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

27-Oct-2016

English - Or. English

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

GUIDANCE DOCUMENT ON THE REPORTING OF DEFINED APPROACHES AND INDIVIDUAL INFORMATION SOURCES TO BE USED WITHIN INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA) FOR SKIN SENSITISATION

Series on Testing & Assessment
No. 256

The corresponding annexes are available in the following cotes:
ENV/JM/MONO(2016)29/ANN1 and ENV/JM/MONO(2016)29/ANN2

JT03403923

Complete document available on OLIS in its original format

ENV/JM/MONO(2016)29
Unclassified

English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing & Assessment

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IOMC

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Paris, 2016

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FOREWORD

With a view to assisting the evaluation of integrated approaches to testing and assessment (IATA) in regulatory decision-making within OECD Member Countries, this guidance document provides guidance on the reporting of defined approaches to testing and assessment. A defined approach consists of a fixed data interpretation procedure (DIP) (e.g. statistical, mathematical models) applied to data (e.g *in silico* predictions, *in chemico*, *in vitro* data) generated with a defined set of information sources to derive a prediction. In contrast to the assessment process within Integrated Approaches to Testing and Assessment (IATA), that necessarily involves some degree of expert judgment, predictions generated with defined approaches are rule-based and can either be used on their own if they are deemed to fit-for-purpose or considered together with other sources of information in the context of IATA.

The template for reporting defined approaches to testing and assessment based on multiple information sources and the template for reporting individual information sources are provided in guidance document ENV/JM/MONO(2016)28 and they have been used by an ad-hoc expert group to document a number of defined approaches developed in the area of skin sensitisation using the adverse outcome pathway (AOP) as a conceptual framework. These defined approaches are proposed for hazard and/or potency prediction. It is not the intent of this document to seek for endorsement of any specific defined approach provided in the case studies, but rather provide a perspective of how individual information sources and defined approaches developed for skin sensitisation assessment should be reported in a harmonised way and to illustrate what forms these may take, whether they are statistically derived, or qualitative in nature, and serve different purposes (i.e. hazard versus potency prediction). A harmonised approach in the reporting of the different elements used within IATA is critical to ensure consistency in the use of IATA-derived predictions/assessments for regulatory decisions and to promote mutual acceptance of such assessments. The present document was endorsed by the Task Force on Hazard Assessment in June 2016.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, which has agreed that it be declassified and made available to the public.

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1. INTRODUCTION

This document provides guidance on the reporting of defined approaches to testing and assessment. A defined approach consists of a fixed data interpretation procedure (DIP) (e.g. statistical, mathematical models) applied to data (e.g *in silico* predictions, *in chemico*, *in vitro* data) generated with a defined set of information sources to derive a prediction. In contrast to the assessment process within Integrated Approaches to Testing and Assessment (IATA), that necessarily involves some degree of expert judgment, predictions generated with defined approaches are rule-based and can either be used on their own if they are deemed to fit-for-purpose or considered together with other sources of information in the context of IATA.

This document is not intended to endorse any specific defined approach exemplified in the case studies. The case studies are provided as examples of the level of information needed to facilitate a harmonised approach to the reporting of defined approaches that can be used as elements within IATA specifically in the field of skin sensitisation. A harmonised approach in reporting the different IATA elements is critical to ensure consistency in the use of IATA-derived predictions/assessments for regulatory decisions and to promote mutual acceptance of such assessments.

2. BACKGROUND

Allergic contact dermatitis (ACD) is the clinical manifestation of a changed responsiveness of the adaptive immune system following repeated exposure to a sensitising substance. The development of ACD is characterised by two distinct phases: 1) the induction of specialised immunological memory following the initial exposure to the allergen, termed sensitisation and 2) elicitation of the visible, clinical allergic response following subsequent exposure to the allergen.

Historically, predictive tests to identify and characterise substances causing ACD have used animals. The standard and accepted skin sensitisation test methods, for which OECD guidelines are available, include the guinea-pig maximisation test (GPMT) according to Magnusson and Kligman and the occluded patch test of Buehler (TG 406), where the endpoint measured is elicitation (i.e. the organism response/adverse outcome); and the mouse local lymph node assay (LLNA; TG 429) and its non-radioactive variants (TG 442a and TG 442b) where the endpoint measured is cell proliferation in the lymph node (i.e. organ response/induction).

There is general agreement on the key chemical and biological events underlying skin sensitisation (e.g. Karlberg et al., 2008; Vocanson et al. 2009; Adler et al., 2010; Martin et al., 2010; Kimber et al., 2011), and this knowledge has now been summarised by the OECD in the report entitled: "The Adverse Outcome Pathway (AOP) for skin sensitisation initiated by covalent binding to proteins" (OECD, 2012a, 2012b) to facilitate the development of toxicological assays and strategies to assess this toxicological endpoint.

The skin sensitisation AOP identifies four key events (KEs) with KE_1, the covalent binding to skin proteins (termed haptenation) either of the parent substance or of its reactive derivatives following abiotic/metabolic activation, which is postulated to be the molecular initiating event (MIE), followed by KE_2, the activation of epidermal keratinocytes, KE_3, the activation (maturation) and mobilisation of Langerhans cell and dermal dendritic cells (DC), and KE_4, the DC-mediated antigen presentation to naïve T-cells and proliferation /activation of allergen specific T-cells (Figure 1).

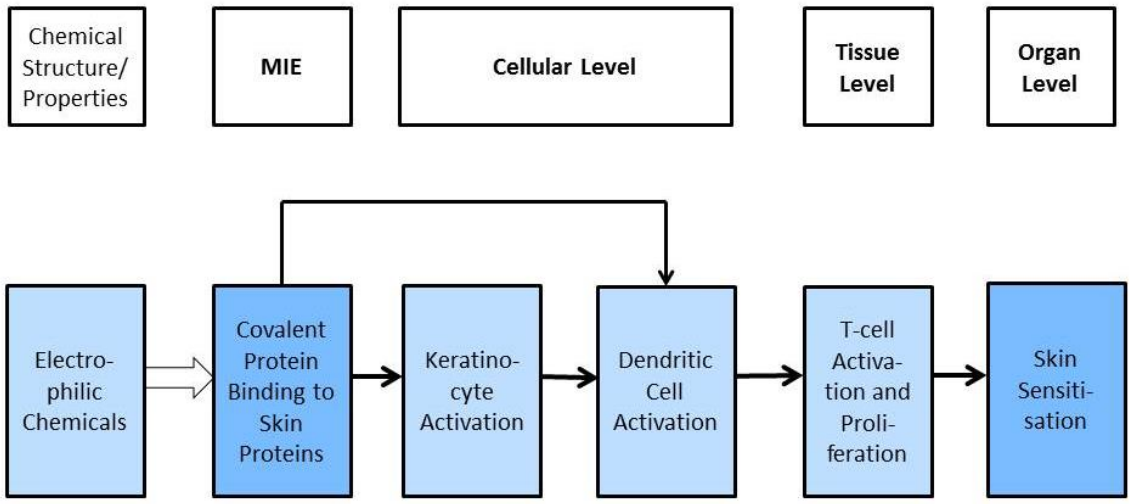

Figure 1: Flow diagram of the pathways and the intermediate steps associated with skin sensitisation (adapted from OECD, 2012a).

Knowledge of the skin sensitisation pathway has prompted the development of alternative methods (*in silico*, *in chemico*, *in vitro*) addressing specific KEs. Information generated by these methods can contribute to the assessment of the skin sensitisation potential and potency of chemicals when used as information sources within defined approaches and IATA. Within such AOP-informed defined approaches/IATA, the different information sources would target KEs along the defined toxicity pathway and the results could be used to inform a regulatory decision.

Non-testing and testing methods are available to estimate penetration, simulate metabolism or abiotic transformation processes as well as identify electrophilic features and quantify their reactivity. *In chemico* and *in vitro* assays are also available to measure reactivity, informing about the ability of a substance to activate the MIE. *In vitro* assays are available to characterise keratinocyte inflammatory responses and to measure markers of dendritic/monocytic cell activation. These methods are able to characterise a number of the KEs in the skin sensitisation AOP and in doing so form the basis of AOP-informed defined approaches and IATA. Exposure considerations and an understanding of bioavailability may also inform the defined approach or IATA, though these components fall outside of the definition of an AOP (OECD, 2013).

The availability of non-animal methods for skin sensitisation favoured in recent years the development of defined approaches to testing and assessments which, in most cases, are designed to predict an existing line of evidence (i.e. responses in animal models or in humans). Within such defined approaches data generated with selected sources of information (i.e. physicochemical properties, *in silico*, *in chemico*, *in vitro* data etc.) are converted into predictions by applying a DIP. Examples of DIP include mathematical and statistical models.

Predictions generated with defined approaches can be used on their own if considered adequate for a specific regulatory application or may be evaluated together with other information sources in the assessment process within IATA. In such a case a defined approach would be considered as an IATA component.

3. MAPPING OF INFORMATION SOURCES THAT CAN BE USED WITHIN DEFINED APPROACHES AND IATA FOR SKIN SENSITISATION BY APPLYING THE AOP AS A FRAMEWORK

Depending on the final purpose (e.g. hazard or risk), the assessment of skin sensitisation can include: consideration of the expected exposure to the substance being evaluated, an understanding of dermal bioavailability including skin penetration and metabolism, information on KEs and any other supporting information, i.e. information from non-testing and testing methods designed to address other health or environmental endpoints that nevertheless may inform skin sensitisation assessment. The possible elements and information sources that can be used within defined approaches and IATA for skin sensitisation assessment are listed in Table 1. Some of the elements, highlighted in grey in Table 1, address KEs within the skin sensitisation AOP. Note that this is not an exhaustive list and does not imply any judgement about the suitability of any of the listed information sources for a specific assessment.

It has to be noted that the elements addressed within a specific defined approach or IATA and the type of information sources used to populate each individual element may vary depending on the scope and the specific regulatory requirement. This implies that for certain regulatory purposes (e.g. hazard identification) the assessment may be conducted by addressing fewer elements than in the case of more complex regulatory needs (e.g. risk assessment). It is therefore envisaged that different defined approaches and IATA solutions may be possible depending on the chemical under investigation, the regulatory need and the specific regulatory requirements in the different regions.

Table 1: Elements and examples of information sources that can be used within defined approaches and IATAs for skin sensitisation

Elements	**Information sources addressing each element**
Exposure consideration	• Applied dose • Frequency of exposure • Formulation effects • *In vitro* to *in vivo* extrapolation
Chemical descriptors	**Chemical structure** **Physico-chemical properties** • Molecular Weight • Physical state • pKa • Log Kow • Evaporation rate/Vapour pressure • Water solubility
Dermal bioavailability − **Skin penetration**	**Non-testing methods** • Characterisation of skin absorption (e.g. physiologically based-pharmacokinetic (PBPK) models **Testing methods** • TG 428 (Skin absorption: *in vitro* method) • TG 427 (Skin absorption: *in vivo* method) • TG 428 modified to include time course (Pendlington et al., 2008; Davies et al., 2011)
− **Skin metabolism**	**Non testing methods:** • *In silico* e.g. structure-metabolism rules encoded in the expert system TIMES-SS, Meteor Nexus, simulators for skin metabolism and autoxidation within the OECD Toolbox **Testing methods** • 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 • Protein binding/reactivity alerts (e.g. OECD Toolbox, Derek Nexus, Toxtree, TIMES-SS)[1] **Testing methods** • TG 442C (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 et al., 2013) − Amino acid Derivative Reactivity Assay (ADRA; Yamamoto et al., 2015) − SH test (Suzuki et al., 2009)
AOP key event 2: events in Keratinocytes	
Activation of biochemical pathways	**Testing methods** • TG 442D (ARE-Nrf2 Luciferase Test Method-KeratinoSens™) • LuSens (Ramirez et al., 2014, 2016) • AREc32 cell line assay (Natsch and Emter, 2008).
Pathways-associated gene expression	• SENS-IS (Cottrez et al., 2015, 2016) • HaCaT gene signature (van der Veen et al., 2013) • SenCeeTox (McKim et al., 2012) • Epidermal Sensitization Assay (EpiSensA; Saito et al., 2013)
Pathways-associated protein expression	• Proteomic signature in keratinocytes (Thierse et al., 2011)
Release of pro-inflammatory mediators	• RhE-IL-18 (Gibbs et al., 2013)
AOP key Event 3: Events in Dendritic cell	
Expression of co-stimulatory and adhesion molecules in dendritic / monocytic cells	**Testing methods** • h-CLAT (Ashikaga et al., 2010; TG 442E) • U-SENS™ (Piroird et al., 2015) • modified MUSST (Bauch et al., 2012) • PBMDC (Reuter et al., 2011)
Pathways-associated protein	• MUTZ SensiDerm (Thierse et al., 2011)

expression in dendritic / monocytic cells **Pathways-associated gene expression in dendritic / monocytic cells**	• 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 • 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** • TG 429 (LLNA) • TG 442A (LLNA: DA) • TG 442B (LLNA: BrdU-ELISA)
AOP Adverse Outcome	
	(Existing) human data • Human Repeat Insult Patch Test (HRIPT) • Human Maximisation Test • Clinical data • Data from occupational exposure • Epidemiological data **(Existing) animal data** • TG 406 (Guinea-pig Maximisation Test; Buehler Test)
Others	• Skin corrosion (e.g. OECD TG 430,431,435, 404) • Skin irritation (e.g. OECD TG 439, 404) • Genotoxicity (e.g. OECD TG 471) (see Wolfreys and Basketter, 2004; Patlewicz et al., 2010; Mekenyan et al., 2010)

[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.

The sorts of (Q)SAR models that are available for skin sensitisation are provided in Table 2 for illustrative purposes. For more information, reviews describing the available *in silico* approaches for skin sensitisation include Patlewicz and Worth (2008) and more recently Sharma et al. (2012).

Table 2: QSARs models for skin sensitisation

Model	Type	Chemical coverage	Availability	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	Published in the literature	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)
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)	Published in the literature	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 e.g. Estrada et al., (2003)	Global models	Mainly based on the Gerberick et al. (2005) dataset hence cover a broad coverage of chemicals	Variable	KE4	Potency categorisation as defined by EC3 values in the LLNA	Hazard identification – semi-quantitative assessment of potency	Many were reviewed in Roberts et al. (2007b)
TOPKAT	Expert system (statistical)	Based mainly on the datasets published by Cronin and Basketter (1994) hence reasonably broad coverage of chemicals	Commercial	AO	Binary model to predict likelihood of sensitisation and additional model to estimate qualitatively the potency as defined in the GPMT	Hazard identification – semi-quantitative assessment of potency	http://www.accelrys.com/products/topkat/
MCASE Suite of models to predict each of the KEs in the AOP	Expert system (statistical)	Broad coverage of chemicals	Commercial	KE1 (MIE), KE2, KE3, KE4, AO	Models to predict the outcome of the DPRA, ARE activation, n-CLAT, EC3 potency bands and overall binary sensitisation outcome	Hazard identification – semi-quantitative assessment of potency	http://www.multicase.com/case-ultra-models#skin_eye_tox_bundle
Derek	Expert	Broad coverage	Commercial	KE4, AO	Qualitative	Hazard	http://www.lhasalimited.org/index.php

Nexus	system (Knowledge based)	of chemicals			likelihood of skin sensitisation potential	identification	
TIMES-SS	Expert system (Hybrid)	Broad coverage of chemicals	Commercial	AO	Based on data from LLNA, GPMT and Human	Hazard identification – semi-quantitative assessment of potency	Dimitrov et al., (2005); Patlewicz et al., (2007, 2014)

(Q)SAR predictions may be gathered from databases (in which the predictions have already been generated and documented) or generated *de novo* through the available models. Most (Q)SARs do not account for transformation of a substance explicitly. Some expert systems such as TIMES-SS incorporate simulators for metabolism so that predictions for parent compounds and their metabolites are considered at the same time in making an overall prediction of activity. Derek Nexus can be linked to its Meteor Nexus metabolism program to make predictions of parent compounds and their estimated metabolites. The OECD toolbox incorporates simulators for metabolism and degradation such that a parent chemical and its expected metabolites can be profiled together for the purposes of forming chemical categories to facilitate data gap filling.

Conclusions about the likely properties of a substance can also be based on the knowledge of the properties of one or more similar chemicals, by applying chemical grouping methods.

The OECD guidance document, Series on Testing and Assessment No. 194 provides information on the use of chemical grouping and read-across approaches (OECD, 2014). As with (Q)SARs, grouping approaches can be used to indicate either the presence or the absence of an effect.

4. DEFINED APPROACHES TO TESTING AND ASSESSMENT AND THEIR ROLE WITHIN IATA FOR SKIN SENSITISATION

In the area of skin sensitisation the availability of a suite of non-animal *in silico*, *in chemico* and *in vitro* methods has prompted the development of defined approaches based on the integration of readouts from these methods. As defined in the OECD guidance document ENV/JM/MONO(2016)28, defined approaches to testing and assessment are based on a fixed set of information sources and a fixed data interpretation procedure (DIP) to convert inputs from the different information sources into a prediction.

The DIP within defined approaches can range from simple rule-based decision steps to mathematical and statistical models. In contrast to the WoE process, in a defined approach the weighting of the different information is fixed and does not leave room for subjective interpretation. The final prediction can be used on its own if fit-for-purpose to satisfy a specific regulatory need or can be used as a component within IATA and thus considered in the WoE assessment together with other relevant information (see Table 1).

In contrast to an IATA that is customised for the chemical/class of chemicals under investigation and the specific regulatory need, defined approaches are generally designed to be applicable to a larger chemical space and most of those available in the area of skin sensitisation have been developed to predict an existing line of evidence (e.g. LLNA hazards or potency).

An overview of the defined approaches, documented in more details in Annex I (ENV/JM/MONO(2016)29/ANN1), is provided in Table 3.

Table 3: Defined approaches to testing and assessment documented in Annex I.

Case study		**Purpose**
I	An Adverse Outcome Pathway-based "2 out of 3" integrated testing strategy approach to skin hazard identification (BASF)	Hazard identification
II	Sequential Testing Strategy (STS) for hazard identification of skin sensitisers (RIVM)	Hazard identification
III	A non-testing Pipeline approach for skin sensitisation (G. Patlewicz)	Hazard identification
IV	Stacking meta-model for skin sensitisation hazard identification (L'Oréal)	Hazard identification
V	Integrated decision strategy for skin sensitisation hazard (ICCVAM)	Hazard identification
VI	Consensus of classification trees for skin sensitisation hazard prediction (EC- JRC)	Hazard identification

VII	Sensitizer potency prediction based on Key event 1 + 2: Combination of kinetic peptide reactivity data and KeratinoSens® data (Givaudan)	Potency prediction
VIII	The artificial neural network model for predicting LLNA EC3 (Shiseido)	Potency prediction
IX	Bayesian Network DIP (BN-ITS-3) for hazard and potency identification of skin sensitizers (P&G)	Potency prediction
X	Sequential testing strategy (STS) for sensitising potency classification based on in chemico and in vitro data (Kao Corporation)	Potency prediction
XI	Integrated testing strategy (ITS) for sensitising potency classification based on in silico, in chemico, and in vitro data (Kao Corporation)	Potency prediction
XII	DIP for skin allergy risk assessment (SARA) (Unilever)	Potency prediction

The intent of this guidance document is to exemplify how these defined approaches and the information sources used within (see Annex II in ENV/JM/MONO(2016)29/ANN2) should be documented to facilitate a harmonised methodology in their reporting, critical to ensure consistency in the use of IATA-derived predictions/assessment for regulatory decisions.

The case studies documented in this guidance document do not imply acceptance or endorsement by any Member Country or OECD. They are intended only to provide a perspective of how individual information sources and defined approaches, used on their own or within an IATA for skin sensitisation, should be reported and to illustrate what forms these may take, whether they are statistically derived, or qualitative in nature, and serve different purposes (i.e. hazard versus potency prediction).

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