Background Document on \textit{in vitro} testing for skin sensitisation

\textit{ECHA, with the support of selected members of the Member State Committee (MSC) and of the Committee for Risk Assessment (RAC), has drafted this document in the context of the preparatory work for the MSC-RAC Joint Workshop that took place on 11-12 October 2018 in Helsinki. The aim of the document was to provide to the workshop participants background information on the Germ Cell Mutagenicity topic in order to prepare for the discussion during the workshop.}

\textit{This document does not represent the official position of the Agency.}

\textbf{I. Background}

Due to the recent developments on \textit{in vitro} methods for skin sensitisation and their subsequent adoption as OECD test guidelines (starting from 2015 with the first test guideline), it was decided to change the REACH information requirements for the skin sensitisation endpoint (Annex VII, section 8.3). The change entered into force on 10 May 2017. However, to this date, there is no internationally agreed way to combine such \textit{in vitro} assays to obtain information whether a substance is a skin sensitiser and not and how to obtain information about the skin sensitisation potency. This is especially linked to the potency relative to hazard classification as skin sensitisier category 1A or 1B. Due to the changes in the REACH standard information requirements, in case new information needs to be generated, the registrant should perform \textit{in vitro} assays as a first step. Hence, ECHA and MSC have to decide whether the results of such \textit{in vitro} tests, potentially as a sole information, can be used to fulfil the standard information requirement for the particular substances. Furthermore, ECHA and RAC have to decide whether a conclusion on classification for skin sensitisation, including potency determination, based on such \textit{in vitro} data can be drawn.

The aim of the revision of the standard information requirements for skin sensitisation of REACH was to replace the former \textit{in vivo} skin sensitisation study i.e. LLNA as much as possible with a combination of results from relevant \textit{in vitro} methods in addition to any other relevant available information.

\textbf{II. REACH standard information requirements}

The revision of the REACH Regulation consisted of two major changes in respect to the former standard information requirements

1) Information on whether a substance is a sensitizer or not needs to be provided. Then if it can be established that the substance is a sensitisier, its potency needs to be assessed.

"8.3. Column 1: Skin sensitisation
Information allowing:

- a conclusion whether the substance is a skin sensitiser and whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and risk assessment, where required."

This applies to all data that has been generated after 10 May 2017 (see column 2 of Annex VII, section 8.3.2).

2) In case new testing needs to be performed, the registrant has to start with \textit{in vitro} testing where, as a starting point, three \textit{in vitro} methods covering the first three key events of the Adverse Outcome Pathway (OECD, 2012) needs to be performed.
The registrant can waive the in vitro methods and immediately proceed with in vivo testing if none of them is suitable for their substance or, the results are not adequate for classification and risk assessment. In addition to the standard information requirement of in vitro methods, the registrant should use any other available relevant and reliable information, including information according to Annex XI of REACH Regulation e.g. in silico methods and read-across, to support their conclusion to fulfil the standard information requirement for skin sensitisation.

In addition, if the registrants based on available information can conclude on classification and risk assessment, where required, relying only on 1 or 2 in vitro/ in chemico test results, they can waive the others (note: currently there are no stand-alone in vitro assays, but methods holding promise of this are under development in the OECD Test Guideline Programme and may become available in future).

As the REACH standard information requirements have changed, the decision on the hazard identification (sensitiser vs non-sensitiser) and potency determination (whether a substance is potent enough under CLP to be categorised into 1A or not) needs to be completed also based on in vitro methods in the future – and as available other relevant and reliable information including non-test information. In case it is not possible to reach a conclusion whether a skin sensitising substance has the potential to produce significant sensitisation (Cat 1A) in humans based on in vitro results only, the registrants shall perform, or ECHA can request via compliance check the registrant to perform a suitable in vivo test. Unless there are specific reasons in regard to the substance in question the test method of choice would in those cases be the Local Lymph Node Assay (LLNA, OECD TG 429). Additional information obtained from e.g. in silico tools or via read-across approach can be used to support the conclusion based on in vitro methods of skin sensitisation potential, if suitable and available.

III. Importance of potency determination

Whether a substance is a skin sensitiser or not, and if it is a moderate, strong or extreme skin sensitiser, significantly influences not only classification and labelling of the substance and mixtures containing the substance, but also the conditions of use. Thus depending on the potency, different risk management measures may need to be applied.

The potency of the skin sensitising substance has an important role when the substance is used in a mixture by determining at which concentration the mixture needs to be labelled as skin sensitising.

IV. Current status of Defined Approaches

In vitro methods have been adopted for the first three key events of the adverse outcome pathway (OECD, 2012) i.e. to assess molecular interaction with skin proteins (key event 1, OECD TG 442C), inflammatory responses in keratinocytes (key event 2, OECD TG 442D) and activation of dendritic cells (key event 3, OECD TG 442E). Each of these key events are described in the standard information requirement under REACH. Currently, none of the adopted test methods are considered to be stand-alone test methods and hence combining the results of them are needed, and it may well be warranted to use such results together with predictions done by in silico methods. In the future, stand-alone non-animal methods with scientific validity may however become available for acceptance in specified types of cases as standard information for skin sensitisation and hence be available for adequate for both REACH and CLP purposes.

Based on the available methods different approaches to combine those methods for both hazard identification and characterisation (potency) have been described in another OECD document (GD 256) and in recent publications (Hoffmann et al., 2018; Kleinstreuer et al., 2018). Those
combinations of different in vitro test methods and/or in silico methods are called “Defined Approaches” (DAs). DAs are prescribed combinations or prediction models employing relevant data generated by in silico and/or in vitro test methods. For skin sensitisation a few different types of DAs have been proposed: i) consisting solely of data generated by in vitro methods, ii) consisting of data generated by in vitro methods and (Q)SAR models / tools e.g. the freely available OECD QSAR Toolbox , or iii) consisting of information relaying solely on in silico predictions. In general combination of different methodologies when used in certain prescribed combinations (DAs) seems to provide similar accuracy¹ as the current “golden” animal standard in vivo method i.e. LLNA (OECD TG 429) when compared to human data.

With this in mind, an OECD project was initiated in 2017 to review the approaches presented in the OECD GD 256. The aim of the project is to evaluate whether specific DAs would allow a conclusion on skin sensitisation potential and potency as described in UN GHS (category 1A and 1B) to be made with at least the same accuracy as the current in vivo LLNA without the need to apply expert judgement. This means that in case such in vitro and in silico methods are applicable for a specific substance, then prescribed combination of such methods (DAs) would provide an output that could be used directly for both hazard identification and hazard characterisation in the form of an appropriate classification. In the cases, where there are e.g. human data, animal data, and/or non-test data (in silico and/or read-across data) in addition to the information elements of an employed DA, such additional information cannot be neglected. Therefore, a weight of evidence assessment of all available information including both the outcome of the DA and such additionally available data need to be applied based on expert judgement to conclude on the skin sensitisation potential and potency.

Issues under further consideration and analysis in the OECD project on Defined Approaches for Skin Sensitisation includes:

- variability in human and animal (e.g. LLNA data),
- how to characterize the applicability domain of in vitro and in silico methods,
- how results from in silico methods are being documented (QMRF/QPRF) in performance based DA TGs,
- whether individual possible DAs for skin sensitisation will have a large overlap of false positives and false negatives – and if not, how to consider this in respect to acceptability or how handle this in regulatory decision making?

As DAs are currently under development but not yet adopted as OECD TGs it is currently not possible to conclude on these issues and the future role of DA e.g. under REACH and for classification and labelling. Use of the results from such DAs as the sole basis for hazard classification (hazard identification and potency considerations) would however probably require certain revisions/ adjustments of the skin sensitisation classification criteria in CLP regulation.

Due to the recent change in the standard REACH information requirements and the importance of potency determination, it is crucial that relevant in vitro methods and DAs for skin sensitisation can be used for CLP purposes including the potency determination and the identification of extreme sensitisers. If the outcome of the review of a proposed DA shows that it does not allow appropriate identification of skin sensitisers, it should not be adopted as an OECD TG. In case the outcome of the review is that it allows appropriate identification of skin sensitisation, however not potency in categories 1A or 1B, this needs to be made clear in the OECD TG i.e. additional information is needed to determine the potency of skin sensitising substance. Off course methods for both identification and potency assessment of skin sensitisers would be most useful. However, methods that adequately can identify skin sensitising substances but not their potency would still be useful in minimizing testing with laboratory animals. If for example 20 to

¹ Accuracy=percentage of correct predictions (sensitivity is the percentage of positives predicted correctly, whereas specificity is percentage of negatives predicted correctly The target of the DAs under development is that they should have at least the same sensitivity and specificity as the LLNA (OECD TG 229) when compared with human data
30% of all REACH substances are skin sensitising, their identification would mean that only on those 20 to 30% further potency information would be warranted.

OECD discussions have recently been initiated to discuss how results from in silico methods like QSAR model predictions and OECD QSAR Toolbox profiler information could be used in the context of DAs for skin sensitisation in addition to the in vitro methods.

V. What can ECHA in collaboration with MSC do when evaluating submitted data from application of Defined Approaches for skin sensitisation?

If a REACH registrant only has provided in vitro data and/or data according to an adopted DA and ECHA does not agree on the conclusion (hazard identification or characterization including classification for skin sensitisation and potency assessment), ECHA can via compliance check in collaboration with the MSC request additional data e.g. LLNA in line with the REACH regulation.

VI. What can ECHA do when the registrant has performed in vivo testing and ECHA disagrees with the in vitro waiver provided?

If there is concern that potentially unnecessary animal testing has been performed for the REACH registration purpose and that such testing was not in accordance with the standard information requirements of REACH i.e. without prior performance of in vitro testing and without proper column 2 adaptation, ECHA can inform the national enforcement authorities of such cases. Similar approaches have been taken when higher tier animal studies have been performed without submitting testing proposals or in vivo irritation testing without the prior performance of in vitro studies.

VII. What can RAC do when evaluating in vitro data and Defined approaches?

The CLP legislation refers to the use of human and animal data to identify and assess the potency of sensitisers. Potency is critical part of the classification and the criteria on how to interpret the LLNA, GMPT (OECD TG 406) and Buehler (OECD TG 406) in vivo assays are specified in the legislation. However, currently the CLP Regulation does not contain specific criteria on how to classify a substance based on DAs containing in vitro and/or in silico methods. However, it is according to the CLP regulation possible to use in vitro and in silico results for classification by applying a weight of evidence approach by use of expert judgement.

As there are currently no specific in silico and in vitro based classification criteria for skin sensitisation (including categorization of skin sensitisers in category 1 A or 1 B, or setting SCL), the consideration for classification needs to be done on a case by case basis. A project to investigate possibilities for inclusion of in silico, read-across and in vitro criteria in the GHS has been initiated.
References:
REACH Regulation: https://echa.europa.eu/regulations/reach/legislation

CLP Regulation: https://echa.europa.eu/regulations/clp/legislation

Guidance on application of CLP criteria:


OECD AOP skin sensitisation, available at:

OECD 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, available at: https://www.oecd-ilibrary.org/docserver/9789264279285-en.pdf?expires=1536914130&id=id&accname=guest&checksum=1A0CC4A485D582CCFD582E9D77491146