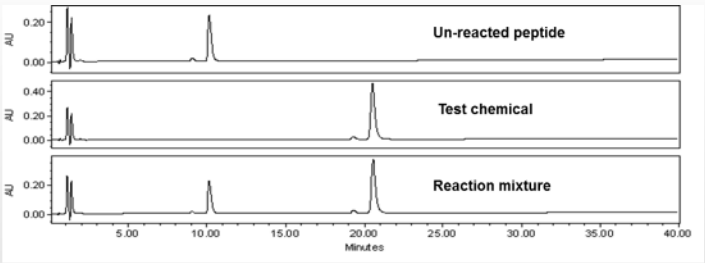
The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins; Part 1: Scientific Evidence Series on Testing and Assessment No.168 ENV/JM/MONO(2012)10/PART1

Toolbox of non-animal methods



Direct Peptide Reactivity Assay (DPRA) -1





Mechanistic basis: addresses the mechanism of haptenation, the Molecular Initiating Event (MIE) of the skin sensitisation AOP

Test system: synthetic heptapetides containing either cysteine or lysine

Endpoints measured: cysteine and lysine peptide % depletion

Protocol: cysteine and lysine peptide solutions incubated with the test chemical at 1:10 and 1:50 ratio respectively for 24h at room temperature. Relative peptide concentration measured by HPLC with gradient elution and UV detection at 220 nm

Controls: positive (cinnamic aldehyde), negative (peptide solutions)

Direct Peptide Reactivity Assay (DPRA) -2

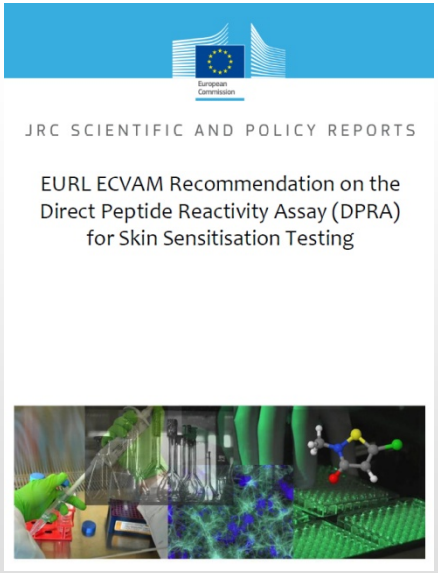
DPRA Cysteine 1:10/Lysine 1:50 Prediction Model

Mean of cysteine and lysine % depletion	Reactivity Class	DPRA Prediction
0% ≤ mean % depletion ≤ 6.38%	No or minimal reactivity	Negative
6.38% < mean % depletion ≤ 22.62%	Low reactivity	Positive
22.62% < mean % depletion ≤ 42.47%	Moderate reactivity	
42.47% < mean % depletion ≤ 100%	High reactivity	

Prediction model: mean percent cysteine and lysine peptide depletion value of 6.38 is used as threshold to discriminate between negative and positive predictions

Prediction model based only on cysteine depletion values available in case the test chemical has the same retention time of the lysine peptide

An accurate description of the DPRA including the prediction model is available in the DB-ALM protocol 154 accessible at http://ecvam-dbalm.jrc.ec.europa.eu/



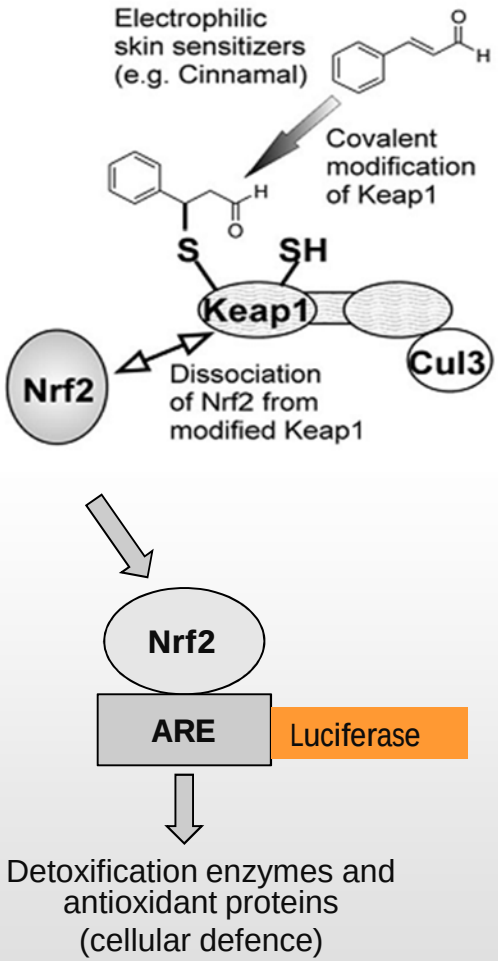
Applicability and limitations:

§ Not applicable for the testing of metals, oxidizers (cysteine dimerisation), highly hydrophobic substances, complex mixtures of unknown composition and UVCB

§ No metabolic competent activation system (i.e. pro-haptens not detected)

Status: validated by EURL ECVAM for transferability and reliability, OECD accepted (Test Guideline 442c)

KeratinoSens™ -1



Mechanistic basis: addresses responses in keratinocytes (key event 2 of the skin sensitisation AOP) by measuring activation of the antioxidant/electrophile response element-dependent pathway (Keap1-Nrf2-ARE)

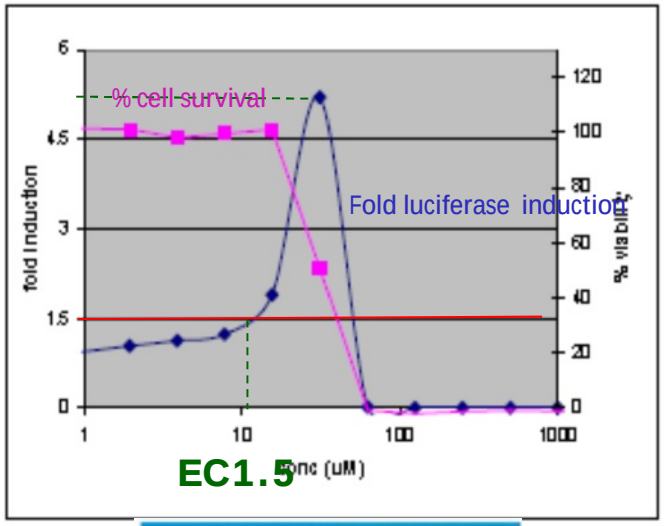
Test system: human keratinocyte-derived cell line with a stable insertion of a luciferase gene under the control of an ARE element

Endpoints measured: luciferase gene fold induction and cytotoxicity (MTT assay)

Protocol: Cells exposed for 48h to 12 concentrations of test chemical (dose-response information). Luciferase fold induction relative to induction in vehicle controls quantified by luminescence analysis

Controls: positive (cinnamic aldehyde), negative (DMSO used as vehicle)

KeratinoSens™ -2



Prediction model: a test chemical is rated positive if the luciferase activity is 1.5 fold higher and statistically significantly different as compared to the solvent control at a concentration with > 70% cell viability in at least two of three independent repetitions

An accurate description of the KeratinoSens™ including the prediction model is available in the DB-ALM protocol 155 accessible at http://ecvam-dbalm.jrc.ec.europa.eu/

Applicability and limitations:

- Not applicable to test chemicals not soluble in water or DMSO
- Designed to detect sensitising chemicals with selective reactivity towards nucleophilic cysteine sulfhydryl groups
- Limited metabolic capacity (e.g. pro-haptens requiring P450 activation not detected)

Status: validated in an industry-led ring trial for transferability and reliability and peer-reviewed by the ESAC, OECD accepted (Test Guideline 442d)

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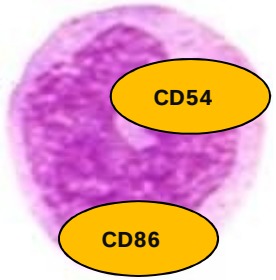
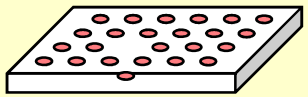
human Cell Line Activation Test (h-CLAT) -1



THP-1cells
treatment 24 hours



Cell staining
CD54/CD86
Propidium iodide

Measurement of cell viability and cell activation by flow cytometry

Mechanistic basis: addresses responses in dendritic cells (DC) (key event 3 of the skin sensitisation AOP) by measuring modulation of the expression of co-stimulatory and adhesion molecules

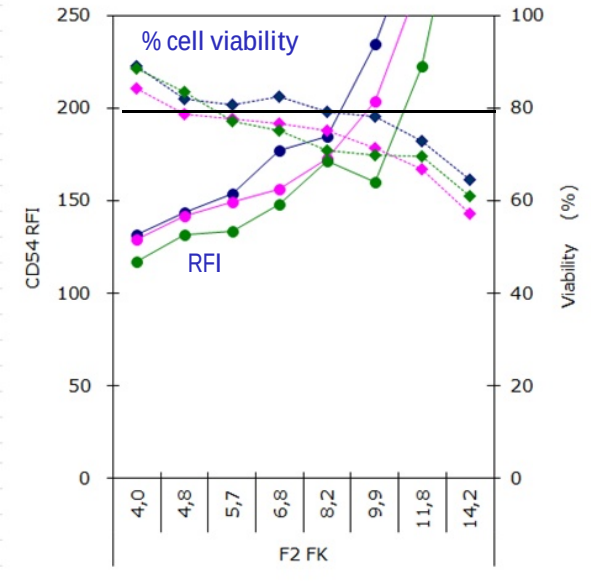
Test system: human monocytic leukemia cell line (THP-1)

Endpoints measured: relative fluorescence intensity (RFI) of CD86 and CD54 and cytotoxicity (propidium iodide)

Protocol: Cells exposed for 24h to 8 concentrations of test chemical (dose-response information). RFI of CD86 and CD54 compared to vehicle controls quantified by flow cytometry

Controls: positive (DNCB), negative (medium, saline or DMSO used as vehicle)

human Cell Line Activation Test (h-CLAT) -2



Prediction model: A chemical is rated positive if the RFI of CD86 is ≥ 150% and/or if the RFI of CD54 is ≥ 200% at any tested dose (≥ 50% of cell viability) in at least two independent repetitions

An accurate description of the h-CLAT including the prediction model is available in the DB-ALM protocol 158 accessible at http://ecvam-dbalm.jrc.ec.europa.eu/

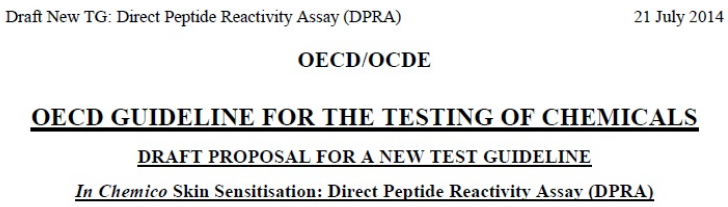
Applicability and limitations:

- Not applicable to chemicals with low solubility in the prescribed solvents
- Limited metabolic capacity (i.e pro-haptens not detected)
- Risk of false negative results with test chemicals with logKow greater than 3.5

Status: validated by EURL ECVAM for transferability and reliability. EURL ECVAM Recommendation in publication, Development of a TG under discussion at the OECD

OECD TG 442c (DPRA) and TG 442d (KeratinoSens™)

Final versions publicly available on the OECD web site as from the 4th of February



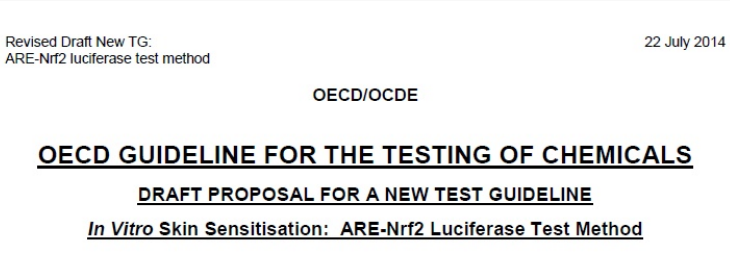
Draft New TG: Direct Peptide Reactivity Assay (DPRA) 21 July 2014

OECD/OCDE

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW TEST GUIDELINE

In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)



Revised Draft New TG:
ARE-Nrf2 luciferase test method 22 July 2014

OECD/OCDE

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW TEST GUIDELINE

In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method

- To support the discrimination between skin sensitisers (i.e. UN GHS Category 1) and non-sensitisers in combination with other complementary information (i.e. in the context of an IATA)
- Depending on the regulatory framework, positive results may be used on their own to classify a chemical to UN GHS Category 1
- The TGs cannot be used on their own to sub-categorise skin sensitisers into UN GHS subcategories 1A and 1B or to predict potency for safety assessment decisions

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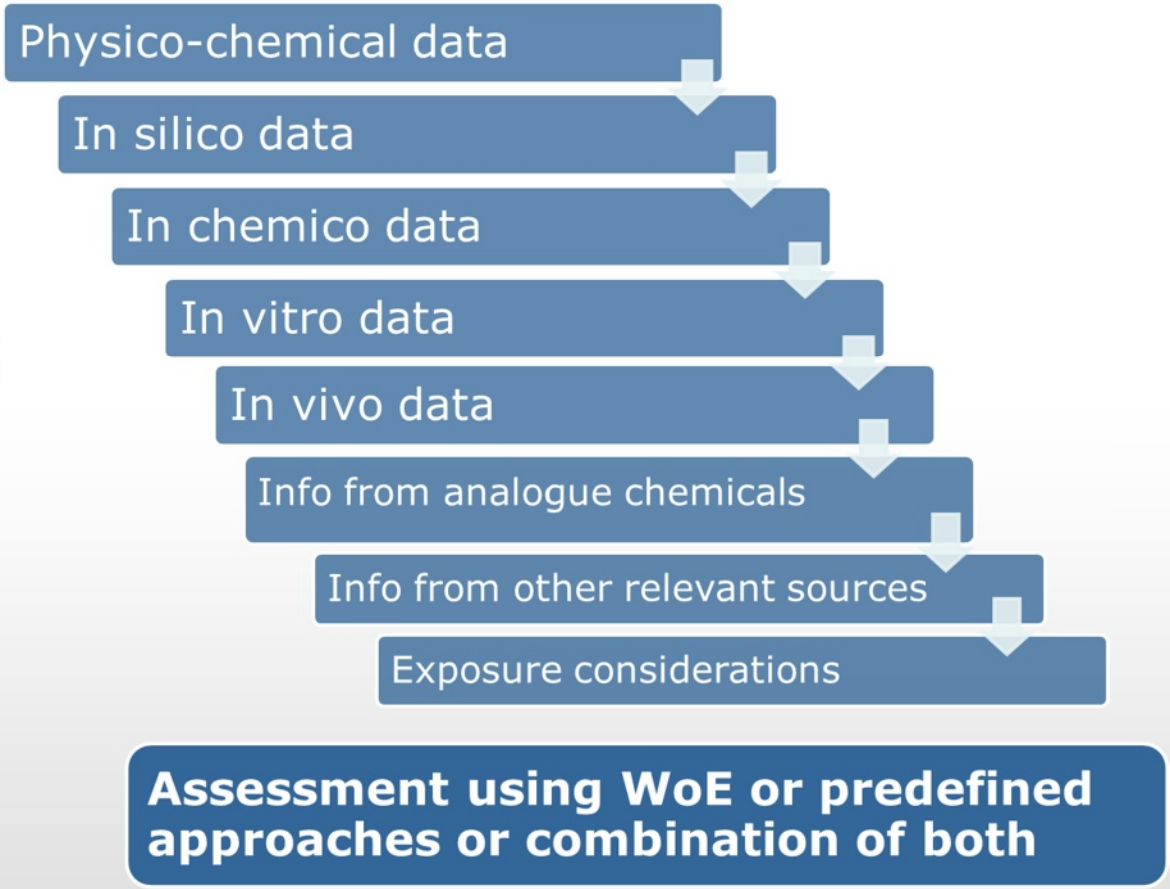
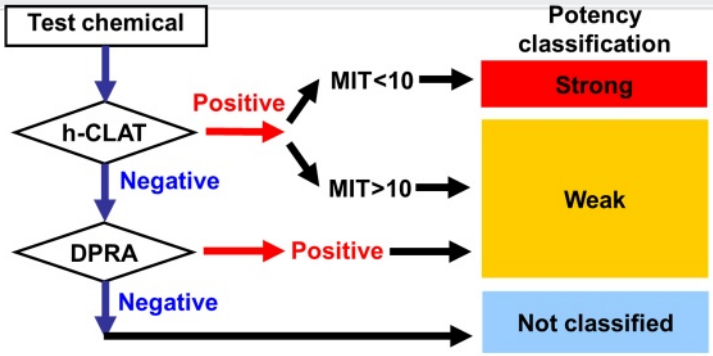
Integrated Approaches to Testing and Assessment (IATA)

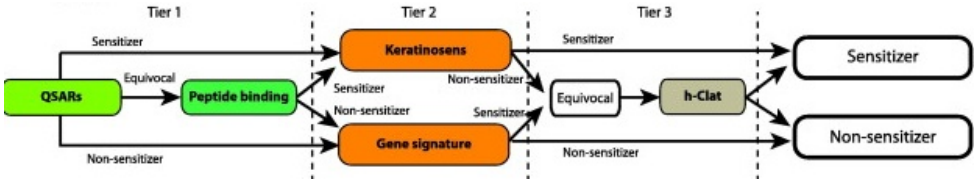
"A structured approach which integrates and weights all relevant existing data and inform about additional data needs to enable (regulatory) decisions"



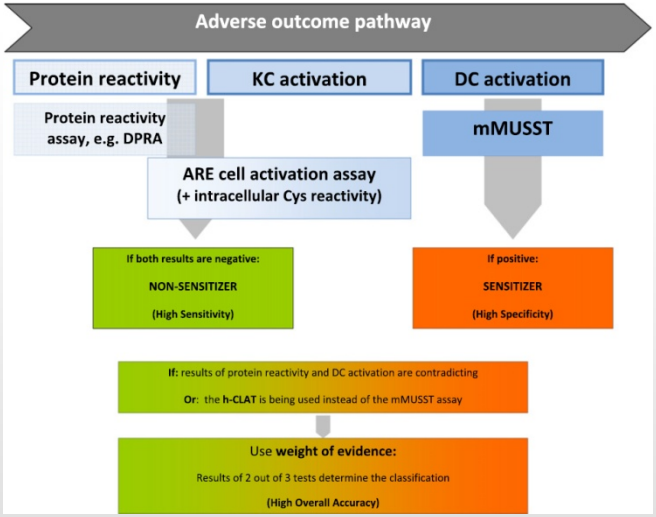
Examples of published data integration strategies for skin sensitisation



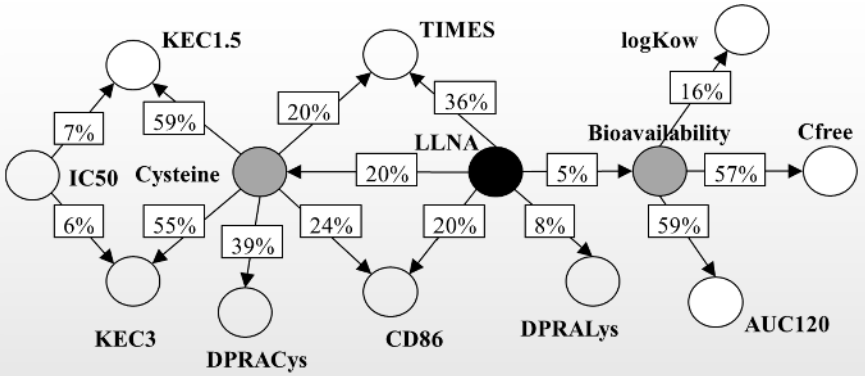
Nukada et al. (2013) Toxicology in Vitro 27, 609-618



Van der Veen et al. (2014) Regulatory Toxicology and Pharmacology 69, 371-379.



Bauch et al. (2012) Regulatory Toxicology and Pharmacology 63, 489-504



Jaworska et al. (2013) Journal of Applied Toxicology 33, 1353-1364

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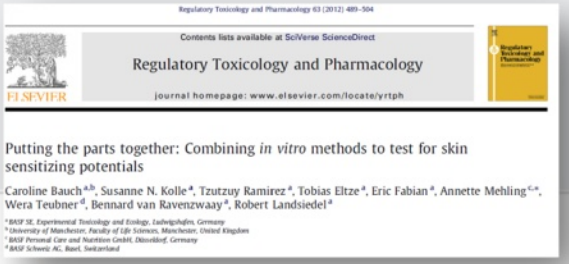
Two out of three: 54 substances (Bauch et al, 2012)


Regulatory Toxicology and Pharmacology 63 (2012) 489–504

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Putting the parts together: Combining *in vitro* methods to test for skin sensitizing potentials

Caroline Bauch[a,b], Susanne N. Kolle[a], Tzutzuy Ramirez[a], Tobias Eltze[a], Eric Fabian[a], Annette Mehling[c,*], Wera Teubner[d], Bennard van Ravenzwaay[a], Robert Landsiedel[a]

[a] BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany
[b] University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom
[c] BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany
[d] BASF Schweiz AG, Basel, Switzerland

- 59 test substances including LLNA performance standards
  - Additives/ stabilizers/ detergents 30%
  - Fragrances 24%
  - Cosmetic preservatives 22%
  - Cosmetic solvents 11%
  - Cosmetic dyes 7%
- 5/59 substances initially selected turned out not to be applicable in all 5 tests due to technical reasons
- **54 substances** with available **LLNA** and **human** skin sensitization information were evaluated in the 4 in vitro/in chemico assays in the validation process (DPRA, KeratinoSensTM, h-CLAT, mMUSST) along with the LuSens assay (similar to the KeratinoSensTM)

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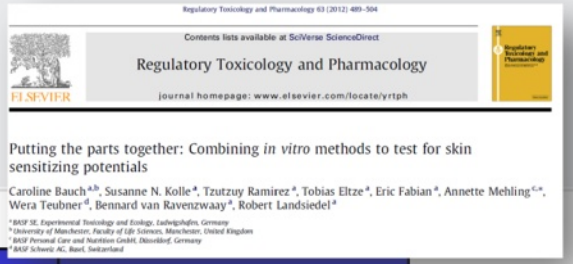
Predictivity of assays and their combinations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[b] University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom
[c] BASF Personal Care and Nutrition GmbH, Düsseldorf, Germany
[d] BASF Schweiz AG, Basel, Switzerland

Compared to human		Accuracy
In vivo standard	LLNA	89 %
Individual assays	DPRA	87 %
	LuSens	82 %
	mMUSST	85 %
	h-CLAT	78 %
Combinations (one of two is positive)	DPRA and LuSens	85 %
	DPRA and mMUSST	81 %
	DPRA and h-CLAT	83 %
	LuSens and mMUSST	80 %
	LuSens and h-CLAT	82 %
Prediction model	DPRA, LuSens and mMUSST	94 %

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Two out of three: 145 substances (Natsch et al, 2013)


Research Article

Applied Toxicology

(wileyonlinelibrary.com) DOI 10.1002/jat.2868

A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation

Andreas Natsch[a]*, Cindy A. Ryan[b], Leslie Foertsch[b], Roger Emter[a], Joanna Jaworska[c], Frank Gerberick[b] and Petra Kern[c]

Cooper statistics compared to LLNA and for WoE 'positive if 2 of 3 tests positive'

	U937-CD86 Test	DPRA	Keratino - Sens™ Assay	WoE (2 of 3 tests) LLNA
Sensitivity	71	82	79	82
Specificity	70	74	72	77
Accuracy	71	80	77	81
n	141	145	145	145

- 43 non-sensitizers according to the LLNA, 33 weak, 39 moderate, 19 strong and 11 extreme sensitizers
- cLogP: majority ranged between 0 and 4
- Molecular weight: majority ranged between 100 and 200 Da

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Two out of three: 54 and 145 substances

Assay		Accuracy 54 chemicals (Bauch et al., 2012) compared to human data	Accuracy 54 chemicals (Bauch et al., 2012) compared to LLNA data	Accuracy 145 chemicals (Natsch et al., 2013) compared to LLNA data
Individual assays	DPRA	87%	79%	80%
	ARE reporter gene assay; LuSens or KeratinoSens	82%	81%	77%
	U937/CD86 Test (MUSST-like test)	85%	74%	71%
2 of 3	DPRA, ARE-based assay and U937/CD86 Test	94%	83%	81%

- Similar accuracy between both studies despite the extended data set
- Additional data from human studies was not available for all 145 substances; accuracy compared to human data was not determined

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Two out of three: 213 substances

Chemical set and reference data	All compared to LLNA data		Subset: LLNA and human data available			
	LLNA data		human data		LLNA data	
Cooper statistics	Acc [%]	n	Acc [%]	n	Acc [%]	n
'2 of 3' WoE approach	79	180	90	101	82	103
DPRA	75	194	84	102	79	105
KeratinoSens	73	188	82	102	74	103
h-CLAT	76	166	82	98	81	101
LuSens	76	78	82	61	75	63
(m)MUSST	73	150	78	85	75	87
LLNA	-	-	82	111	-	-


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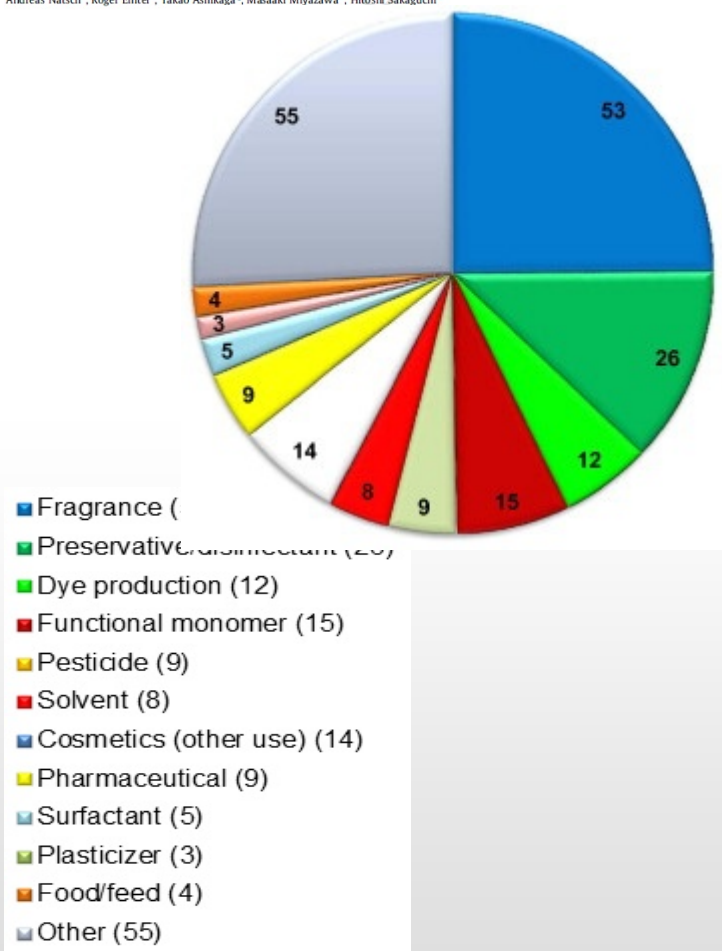
Assessing skin sensitization hazard in mice and men using non-animal test methods

Daniel Urbisch[a], Annette Mehling[b], Katharina Guth[a], Tzutzuy Ramirez[a], Naveed Honarvar[a], Susanne Kolle[a], Robert Landsiedel[a,*], Joanna Jaworska[c], Petra S. Kern[d], Frank Gerberick[e], Andreas Natsch[f], Roger Emter[f], Takao Ashikaga[g], Masaaki Miyazawa[h], Hitoshi Sakaguchi[h]



Two out of three using “real life” chemicals

- **Real-life substances** and **formulations** generally have a lower purity and contain some other byproducts

- Plant extracts and formulations were tested using gravimetric approaches instead of MW

- 24 sensitizers, 16 non-sensitizers (either LLNA or GPT)

- 7 isocyanates (acylating agents)
- 5 acrylates (Michael acceptors)
- 5 agrochemical formulations
- 3 polyethylene imine polymers
- 6 surfactants
- 6 other cosmetic ingredients
- 7 plant extracts
- 1 peptide

- no known pre/pro-haptens

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Two out of three using “real life” chemicals

	BASF in-house post-validation						Bauch, 2012			Natsch, 2013
	WoE I	WoE II	WoE I w/o PEI, AF	WoE II w/o PEI, AF	WoE I w/o PEI, AF, PE	WoE II w/o PEI, AF, PE	WoE I	WoE I	LLNA	WoE III
n	38	35	24	21	24	21	50	53	54	145
vs.	LLNA/ GPMT	LLNA/ GPMT	LLNA/ GPMT	LLNA/ GPMT	LLNA/ GPMT	LLNA/ GPMT	human	LLNA	human	LLNA
sensitivity	71	75	88	94	93	93	93	81	96	82
specificity	86	73	85	70	90	86	95	88	81	77
accuracy	76	74	87	85	92	90	94	83	89	81

- **WoE I**: DPRA, LuSens, mMUSST; **WoE II**: DPRA, LuSens, h-CLAT; **WoE III**: DPRA, KeratinoSens, (m)MUSST
- AF: agrochemical formulation; PEI: polyethylene imine; PE: plant extract

- The protocols for the test methods are intended for defined substances (e.g. require use of molar equivalents)
- Agrochemical formulations and polyethylene imine based polymers were not well predicted by the in vitro strategy indicating a need to adapt the methods

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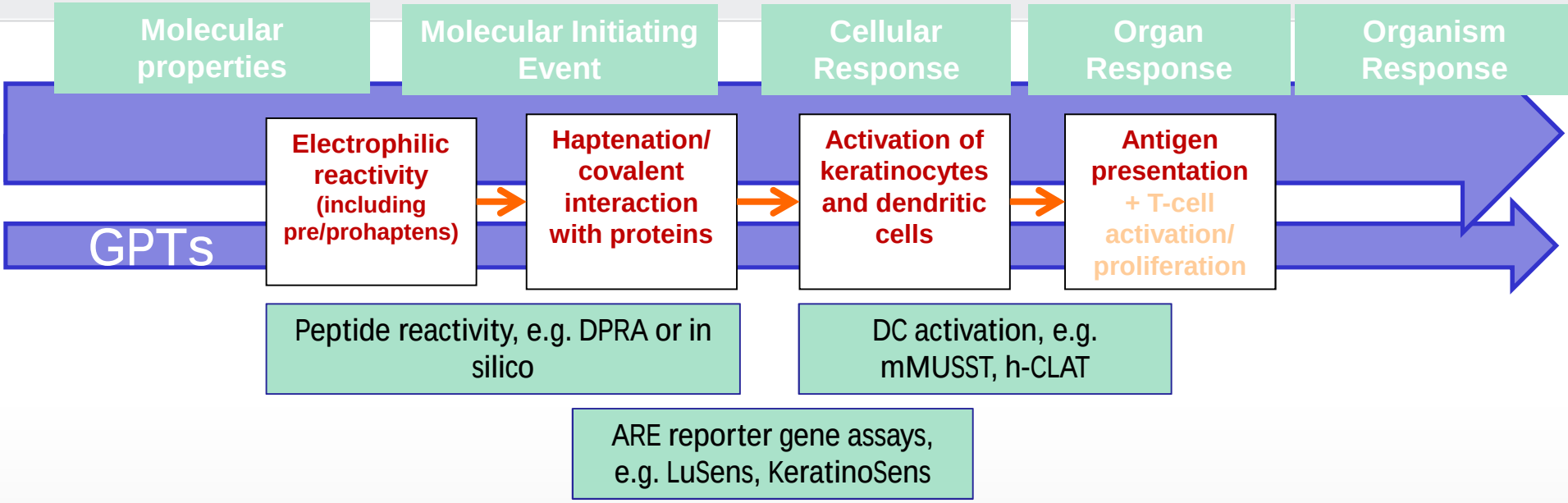
The accuracy of two out of threes



	Accuracy chemicals (n=54, Bauch et al., 2012) compared to LLNA/ human data	Accuracy chemicals (n=145 Natsch et al., 2013) compared to LLNA data	Accuracy „real life“ chemicals compared to animal data (n= 35; w/o polymers + formulations)	Accuracy of in silico/in vitro combo compared to LLNA/human data	Accuracy ongoing collaborative work all LLNA (n=180)/subset human (n=101)/ subset LLNA (n=103)
Accuracy	83 / 94%	81%	77% / 87%	81% / 86 %	79% / 90% / 82%

Limitations of the two out of three

Substances may be incorrectly predicted if they:

- Have a high cytoxicity
- Have a low solubility in aqueous media (cell cultures)
- Are not stable at high pH (DPRA)
- Are pre- or prohaptens

The strategy is not yet applicable

- To determine the potency
- To assess complex mixtures/substances such as polymers and formulations

Use of alternative methods in read-across approaches: Data matrix for a grouping of glycerides

Fatty acid, saturated	Tri-C7	C8-18	C8-21	C14-18 mono and di	C18	C18
Fatty acid, unsat.		C18	C8-21	C16-18/C18 hydroxy		C18
Acetate			Mono and di			Mono and di
In vivo	Negative	Negative	Negative	No data	Negative	Negative
OECD Toolbox PB alert	No alert	No alert	No alert	No alert	No alert	No alert
DPRA				Negative		

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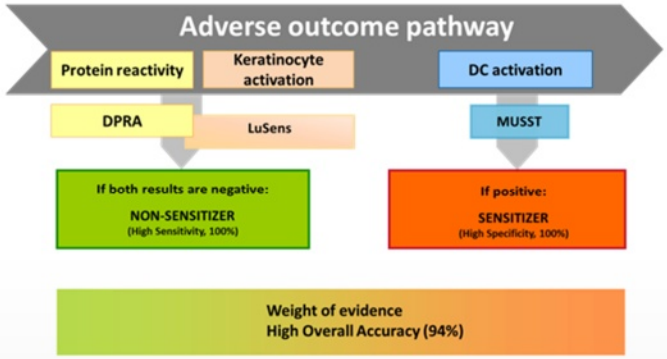
Use of alternative methods in read-across approaches: Data matrix for a grouping of acrylates

Alkyl chain	Methyl-	Ethyl-	n-butyl	2-ethylhexyl-	2-propyl-heptyl
In vivo	Positive	No data	Positive	Positive	No data
OECD Toolbox PB alert	Michael acceptor	Michael acceptor	Michael acceptor	Michael acceptor	Michael acceptor
DPRA	Positive	Positive	Positive	Positive	Positive
LuSens				Positive	Positive
mMUSST				Positive	Positive

Economy and animal welfare

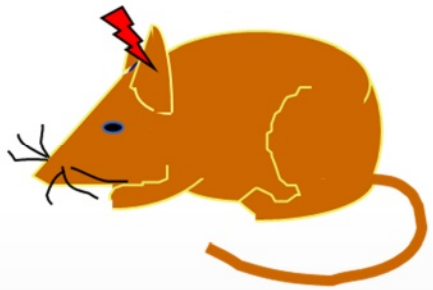
In vitro

Two out of Three



Costs	Number of animals
~ 6850 €	0

In vivo



Costs	Number of animals
~ 7600 €	~ 25

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Strategy for potency: An example Nukada et al. 2013


Toxicology in Vitro 27 (2013) 609–618

Contents lists available at SciVerse ScienceDirect

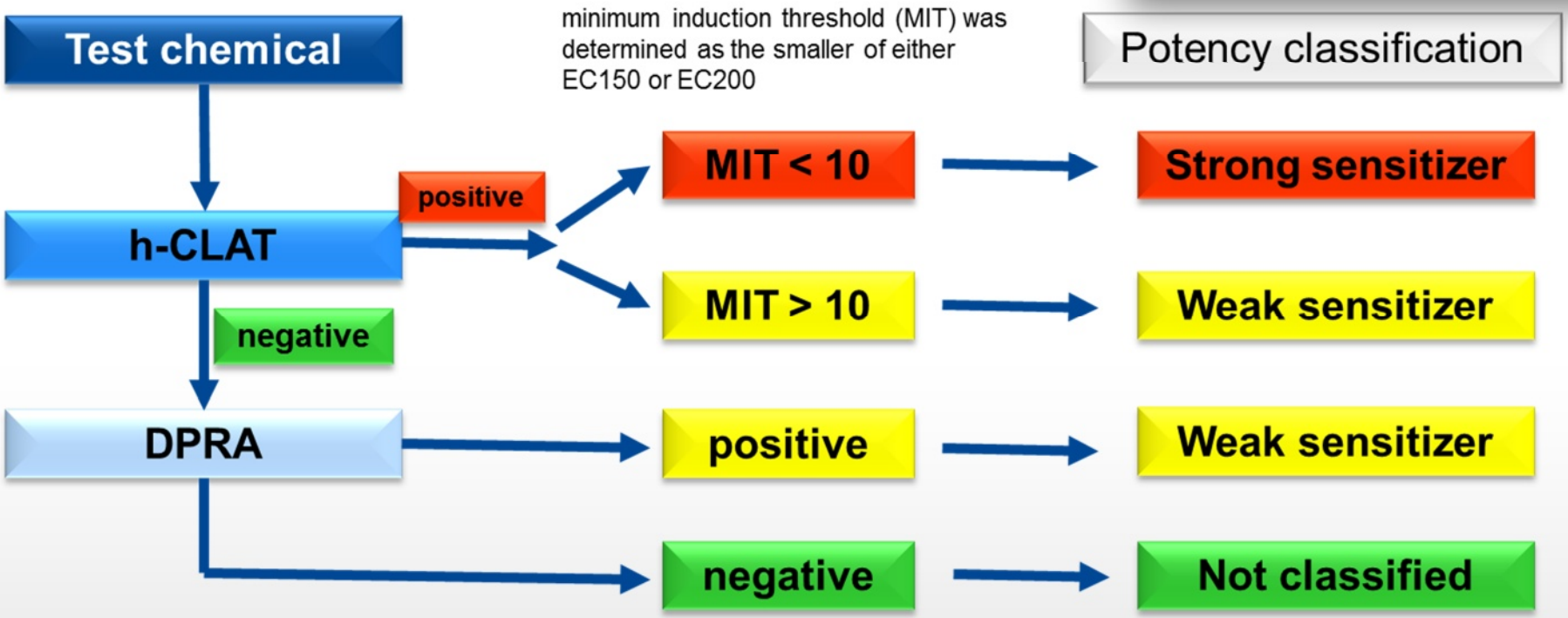
Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Data integration of non-animal tests for the development of a test battery to predict the skin sensitizing potential and potency of chemicals

Yuko Nukada, Masaaki Miyazawa *, Saitou Kazutoshi, Hitoshi Sakaguchi, Naohiro Nishiyama

Safety Science Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai-Machi, Haga-Gun, Tochigi 321-3497, Japan



Sensitizer	76
Nonsensitizer	25

Sensitivity	96%
Specificity	56%
Accuracy	86%

Compared to **LLNA**

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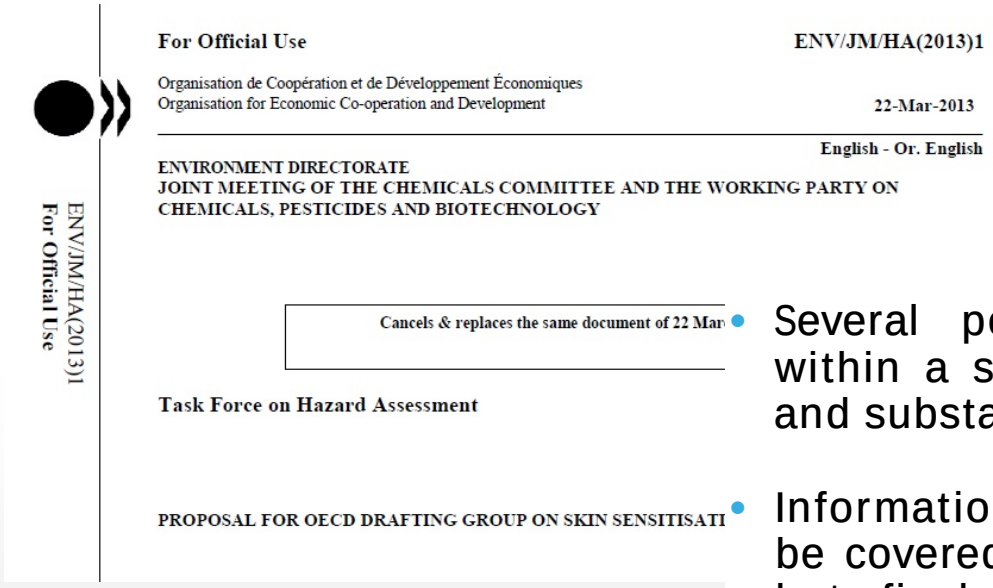
EC–lead OECD Project on the Development of a Guidance Document on the Evaluation and Application of IATA for Skin Sensitisation



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Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development 22-Mar-2013

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 22 Mar

Task Force on Hazard Assessment

PROPOSAL FOR OECD DRAFTING GROUP ON SKIN SENSITISATI

ENV/JM/HA(2013)1
For Official Use

- Several possibilities of combining information within a skin sensitisation IATA (context-specific and substance-tailored)
- Information generated by some of the sources will be covered by Mutual Acceptance of Data (MAD), but final decision will not since, unlike Test Guidelines, IATA fall outside the scope of MAD
- There is therefore a risk of inconsistency in the reporting and evaluation of IATA between OECD Member Countries

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Aims of the OECD project on skin sensitisation IATA

- Definition of a set of principles to promote regulatory consideration of IATA
- To provide guidance to facilitate a harmonised approach for the reporting of IATA to promote consistent evaluation and application within OECD member countries
- Harmonised templates for reporting individual information sources and structured approaches for data integration used within IATA
- Examples of compiled case studies

Release to the OECD HATF foreseen before summer 2015

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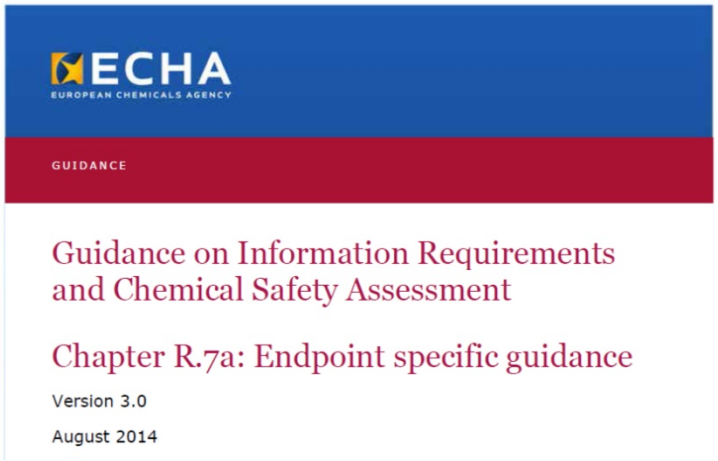
REACH Guidance on IR&CSA



Guidance on Information Requirements and Chemical Safety Assessment, Endpoint specific guidance (Chapter R.7a), **Section R.7.3 on skin and respiratory sensitisation**

- Provides guidance on how to fulfil REACH information requirements using different types of information, existing or newly generated with testing and non-testing methods
- Includes a general Integrated Testing Strategy
- A draft revised version is currently under preparation to take the new developments (AOP, IATA and *in vitro* methods etc.) into account
  - PEG consultation foreseen in summer 2015
  - Public release foreseen before summer 2016

EPAA/LRI/ECHA Workshop Series

- EPAA/LRI/ECHA Workshop (2013): Provided a platform for cross-industry and regulatory dialogue on acceptability of in vitro based ITS/IATA
- Follow-up workshop to be held in April 2015 where a number of proposed ITS/IATA will be discussed


Regulatory Toxicology and Pharmacology xxx (2013) xxx–xxx

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Workshop Report

Skin sensitisation – Moving forward with non-animal testing strategies for regulatory purposes in the EU ☆

David Basketter [a,*], Nathalie Alépée [b], Silvia Casati [c], Jonathan Crozier [d], Dorothea Eigler [e], Peter Griem [f], Bruno Hubesch [g,h], Joop de Knecht [i], Robert Landsiedel [j], Kimmo Louekari [k], Irene Manou [d], Gavin Maxwell [l], Annette Mehling [m], Tatiana Netzeva [k], Thomas Petry [n], Laura H. Rossi [k]

Summary

- The current standard data requirement for REACH is the LLNA, however, tests on animals must only be conducted as a last resort and ANNEX XI describes how standard data requirements can be adapted
- So far, OECD or EU adopted non-animal test method for skin sensitisation were not available
- Given the limited mechanistic coverage and inherent limitations of available methods, combinations of different non-animal methods (*in silico, in chemico, in vitro*) are needed especially to support negative conclusions
- Based on the extensive comparative studies conducted (currently n=180), the 2 out of 3 weight of evidence approach affords high predictivity for skin sensitization hazard identification (slightly better than LLNA). This is in-line with what has been shown in the published literature for other non-animal integration approaches, i.e. they are more predictive than the animal test
- Non-animal methods can be integrated in read across approaches
- Pre- or pro-haptens, highly lipophilic, cytotoxic substances, etc. are challenging; potency assessments remain a challenge
- Well documented integrated approaches may be acceptable for ECHA for substances shown to be in the domain of such approach (peer reviewed publications essential for non-adopted methods). Ongoing OECD activities aim to facilitate the regulatory consideration of ITS/IATA
- No toxicological test is perfect – including the animal tests – it is important to know their strengths and limitations

Some Useful References - 1

OECD Guidance Document No. 168: The adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins: Part 1 and Part 2 available at http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)10/part1&doclanguage=en and http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)10/part2&doclanguage=en

Draft OECD TG442c: In chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA) available at http://www.oecd.org/chemicalsafety/testing/Draft_DPRA_TG_final_15May2014.pdf

Draft OECD TG442d: In vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method available at http://www.oecd.org/chemicalsafety/testing/Draft_Keratinosens_TG_16May_final.pdf

ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance available at http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

ECHA Report 2014 The Use of Alternatives to Testing on Animals for the REACH Regulation: Second report under Article 117(3) of the REACH Regulation available at http://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf

Alternative methods for regulatory toxicology state-of-the-art review available at: http://newsletter.echa.europa.eu/home/-/newsletter/entry/5_14_alternative-methods-to-avoid-testing-on-animals-an-important-new-review

Some Useful References - 2

Basketter D, Alépée N, Casati S, Crozier J, Eigler D, Griem P, Hubesch B, de Knecht J, Landsiedel R, Louekari K, Manou I, Maxwell G, Mehling A, Netzeva T, Petry T, Rossi LH. Skin sensitisation--moving forward with non-animal testing strategies for regulatory purposes in the EU. Regul Toxicol Pharmacol. 2013 Dec;67(3):531-5.

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Maxwell G, MacKay C, Cubberley R, Davies M, Gellatly N, Glavin S, Gouin T, Jacquoilleot S, Moore G, Pendlington R, Saib O, Sheffield D, Stark R, Summerfield V. 2014. Applying the skin sensitisation adverse outcome pathway (AOP) to quantitative risk assessment. Toxicology In Vitro 28: 8-12.

Natsch A, Ryan CA, Foertsch L, Emter R, Jaworska J, Gerberick F, Kern P. A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. J Appl Toxicol. 2013 Nov;33(11)

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Nukada Y, Miyazawa M, Kazutoshi S, Sakaguchi H, Nishiyama N. Data integration of non-animal tests for the development of a test battery to predict the skin sensitizing potential and potency of chemicals. Toxicol In Vitro. 2013 Mar;27(2):609-18.

Some Useful References - 3

Patlewicz G, Kuseva C, Kesova A, Popova I, Zhechev T, Pavlov T, Roberts DW, Mekenyan O. 2014. Towards AOP application – Implementation of an integrated approach to testing and assessment (IATA) into a pipeline for skin sensitization. Regulatory Toxicology and Pharmacology 69: 529-545.

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van der Veen JW, Rorije E, Emter R, Natsch A, van Loveren H, Ezendam J. Evaluating the performance of integrated approaches for hazard identification of skin sensitizing chemicals. Regul Toxicol Pharmacol. 2014 Aug;69(3):371-9.

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What did you think about the webinar? Please take part in our email survey (in your inbox now)

A downloadable recording of this presentation (with slides) will be available shortly.

If you have any questions, please contact Lorna (lorna@chemicalwatch.com)

NEXT

Alternative approaches to mammalian acute toxicity testing, 5 March, 4pm (UK time)
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