

Evaluating Defined Approaches to Testing and Assessment of Skin Sensitization Potential

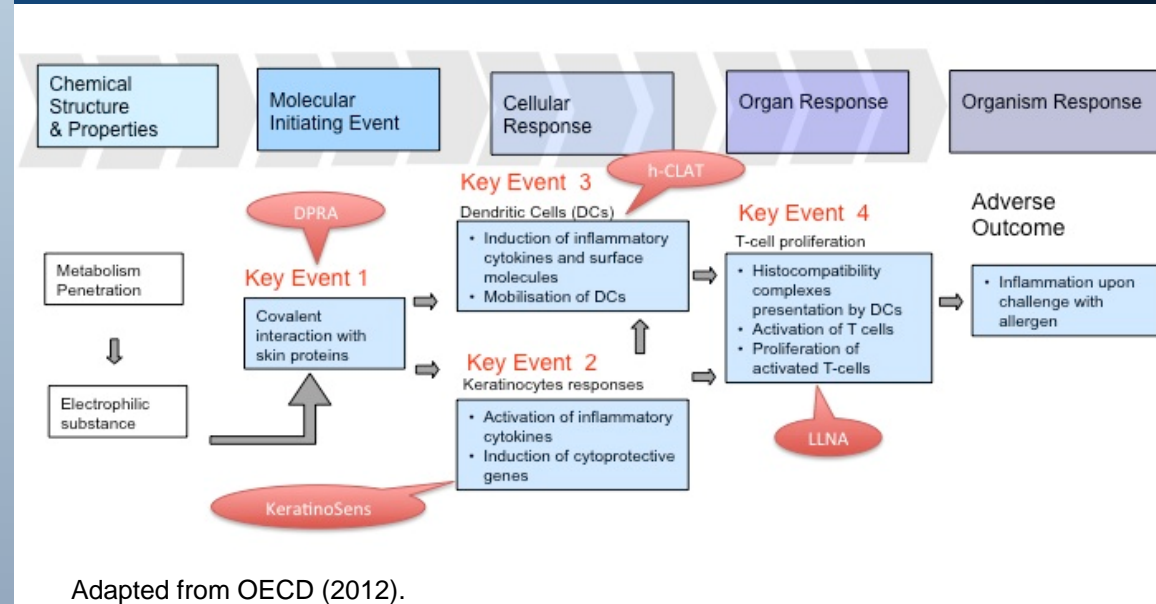
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Introduction

- Skin sensitization is a toxicity endpoint of widespread concern, and testing for skin sensitization potential is required for many chemical products. Testing and data requirements vary by country and regulatory authority. The murine local lymph node assay (LLNA), which uses up to 20 animals per substance tested, is currently the preferred test for most regulatory applications.
- The mechanistic understanding of the biological process involved in skin sensitization has evolved to support the development of methods that predict skin sensitization potential of chemicals without using animals (**Figure 1**).
- Defined approaches to testing and assessment built on this mechanistic understanding can combine multiple information sources to produce predictions of skin sensitization potential that can be more accurate than a prediction derived from a single test method.
- In this poster, we present an overview of an international workshop that evaluated the suitability of non-animal defined approaches to assess the skin sensitization potential of chemicals.

Figure 1. Adverse Outcome Pathway for Skin Sensitization Produced by Substances That Covalently Bind to Proteins



ICATM Skin Sensitization Workshop

- The International Cooperation on Alternative Test Methods (ICATM) (**Table 1**) was established by international validation organizations to enhance international cooperation in validation and promotion of alternative test methods and strategies for regulatory use.
- ICATM members organized a workshop on the International Regulatory Applicability and Acceptance of Alternative Approaches to Skin Sensitization Assessment of Chemicals, which was hosted by the Joint Research Centre of the European Commission on October 4-5, 2016. The objectives of the workshop were to:
 - Facilitate a common understanding of the non-animal approaches (i.e., in vitro, in chemico, in silico and read-across) that are available and their current proposed use (i.e., within defined approaches and integrated approaches to testing and assessment [IATA])
 - Identify the current regulatory requirements for skin sensitization in different regions and countries by chemical sector that could potentially be satisfied with the use of non-animal approaches
 - Identify the obstacles that hamper the use of non-animal approaches in certain regulatory areas and regions and define what steps should be taken to support their regulatory application
 - Discuss the evaluation and acceptance processes associated with the use of defined approaches and IATA
 - Define a set of performance-based criteria for regulatory use of defined approaches
 - Issue recommendations on the use of defined approaches for specific regulatory applications for specific chemical sectors
- The workshop drew over 40 attendees representing ICATM partner and participant groups and regulatory authorities from Austria, Brazil, Canada, China, Czech Republic, Denmark, European Union, Germany, Japan, South Korea, United Kingdom, and United States.
- Workshop participants surveyed the specific regulatory needs and uses for skin sensitization information for seven countries/regions (**Table 1**).

Table 1. Regulatory Requirements for Skin Sensitization Hazard Evaluation, Potency Classification, and Risk Assessment

Chemical Sector	Canada ^a	European Union ^a	Japan ^a	South Korea ^a	United States ^a	Brazil	China
Pesticides	Hazard	Hazard	Hazard	Hazard	Hazard	Hazard	Potency
Pharmaceuticals	Hazard, risk	Hazard	Not specified	Hazard, potency	Potency	Hazard	NI
Cosmetics and Personal Care Products	Risk ^b	Hazard, potency, risk	Hazard, potency	Hazard	Not required	Hazard	Potency
Household Substances and Art Materials	NI	NI	NI	NI	Hazard, potency	NI	NI
Workplace Chemicals	Potency	NI	NI	Hazard	Hazard, potency	NI	Not specified
Industrial Chemicals	Potency, risk	Potency, risk	NI	Hazard, risk	Risk	NI	Hazard, potency, risk

NI = participants provided no information on the requirements for the chemical sector in this country/region.
^aICATM member countries; Brazil and China participate in ICATM activities but are not formal members.
^b Skin sensitization data are typically not required, but risk assessment is required for prohibited or restricted substances.

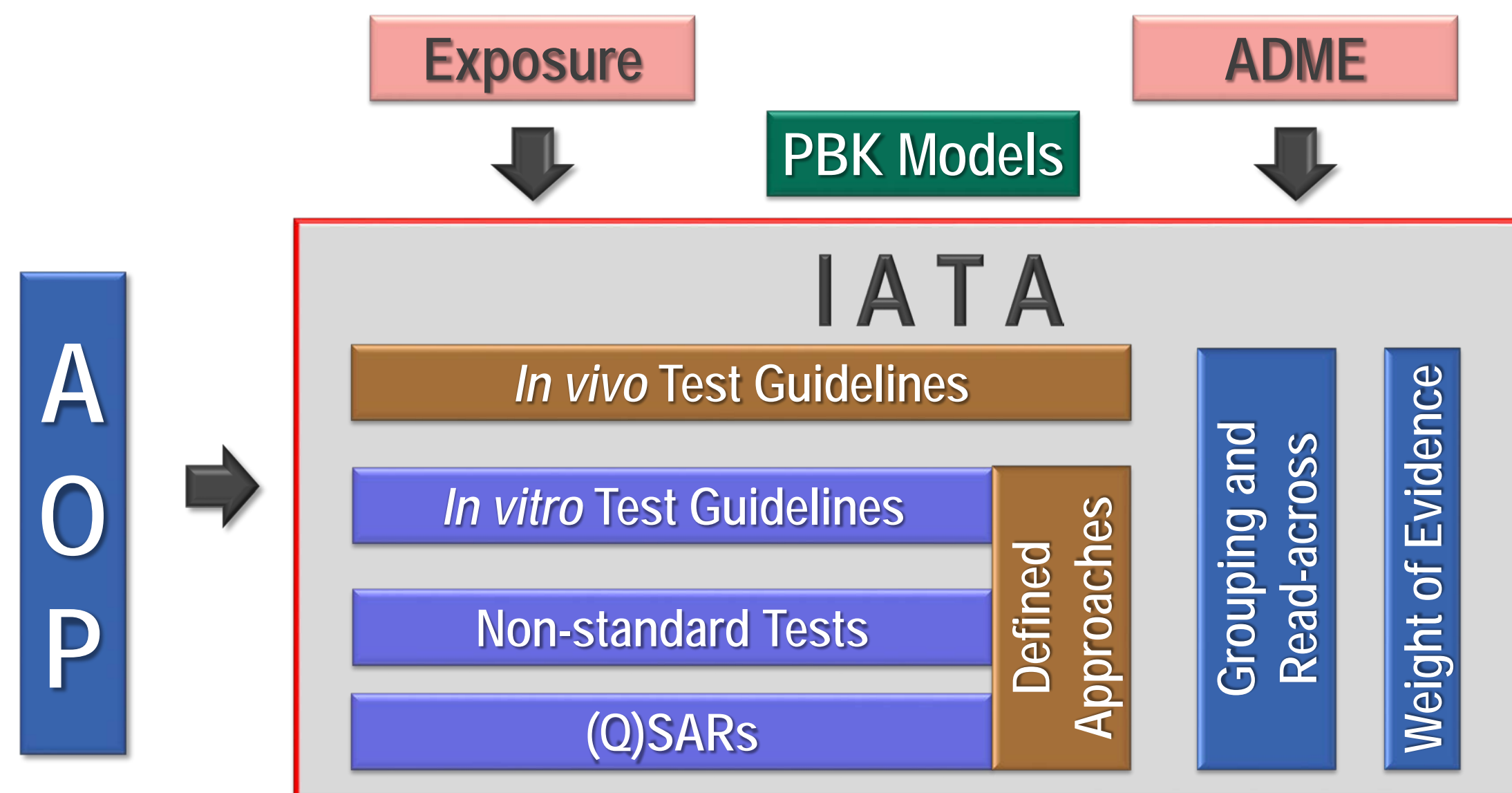
Available Non-Animal Test Methods

- The Organisation for Economic Co-operation and Development (OECD) has issued test guidelines for three non-animal in chemico or in vitro skin sensitization test methods that align with the adverse outcome pathway (AOP) for skin sensitization for substances that bind covalently to skin proteins (OECD 2012) (**Figure 1**).
 - Test Guideline 442C: The direct peptide reactivity assay (DPRA) measures covalent interaction with proteins (Key Event 1) (OECD 2015a).
 - Test Guideline 442D: The ARE-Nrf2 luciferase test method (i.e., KeratinoSens™ assay) measures activation of cytoprotective genes in keratinocytes (Key Event 2) (OECD 2015b).
 - Test Guideline 442E: The human cell line activation test (h-CLAT) measures activation and mobilization of dendritic cells in the skin (Key Event 3) (OECD 2016a).
- All three methods are recommended for use in integrated strategies, rather than as stand-alone tests, to classify substances for skin sensitization hazard.

Defined Approaches for Skin Sensitization

- A defined approach may incorporate data from multiple sources to predict the skin sensitization hazard or potency posed by a chemical using:
 - A fixed data interpretation procedure (based on, for example, a statistical or mathematical model) applied to**
 - Data, such as in chemico or in vitro data or in silico predictions, generated with**
 - A defined set of information sources (such as specific assays or computational methods)**
- In contrast to IATA (**Figure 2**), which utilize expert judgment, predictions generated with defined approaches are rule-based. These predictions can either be used on their own to predict chemical hazard or potency or considered with other sources of information.

Figure 2. Relationship of IATA to Defined Approaches and Other Elements of Chemical Safety Assessment



ADME = absorption, distribution, metabolism, and excretion; PBK = physiological biokinetic; (Q)SAR = quantitative structure-activity relationship

Available Defined Approaches

- A number of defined approaches have been published (Asturiol et al. 2016; Gomes et al. 2014; Hirota et al. 2015; Jaworska et al. 2015; Natsch et al. 2015; Patlewicz et al. 2014; Strickland et al. 2016; Takenouchi et al. 2015; Urbisch et al. 2015; Van der Veen et al. 2014). These defined approaches:
 - Are included in a guidance document from OECD as case studies to illustrate how defined approaches for skin sensitization assessment and the information sources used therein should be documented (OECD 2016b,c)
 - Use the AOP (**Figure 1**) as a conceptual framework and include data from one or more of the non-animal OECD test guidelines, along with other information

Evaluation of Defined Approaches

- Workshop participants agreed that the performance of defined approaches should be evaluated against the performance of the current animal models used for regulatory purposes.
 - The available evaluations of accuracy and reproducibility use different sets of chemicals.
- Accuracy of the current animal models for predicting human sensitizer/nonsensitizer outcomes:
 - For the LLNA, ICCVAM (1999) reported accuracy of 72% (41/57), Urbisch et al. (2015) reported 82% (91/111) accuracy, and Strickland et al. (2017) reported 84% (81/96) accuracy.
 - For the guinea pig maximization and the Buehler tests, accuracy was 72% (41/57) (ICCVAM 1999).
- Accuracy of the current animal models for predicting human skin sensitization potency classification in three categories (1A sensitizer, 1B sensitizer, and nonsensitizer) used by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS; UN 2015):
 - For the LLNA, accuracy has been reported from 54% (74/136) (ICCVAM 2011) to 69% (60/87) (Zang et al. 2017).
 - For the guinea pig maximization and the Buehler tests, accuracy was 59% (33/56), which was similar to that of the LLNA (61% [34/56]) for the same set of chemicals (ICCVAM 2011).
- A number of evaluations of the variability of the LLNA EC3 (effective concentration at the stimulation index of three, the threshold for a positive response) show that it may vary by four to five-fold (e.g., Jowsey et al. 2008). A recent evaluation of LLNA reproducibility for predicting response categories reported:
 - For sensitizer/nonsensitizer outcomes, 78% (68/87) of the chemicals evaluated had concordant responses when the solvents were the same for each test of each chemical and 68% (63/93) of the chemicals had concordant responses when the solvents were not the same for each test of each chemical (Dumont et al. 2016).
 - For placing chemicals in GHS potency categories, the concordance of LLNA outcomes was 62% (53/85) when the solvents were the same and 49% (43/87) when the solvents were not necessarily the same for each test of each chemical (Dumont et al. 2016).
- There are few reports on the reproducibility of guinea pig tests:
 - Basketter et al. (1993) reported that the guinea pig maximization test and the Buehler test produced concordant positive results for two chemicals in two laboratories.
 - Basketter and Gerberick (1996) reported that the Buehler test produced consistent sensitizer/nonsensitizer results for three chemicals tested in two laboratories.

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Discussion and Conclusion

- Workshop attendees agreed that there are non-animal defined approaches that are sufficiently predictive to be used instead of the current animal tests.
- Workshop participants cited a number of obstacles to widespread regulatory acceptance of non-animal approaches to skin sensitization assessment. Needs to be addressed include training in both conducting the approaches and interpreting output, as well as commercial availability of alternative tests. Clear guidance for the application of multiple defined approaches is also needed because it is unlikely that a single defined approach will be applicable to all chemical sectors.
- As a follow-up activity to the workshop, ICATM proposed a new performance-based test guideline to the OECD Test Guidelines Programme. The proposed test guideline will:
 - Define performance criteria for non-animal methods for the assessment of skin sensitization
 - Include non-animal methods and strategies that meet the criteria
- The OECD Working Group of National Coordinators will consider development of the proposed performance-based test guideline in April 2017.

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