Non-testing, testing methods and defined approaches for potency assessment

Silvia Casati

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Joint Research Centre
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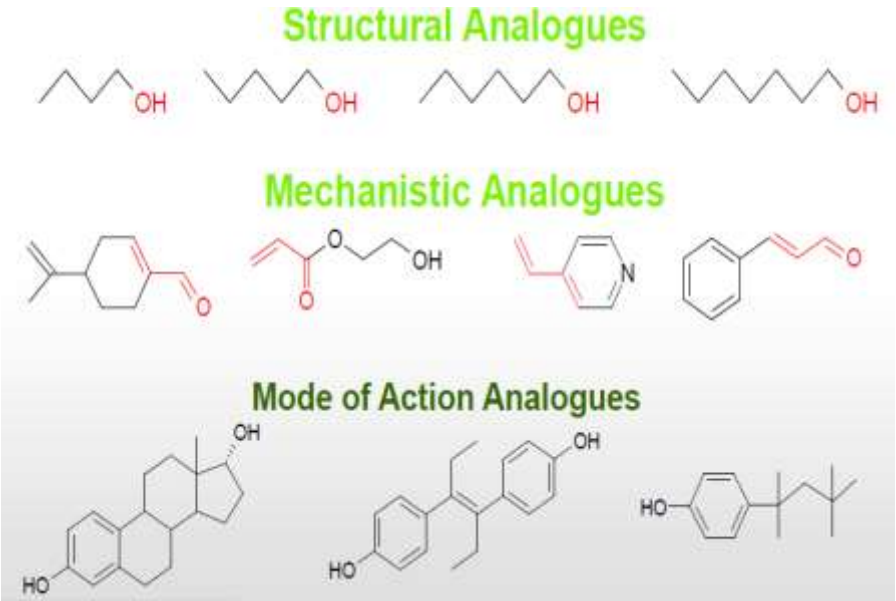
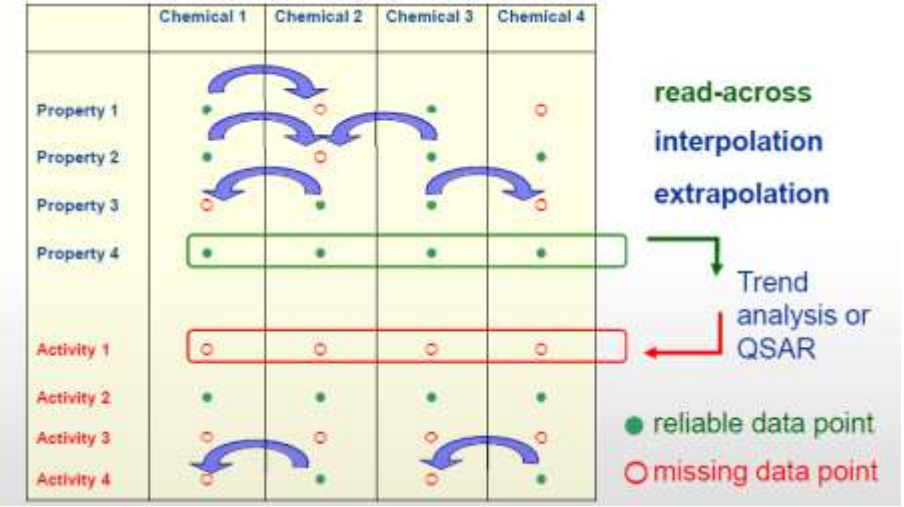
QSARs models

Model	Type	Chemical coverage	Anchor point in the AOP	Endpoint predicted	Role in IATA	References	
Relative alkylation index (RAI) approach	Local QSAR approach	Various RAI derived for specific chemical classes e.g. sulfonate esters, sulfones, primary alkyl bromides, acrylates, aldehydes and diketones	KE4, AO	Most of the RAI models aim to predict the EC3 value in the LLNA, a few predict the outcome in guinea pig tests	Hazard identification and characterisation	Examples include: Roberts and Williams (1982), Roberts et al., (1983, 1991, 2007a), Roberts (1987, 1995), Roberts and Basketter, (1990, 1997, 2000), Patlewicz et al., (2002), Patlewicz et al., (2004), Roberts et al., (1999), Roberts and Patlewicz (2002)	Do not account for metabolism Few chemicals in specific classes with in vivo data
QMM approach which is an extension of the RAI approach	Local QSAR approach	Developed on the basis of Reaction mechanistic domains (Schiff base formers, Michael addition, Acylating agents, SN2)	KE4	EC3 in the LLNA	Hazard identification and characterisation	Examples are : Roberts et al (2006, 2011), Roberts and Natsch (2009); Roberts and Aptula, (2014).	
Various	Global models and expert systems (e.g. MCASE, TOPKAT , TIME-SS)	Broad coverage of chemicals	KE4		Hazard identifiation – semi-quantitative assessment of potency	Various some of them are commercial models	Few of them (e.g. TIME-SS) incorporate simulators for metabolisms

***OECD ENV/JM/HA(2016)11* (draft)**

Grouping and Read-across





Rely on availability of in vivo data

OECD Adopted/Proposed *In Vitro* Methods

Method	Endpoint	Data Interpretation (OECD TG)	Data Interpretation for Potency Assessment
DPRA (TG 442C)	Cys/Lys peptid depletion (0-100	Depletion≤6.38%: NS epletion>6.38%: S	0%≤depl .38%: Minimal reactivity 6.38% ≤22.62%: Low reactivity 22 ≤42.47%: Moderate reactivity 00%: High reactivity
KeratinoSens (TG 442D) (LuS		emical is S if: ase fold on is h , and $EC_{1.5}$	m 12 concentra d to es, which
	4	Tes - RF - RFI_{CL} (viability	e data EC_{150} for CL $_{150}$ or EC_{200}≤13µg, EC_{150} and EC_{200}>13µg,
U-S	duction of CD86	Test chemi - SI_{CD86}≥15 (viability≥70%	Dose-response data from 4 is used to calculate EC_{150} & CV70: - EC_{150}≤40µg/mL and CV70≤ g/mL: cat 1A - EC_{150}>40 µg/mL or CV70>10µg/mL: cat 1B

In vitro vs LLNA
Sensitivity : 74-86% (81%)
Specificity : 68-85% (83%)
Accuracy : 74-81% (82%)

In vitro vs human
Sensitivity : 72-89% (90%)
Specificity : 64-84% (90%)
Accuracy : 82-84% (90%)

LLNA vs human
Sensitivity : 91%
Specificity : 64%
Accuracy : 82%

Urbisch et al. (2015) Regul. Toxicol. Pharmacol. 71, 337-351

93% accuracy in predicting 5 LLNA classes (150 chemicals)

General limitation of in chemico/in vitro tests

- Only address a specific mechanism of the skin sensitisation AOP
- Test substances need to be soluble in the prescribed vehicle(s) - problems with the testing of highly hydrophobic chemicals (not an issue for methods based on reconstituted skin models)
- Some of them technically applicable to the testing of polymers and mixtures (but limited experience available)
- Insufficient metabolic capacity of the test system (i.e. pre- and pro-haptens give false negative results)

Abiotic/metabolic activation

Analysis based on 127 chemicals with LLNA and in vitro data (DPRA, h-CLAT and KeratinoSens™)

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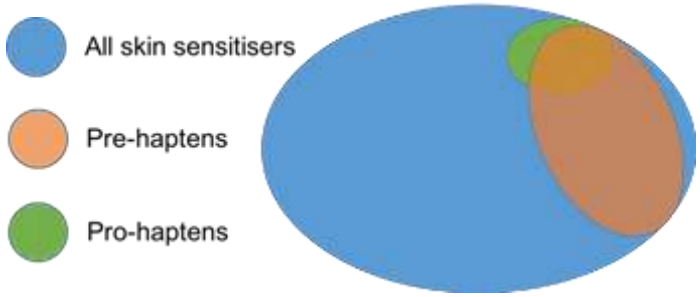
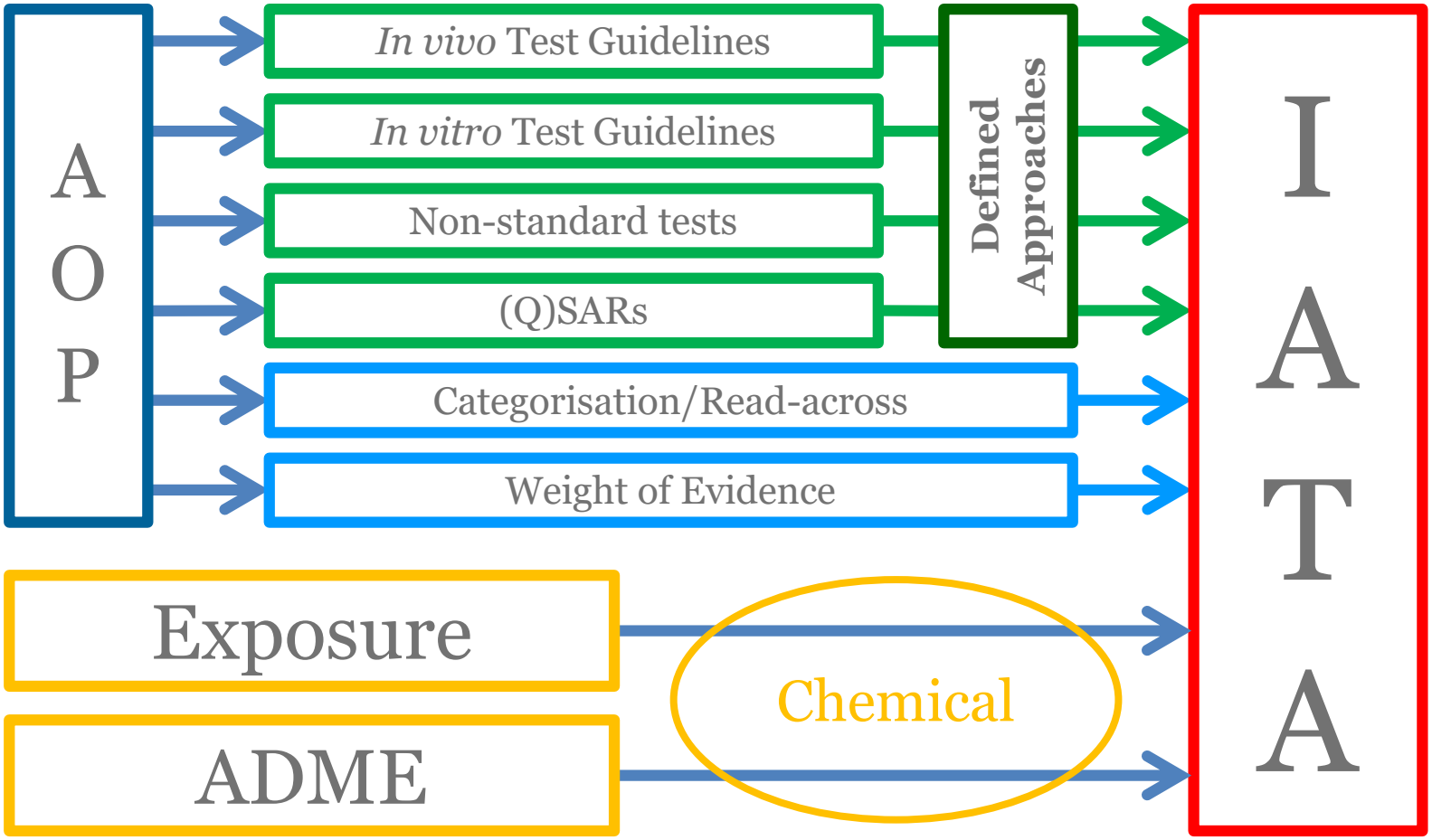
Metabolic / Abiotic Activation

- Approximately 25% of sensitising substances are reported to be pre- or pro- haptens (LLNA data for 319 chemicals; Kern et al., 2010)
- Great majority are pre-haptens



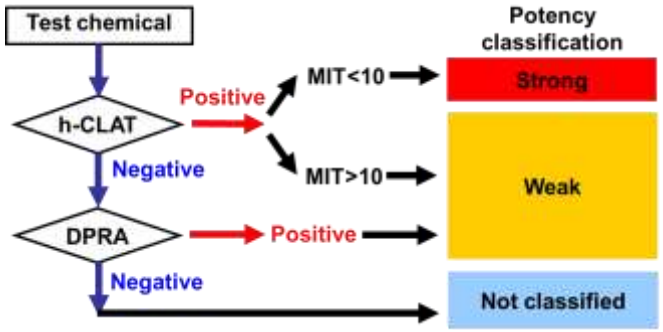
- 22% pre- pro-haptens in the analysed dataset
- Pre-haptens are generally correctly predicted by *in vitro* methods
- Slow oxidisers may not be correctly predicted, just as they would fail to be detected by the *in vivo* methods
- Only 5 chemicals identified as being exclusively pro-haptens
  - ❖ 4 not identified by the DPRA
  - ❖ Correctly predicted by cell-based assays, with h-CLAT detecting the majority
- >90% of pre- and pro-haptens are correctly predicted by *in vitro* methods

Elements within IATA

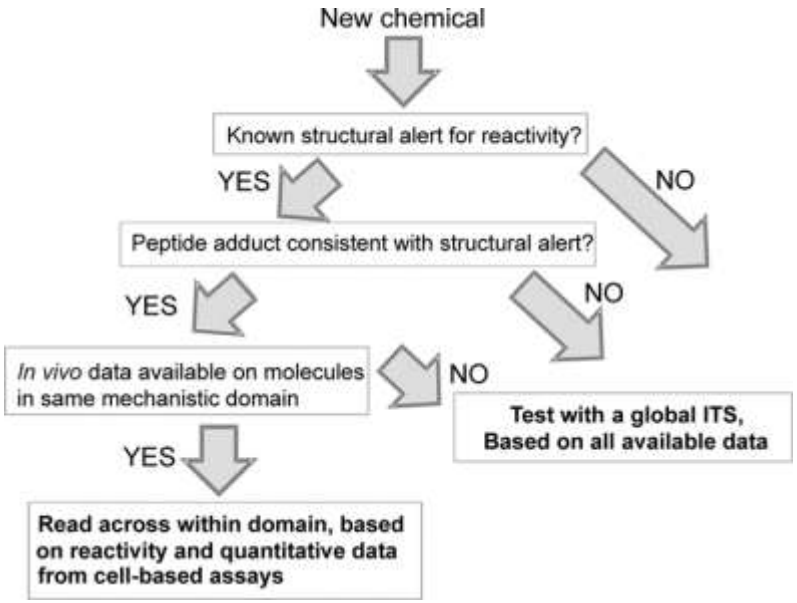


Modified from OECD STA No. 215

Many possibilities of combining information



Takenouchi et al. (2015) J. Appl. Toxicol.: STS & ITS

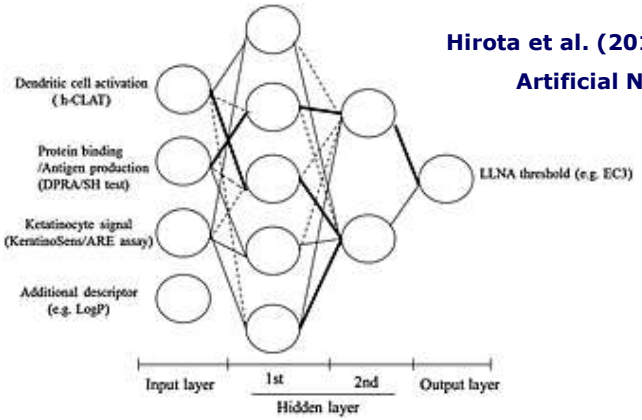


Natsch et al. (2015) Toxicological Science
Global/domain-based asssessment

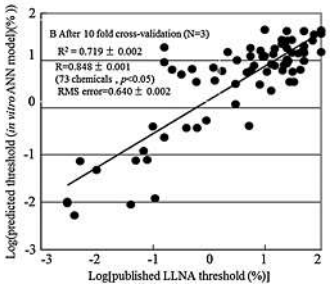
Score	h-CLAT MIT	DPRA depletion	DEREK
3	≤10 µg/mL	≥42.47%	-
2	>10, ≤150 µg/mL	≥22.62, <42.47%	-
1	>150, ≤5000 µg/mL	≥6.376, <22.62%	Alert
0	not calculated	<6.376%	No alert

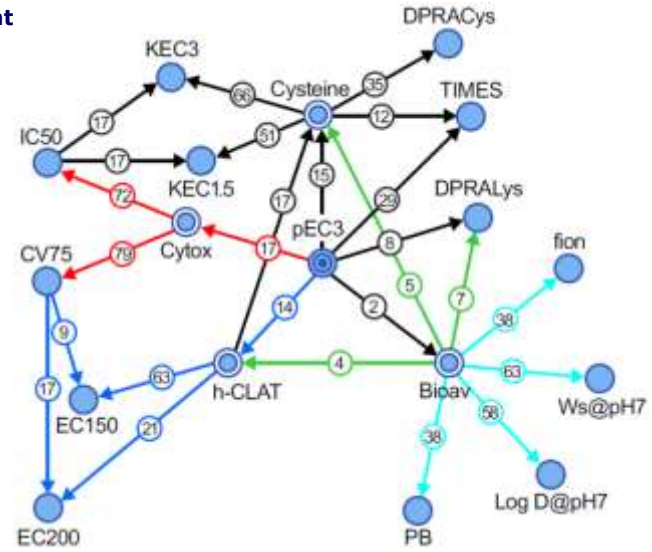
Potency: Total battery score

Strong :	7
Weak :	2-6
Not classified :	0-1



Hirota et al. (2015) J. Appl. Toxicol.:
Artificial Neural Network





Jaworska et al. (2015) Arch. Toxicol.:
Bayesian Network

IATA GD reported case studies

	Case Study	Bioavailability	Phys-chem properties	*In silico*	Protein binding /reactivity	Events in Keratinocytes	Events in DC	Events in T cells	Adverse effect	Others
1	Sensitiser potency prediction Key event 1+2 (**Givaudan**)		X	TIMES SS	Cor1C420-assay	TG 442D				
2	The artificial neural network model for predicting LLNA EC3 (**Shiseido**)		X		SH Test	AREc32 assay	h-CLAT			
3	ITS/DS for hazard and potency identification of skin sensitisers (**P&G**)	penetration (PBPK model)	X	TIMES SS	TG 442C	TG 442D	h-CLAT U937 test	TG 429		
4	Tiered system for predicting sensitising potential and potency of a substance (STS) (**Kao Corporation**)				TG 442C		h-CLAT			
5	Score-based battery system for predicting sensitising potential and potency of a substance (ITS) (**Kao Corporation**)			DEREK Nexus	TG 442C		h-CLAT			
6	IATA for skin sensitisation risk assessment (**Unilever**)	penetration modified OECD TG428			modified OECD TG428					
7	Weight of evidence in vitro ITS for skin hazard identification (**BASF**)				TG 442C	TG 442D LuSens	h-CLAT m-MUSST			
8	STS for hazard identification of skin sensitisers (**RIVM**)			Various	TG 442C	TG 442D HaCaT gene signature	h-CLAT			
9	IATA (**Dupont**)		X	Various	TG 442C glutathione depletion assay	TG 442D	h-CLAT U937	TG 429	TG 406	E.g. Skin Irr/Corr, Ames
10	Decision strategy (**L'Oréal**)		X	Various	TG 442C	TG 442D ARE-Nrf2 Assay	U-SENS™ PGE2 Assay			
11	Integrated decision strategy for skin sensitisation hazard (**ICCVAM**)		X	OECD Toolbox			h-CLAT			
12	Consensus decision tree model for skin sensitisation hazard prediction (**EC JRC**)			TIMES SS Dragon						

TG 442C : DPRA ; TG 442D : KeratinoSens™

Defined approaches for potency prediction

71-96% accuracy for
NS vs 1B vs 1A

Case-studies for predicting (probability distribution) LLNA potency classes or EC3 values

✓Jaworska et al. (2015) Arch. Toxicol., 89, 2355-2383	probability ditribution of potency (4 classes)
✓Hirota et al. (2015) J. Appl. Toxicol. 35, 1333-1347	potency classification (3 classes)
✓Takenouchi et al. (2015) J. Appl. Toxicol. 35, 1318-1332	potency classification (3 classes)
✓Natsch et al. (2015) Toxicol. Sci. 143, 319-332	EC3 or human $DSA_{0.5}$
✓(Maxwell et al. (2014) Toxicol. In Vitro 28, 8-12)	dose-response of human naïve CD 8^+ T cell receptor triggering

Understanding uncertainties

11. 1 Sources of uncertainty

Describe the uncertainties which are considered to be associated with the application of the defined approach by capturing the sources of uncertainty that for example may result from:

1. *The DIP's structure,*
   - *What are the uncertainties related to chosen DIP's structure?*
   - *How does the DIP's coverage or weighting of exposure/toxicokinetic information and/or AOP key events affect your confidence in the overall prediction?*
   - *How does one's confidence in the DIP's prediction vary between different chemicals?*

2. *The information sources used within the defined approach,*
   - *How does variability of the information source's data for a given chemical (i.e. reproducibility) affect one's confidence in the DIP's prediction?*

3. *Benchmark data used,*
   - *How does the reliability and relevance of the reference data for the target of the evaluation (e.g. human, environment) affect one's confidence in the DIP's prediction?*

4. *Others sources*

11.2 Impact of uncertainty on the DIP's prediction

Consider how the individual sources of uncertainty affect the overall uncertainty in the final prediction in the context of the defined approach's application.

***OECD ENV/JM/HA(2016)10* (draft)**

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Variability of reference data

Dumont C, Barroso J, Matys I, Worth A, Casati S.

Analysis of the local lymph node assay (LLNA) variability for assessing the prediction of skin sensitisation potential and potency of chemicals with non-animal approaches.

Toxicol In Vitro.

2016 Apr 13. pii: S0887-2333(16)30075-3.

Uncertainty of LLNA Data

Distribution of LLNA studies (no cat/cat 1B/cat 1A)

GHS Classification				
Group	**No. of chemicals**	**Study distribution (%)**		
		NEG	**cat 1B**	**cat 1A**
NEG group	28 (35)	66 (52)	23 (35)	11 (13)
cat 1B group	50 (65)	16 (18)	68 (68)	16 (14)
cat 1A group	41 (36)	6 (8)	15 (23)	79 (69)

X (Y)

X: solvent effect considered

Y: solvent effect not considered

NEG group: chemicals with at least 1 negative study
cat 1B group: chemicals with at least 1 Cat 1B study
cat 1A group: chemicals with at least 1 Cat 1A study

- **NEG group:** 52-66% of the studies are negative
- **cat 1B group:** 68% of the studies are cat 1B
- **cat 1A group:** 69-79% of the studies are cat 1A

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