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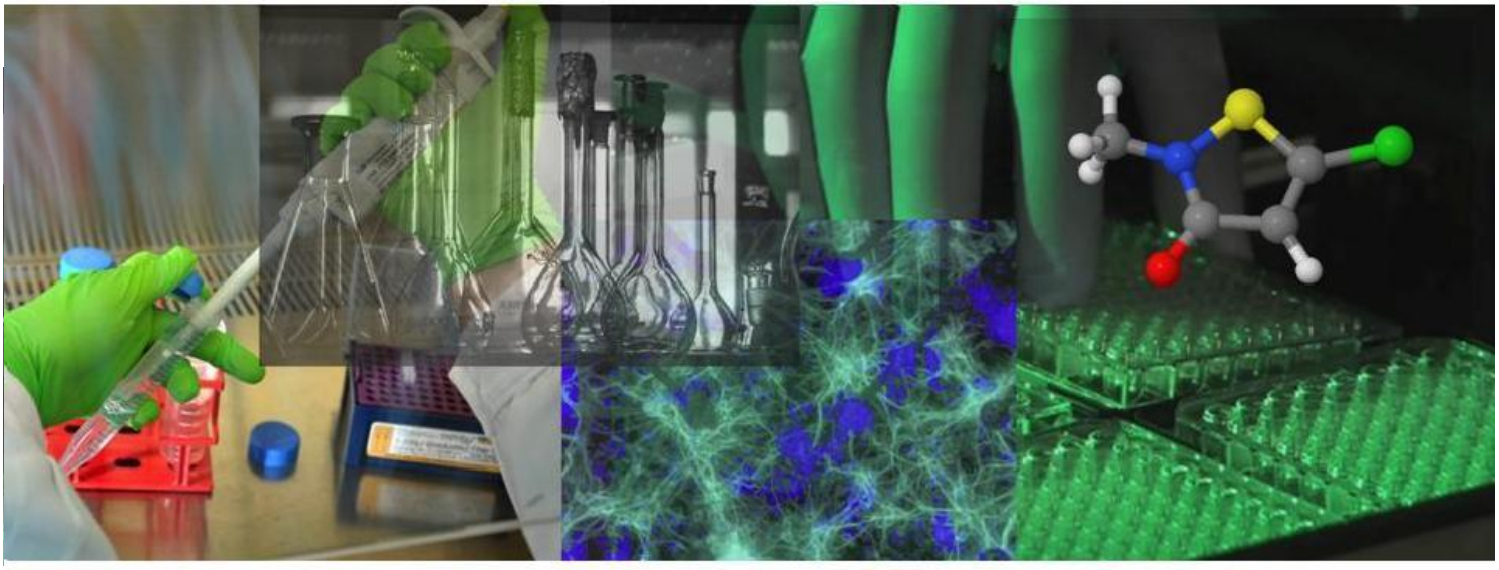

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EURL ECVAM RECOMMENDATION

on the Direct Peptide Reactivity Assay (DPRA) for Skin Sensitisation Testing

November 2013

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This Recommendation was prepared by the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), part of the Institute for Health and Consumer Protection (IHCP), Directorate-General Joint Research Centre (DG JRC) of the European Commission.

The Recommendation was drafted on the basis of the ESAC Opinion and ESAC Working Group Report summarising the detailed scientific peer review of the EURL ECVAM-coordinated study on the Direct Peptide Reactivity Assay (DPRA). The Recommendation further benefitted from comments and suggestions received from members of PARERE (EURL ECVAM's advisory body for Preliminary Assessment of Regulatory Relevance that brings together representatives of Member State regulatory bodies as well as EU agencies including ECHA, EFSA and EMA), and ESTAF (EURL ECVAM's Stakeholder Forum). Input was also provided by partner organisations of EURL ECVAM in the framework of the International Collaboration on Alternative Test Methods (ICATM), and by the general public.

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BACKGROUND TO EURL ECVAM RECOMMENDATIONS

The aim of a EURL ECVAM Recommendation is to provide EURL ECVAM views on the validity of the test method in question, to advise on possible regulatory applicability, limitations and proper scientific use of the test method, and to suggest possible follow-up activities in view of addressing knowledge gaps.

During the development of its Recommendation, EURL ECVAM consults with its advisory body for Preliminary Assessment of Regulatory Relevance (PARERE) and its EURL ECVAM Stakeholder Forum (ESTAF). Moreover, EURL ECVAM consults with other Commission services and its international validation partner organisations of the International Cooperation on Alternative Test Methods (ICATM). Before finalising its recommendations, EURL ECVAM also invites comments from the general public and, if applicable, from the test method submitter.

EXECUTIVE SUMMARY

EURL ECVAM fully endorses the ESAC opinion (Annex I) on the ECVAM-coordinated validation study of the Direct Peptide Reactivity Assay (DPRA) that assessed mainly protocol transferability and within- and between-laboratory reproducibility. The study was conducted in view of the DPRA's possible use as a component of an integrated approach for testing the potential of chemicals to cause skin sensitisation resulting in Allergic Contact Dermatitis (ACD). On the basis of the ESAC Opinion on the DPRA study, EURL ECVAM makes the following Recommendations:

(1) Haptenation, i.e. the covalent binding of low-molecular weight substances ("haptens") to proteins present in skin is considered a prominent mechanism through which chemicals or their metabolites become antigenic. Haptenation has been described as a "molecular initiating event" in the OECD Adverse Outcome Pathway (AOP) for skin sensitisation which summarises the key events known to be involved in chemically-induced ACD (OECD, 2012). Therefore, information from peptide reactivity assays such as the DPRA is relevant for the assessment of the skin sensitisation potential of chemicals.

(2) The EURL ECVAM study showed that the DPRA is transferable to suitably equipped laboratories that are proficient in high performance liquid chromatography (HPLC) analysis and the results obtained demonstrated within- and between-laboratory reproducibility of 87% and 75%, respectively.

(3) Full evaluation of the predictive capacity and applicability domain of the DPRA were outside the scope of the EURL ECVAM study. However, based on the study results and excluding metal compounds for which the test is not applicable, the accuracy of the DPRA for distinguishing sensitisers from non-sensitisers was 82% (sensitivity of 76%, specificity of 92%) which is in agreement with published information from previous studies (Gerberick *et al*., 2007; Bauch et al., 2012; Natsch et al., 2013).

(4) In addition to supporting identification of sensitisers/non-sensitisers, the DPRA may also be able to contribute to the assessment of sensitising potency, e.g. by supporting, within an integrated approach, the subcategorisation of sensitisers according to the United Nations Globally Harmonised System of Classification and Labelling of Chemicals (UN GHS; UN, 2011). More work however is required to determine to which extent DPRA results relate to potency categories.

(5) As the DPRA is an *in chemico* test method lacking metabolic capacity, substances that require metabolic (pro-haptens) or abiotic activation (pre-haptens) may not be detected by the DPRA. In addition, the DPRA specifically detects peptide reactivity associated with lysine and cysteine amino acids. These factors should be taken into account when considering negative results.

(6) In view of the mechanistic complexity of skin sensitisation, DPRA data should always be considered in combination with other information in the context of integrated approaches such as Weight of Evidence (WoE) or Integrated Testing Strategies (ITS). Complementary information may be derived from test methods addressing other key events involved in skin sensitisation (OECD 2012) as well as non-testing methods including read-across information. Thus, EURL ECVAM recommends the development of integrated approaches for identifying and characterising skin sensitisation hazard (EURL ECVAM 2013) and potency. These approaches should be tailored to specific needs (e.g. depending on various sectorial and regulatory requirements).

(7) To support development of integrated approaches employing peptide reactivity assays (such as DPRA) and other sources of information, the applicability of the DPRA should be further characterised, e.g. through a retrospective analysis of existing data and, in case of identified data gaps, by generating additional information through targeted prospective testing. In particular, as pre-haptens are not consistently misclassified by the DPRA, the assay's applicability to these substances should be further investigated. Additionally, attention should be given to substances with electrophilic residues that react preferentially with amino acids other than cysteine or lysine.

(8) Respecting the provision of Directive 2010/63/EU (EU, 2010) on the protection of animals used for scientific purposes, before embarking on animal experiments to identify substances with skin sensitisation potential, data from the DPRA test method should be considered in combination with complementary information in order to reduce and possibly avoid animal testing. In agreement with the provision of Annex XI point 1.2 of the REACH Regulation (EU, 2006) data from non-standard testing methods, such as the DPRA, may be used to adapt the standard information requirements in the context of Weight of Evidence judgments.

1. Introduction

1) The assessment of skin sensitisation potential is an important component in the safety evaluation of substances and represents a standard requirement of legislation on chemicals in the EU. These include: the Classification Labelling and Packaging of substances and mixtures (CLP) Regulation (EU, 2008a), the REACH Regulation (EU, 2006), the Plant Protection Products (PPP) Regulation (EU, 2009a), the Biocides Regulation (EU, 2012) and the Cosmetics Directive (EU, 2009b). As outlined in the EURL ECVAM Strategy for Replacement of Animal Testing for Skin Sensitisation Hazard Identification and Classification (EURL ECVAM, 2013), determining the skin sensitisation hazard properties of substances is a key requirement satisfying already the majority of regulatory needs, e.g. under the CLP and REACH Regulations in the EU. Other regulatory contexts can require an understanding of the relative potency of skin sensitisers with regard to both induction as well as elicitation of contact dermatitis in order to support a full risk assessment and appropriate risk management measures (e.g. setting of appropriate thresholds).

2) Currently there are only *in vivo* regulatory accepted test methods to generate data satisfying regulatory requirements on skin sensitisation. For instance, in the frameworks of the Organisation for Economic Cooperation and Development (OECD) and the EU Test Methods Regulation (EU, 2008b), there are four accepted guidelines, describing: the Buehler Test and Guinea-pig Maximisation Test (GPMT), TG406 (OECD, 1992; EU test method B.6), the Local Lymph Node Assay (LLNA), TG429 (OECD, 2010a; EU test method B.42) and its non-radio-isotopic variants, the Local Lymph Node Assay: DA and the Local Lymph Node Assay: BrdU Elisa , TG 422A and TG 422B respectively (OECD, 2010b; OECD 2010c).

3) The key mechanistic events underpinning the skin sensitisation process that leads to Allergic Contact Dermatitis (ACD) in humans are well understood and have been recently summarised in the OECD report on "The Adverse Outcome Pathway (AOP) for Skin Sensitisation Initiated by Covalent Binding to Proteins"(OECD 2012). These include 1) the covalent binding of the chemical to the skin protein (haptenation), 2) events in keratinocytes including the production of danger signals and release of pro-inflammatory mediators 3) the maturation and mobilisation of dendritic cells (DC), the immuno-competent cells in the skin, and 5) the antigen presentation to naïve T-cells and the proliferation of memory T-cells. Considerable progress has been made in recent years towards the development of alternative non-animal methods that address these key events. It is plausible that the initial event of haptenation is the major determinant of the skin sensitisation process and thus the protein-binding properties of a chemical should be intrinsically linked to its sensitisation potential and potency (Roberts & Aptula, 2008).

4) There is general agreement within the scientific community that, in the near future, it is unlikely that one single alternative method will be able to provide sufficient information to replace the use of animals for this endpoint (Adler et al., 2011). Instead it is held that information from different alternative testing and non-testing methods used in combination will need to be integrated to address this health endpoint (Jowsey *et al.*, 2006; Adler et al., 2011). These methods should address different key events leading to skin sensitisation thus covering the mechanistic complexity of this endpoint. Nevertheless, it should not be ruled out *a priori* that skin sensitisation testing may, in the future, be addressed by one single test method.

5) EURL ECVAM coordinated a validation study of DPRA following a modular approach (Hartung et al., 2004) which had the following objectives:

- To fully assess the reliability of the DPRA protocol, i.e. its transferability and within- and between-laboratory reproducibility.
- To conduct a preliminary evaluation of the ability of the DPRA to discriminate skin sensitising from non-sensitising chemicals as defined by the Globally Harmonised System (GHS) for the classification and labelling of substances for skin sensitisation and as implemented in the European Union CLP Regulation concerning both substances and mixtures. Characterisation of preliminary predictive capacity was performed in view of determining the potential contribution of the method to contribute to decisions on hazard within integrated approaches.
- To consider the ability of the DPRA to contribute to sub-categorisation of skin sensitising chemicals, e.g. into Sub-category 1A and Sub-category 1B as adopted in the 3rd revised version of the GHS.

6) After completion of the study and finalisation of the Validation Study Report (EC-ECVAM-2012), EURL ECVAM requested the ECVAM Scientific Advisory Committee (ESAC) to provide an ESAC Opinion on the study. An ESAC Working group (WG) was subsequently established which drafted an ESAC WG report, which then formed the basis of the ESAC Opinion (see annex) adopted by the ESAC on 17. 12. 2012.

2. Test Method definition

7) The correlation of protein reactivity with skin sensitisation potential is well recognised (Landsteiner and Jacobs, 1936; Dupuis & Benezra, 1982; Lepottevin et al., 1998). Chemical covalent binding to nucleophilic centres in skin proteins is regarded to be the molecular initiating event in the skin sensitisation AOP (OECD, 2012) without which skin sensitisation would not occur. Thus, chemicals capable of reacting with proteins either directly or after biotic or abiotic transformation may have the potential to act as a contact allergen. It should be noted that, in its current design, the DPRA does not provide a measure of reaction rate constant.

8) The DPRA is an *in chemico* method which addresses peptide reactivity by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following 24 hours incubation with the test substance. Depletion of the peptide in the reaction mixture is measured by HPLC using UV detection. Average peptide depletion data for cysteine and lysine are then interpreted by using a classification model developed on the basis of a dataset of chemicals with known reactivity properties, in which chemicals are classified as having minimal, low, moderate or high reactivity. Substances with low to high reactivity are associated with substances that have skin sensitisation potential while those categorised as having minimal reactivity are considered to lack skin sensitisation potential. Due to the absence of a metabolic competent system in the assay, the DPRA is not suitable for the evaluation of pro-haptens, which require metabolic activation to act as sensitisers. The ability of the DPRA to detect pre-haptens, which require abiotic activation (e.g. through

oxidation by air), is not clear, although some pre-haptens are reported to be correctly identified by the DPRA.

9) As a result of the ECVAM-coordinated study (EC EURL ECVAM, 2012), the standardised protocol was found to be transferable and reproducible within and between laboratories experienced in HPLC analysis. Some minor aspects of the protocol have been refined as a result of the experienced gained in the validation study.

10) EURL ECVAM will publish in its DataBase service for ALternative Methods (DB-ALM, see http://ecvam-dbalm.jrc.ec.europa.eu), a comprehensive protocol including a detailed description of the test method and all necessary technical details needed by an end-user laboratory to implement it in a self-sufficient manner.

3. Overall performance of the Direct Peptide Reactivity Assay

Reference data

A key criterion employed for selecting the validation test chemicals was availability of high quality in vivo testing data from the murine LLNA and the GPMT with concordant classification from these two assays. The set of chemicals used in the study consisted of one third of non-sensitisers and two thirds of sensitisers with a balanced representation of potency classes (weak, moderate strong and extreme). Reference chemicals from the LLNA performance standards (OECD 2010a) were included in the chemical set. Additional details can be found in the Validation Study Report (EC EURL ECVAM, 2012).

When interpreting the data of alternative methods, such as the DPRA, that have been largely developed and validated using animal reference data such as LLNA or GPMT the limitations of the reference data should be kept in mind. For instance, the predictive relevance of reference animal tests may not fully reflect the situation in the species of interest, i.e. humans. Notably, an evaluation of the LLNA in comparison to human data has shown an accuracy of about 72% (Anderson et al., 2011), i.e. there is a risk of false negative and false positive results. Moreover there is indication that the LLNA is deficient in detecting low to moderate sensitisers as well as metals and organometal compounds (EC, 2000).

Transferability and Reproducibility

11) On the basis of the results obtained during the study, it is evident that the DPRA can be readily transferred to new laboratories that are properly equipped and experienced with HPLC instruments and techniques.

12) The assessment of the reproducibility was performed on the basis of concordance in classification (sensitiser/non-sensitiser). The experimental data generated in the study indicate that the within-laboratory reproducibility (ranging from 73% in the lead laboratory to 100% in one of the two naïve laboratory) and the between-laboratory reproducibility (75%) are acceptable for the proposed future use of the DPRA (i.e. in combination with other complementary methods).

Preliminary evaluation of predictive capacity based on the ring trial data

13) Full evaluation of the predictive capacity of the DPRA was not within the scope of the EURL ECVAM study. However, the accuracy of the DPRA for dichotomous classification (sensitiser/non-sensitiser) on the basis of all 24 chemicals tested (including two metals, one pro-hapten, dihydroeugenol, and two pre-haptens, 4-phenylendiamine and R(+)-Limonene), was 79% (sensitivity=71%, specificity=92%). When excluding the two metal compounds (which are considered outside the applicability domain and can be readily excluded from testing during practical application of the assay), the accuracy was 82% (76% sensitivity and 92% specificity). Thus, the predictive capacity determined in the study is consistent with published information from a larger set of data (Gerberick *et al*., 2007). Importantly, substances reported as false negatives in the EURL ECVAM study were generally substances with a low sensitisation potency *in vivo.*

14) In relation to the ability of the DPRA to categorise substances in reactivity classes, data from the validation study does not support the use of the DPRA as a standalone method for potency categorisation. This is consistent with published information. However the study results indicate that the assignment of a chemical to a DPRA reactivity category may have the potential to contribute to the determination of its potency.

4. Limitations

4.1 Technical limitations

15) **Solubility of test substances:** Peptide depletion values for substances with limited solubility in the solvents prescribed by the DPRA SOP cannot be derived with sufficient accuracy. Despite the fact that all the chemicals selected for the EURL ECVAM study were found to be compatible with the test system, limitations with the testing of insoluble chemicals have been reported in the submission to ECVAM.

16) **Co-elution:** In those instances, mainly attributable to specific instrument settings, where the test substance or the reaction products elute at the same time as the peptide (co-elution), an accurate measurement of peptide depletion cannot be made. The DPRA SOP provides instructions on how to approach different instances of co-elution, allowing in certain cases an estimation of the peptide depletion and reactivity class assignment. However there might be circumstances were this type of approximation is not appropriate.

17) **Cysteine dimerisation:** Accurate determination of peptide depletion can also be hampered by substances promoting the oxidation of the thiol group in the cysteine peptide that leads to the formation of the cystine dimer. In such cases depletion of the peptide would be overestimated.

4.2 Limitations with regard to applicability

The following limitations of DPRA should be taken into consideration:

18) **Restriction to lysine and cysteine:** The DPRA is designed to measure reactivity of the electrophile towards two amino acids: the thiol group of *cysteine* and the primary amino groups of *lysine*. As other amino acids are not present in the assay, chemicals with preferential reactivity towards amino acids other than cysteine or lysine (e.g. nucleophilic sites in histidine), may lead to false negative results when tested in the DPRA. However, when considering this limitation, it should be also kept in mind that the relative percentages of substances reacting preferably with amino acids other than cysteine and lysine is at present unclear and that the cysteine and lysine peptides represent softer to harder model nucleophiles (OECD, 2012; Schwöbel et al., 2011) which would cover different reaction mechanisms

19) **Metal compounds:** DPRA is not designed to accommodate the spectrum of reaction mechanisms considered to be associated with sensitising metals. For example Nickel, the most important metal allergen, is postulated to form coordination bonds with nucleophilic residues in histidine. However, metal compounds can be readily excluded from testing based on chemical structure and, therefore, this limitation can be easily addressed by simply avoiding the DPRA for the testing of metal compounds.

20) **Pro-haptens and pre-haptens**: The DPRA is not designed to detect the sensitising properties of pro-haptens which require bioactivation, or pre-haptens which require abiotic transformation. Nevertheless, pre-haptens are in some cases reported to be correctly detected as sensitisers by the DPRA, as was the case in the EURL ECVAM study (n=2). However, the reasons why specific pre-haptens are detected while others are not remain unclear. To address the issue of pre- and pro-haptens, *in silico* expert systems such as TIMES-SS (Patlewicz et al., 2007; Roberts et al., 2007) and the OECD QSAR toolbox (www.qsartoolbox.org) could prove useful. Notably, a variation of the DPRA including an additional protocol step which mimics oxidative activation (co-incubation with horseradish peroxidase and hydrogen peroxide) to detect pre- and pro- haptens is under development. (Gerberick et al., 2009).

21) **Oxidation:** Some substances that have oxidative properties (e.g. oxidative colourants) without necessarily causing haptenation may lead to possible false positive results when tested in the DPRA.

5. Suggested regulatory use

22) Due to the complexity of the mechanisms underlying skin sensitisation, it is likely that information from different methods (in silico, in chemico, in vitro) is needed to reduce or replace the need for animal testing, both for hazard identification and potency characterisation purposes. The DPRA is a reliable test method that provides information on peptide reactivity, which is considered to be the molecular initiating event of skin sensitisation (OECD 2012). Therefore, peptide depletion values generated with the DPRA could be used to support read-across from chemical analogues or combined with information from other non-animal methods in the context of a Weight of Evidence (WoE) approach or Integrated Testing Strategy (ITS). The extent of information needed to complement a DPRA result will depend on the intended application (e.g. hazard

identification, classification or potency assessment) and context (availability and quality of other information).

23) For the purpose of hazard identification (i.e. identifying substances with sensitising potential), it is plausible that an unequivocal depletion value in the DPRA combined with the presence of a structural alert or positive QSAR prediction for skin sensitisation may prove sufficient for decision making, thus justifying the waiving of an animal test. To conclude on the absence of sensitising potential, additional information would be needed to increase confidence, such as *in vitro* data on downstream events. In any case a negative DPRA result should be interpreted with care, taking into consideration the possibility of false negatives due to (1) possible reactivity with amino acid residues other than cysteine and lysine, (2) the lack of metabolic capacity of the assay leading to possible misclassification of pro-haptens as well as (3) the uncertain capacity of the DPRA to correctly pick up pre-haptens. For hazard assessment purposes, possible uses of DPRA data in the context of a WoE or ITS have been reported in several scientific publications (Ball et al., 2011; Bauch et al., 2012).

24) Use of results generated with the DPRA for potency prediction has also been proposed (Jaworska *et al*., 2011; Nukada *et al*., 2012). Results from the EURL ECVAM study showed that the limited number of substances with high DPRA reactivity fell into Category 1A of the UN GHS (UN, 2011), suggesting the potential application of DPRA results for potency sub-categorisation. However, further efforts are required to explore how DPRA data may support potency assessment, possibly in combination with data from other methods.

25) When employed within an integrated approach, the DPRA may be useful to satisfy information requirements for Cosmetics (Regulation EC/1223/2009), Chemicals (Regulation EC/1907/2006), Biocides (EC/528/2012) and Plant Protection Products (Regulation EC/1107/2009).

6. Follow-up activities recommended by EURL ECVAM

(1) In view of further testing with the DPRA, EURL ECVAM recommends that the revised protocol available at EURL ECVAM's DB-ALM service (http://ecvam-dbalm.jrc.ec.europa.eu) be used.

(2) The predictive capacity of the DPRA for assessing the sensitisation potential of substances should be further evaluated in the context of its use as part of integrated approaches to testing and assessment.

(3) DPRA data should be analysed to understand the potential of the method to contribute to the potency assessment of substances, including sub-categorisation according to GHS (i.e. categories 1A and 1B). Use of existing human data and data from the LLNA are likely to be useful for this purpose. A study correlating DPRA data with potency categories derived from LLNA has recently been published (Natsch et al., 2013).

(4) To support ITS development and to increase confidence in the DPRA method, additional prospective testing with the DPRA should be tailored towards better understanding of its applicability domain to better define how the method performs with (a) weak sensitisers, (b) pre-haptens and (c) chemicals that have selective reactivity towards amino acids other than cysteine and lysine. The reason for false positive predictions also deserves further investigation.

(5) EURL ECVAM supports the development of an OECD Test Guideline for the DPRA. A project proposal has already been submitted to the OECD and included in the OECD 2012 work program. As this test is best employed in combination with complementary methods, it should be considered in the current initiative being undertaken at OECD to develop a guidance document on Integrated Approaches for Testing and Assessment (IATA) for skin sensitisation.

(6) EURL ECVAM recommends continued investment in the development of the next generation of peptide reactivity assays which can potentially address some of the limitations of the DPRA method.

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Annex 1 ESAC Opinion

Opinion of the EURL ECVAM Scientific Advisory Committee (EASC) on the ECVAM-led study of the Direct Peptide Reactivity Assay (DPRA)

Ispra, 17 December 2012

Summary of the ESAC Opinion

The ESAC was asked to provide an opinion on an EURL-ECVAM led prevalidation study assessing the transferability and reproducibility (within- and between-laboratories) of the DPRA test method (primary objective of the study) in view of its possible future use as part of a non-animal testing strategy for skin sensitization. The study had also been designed to provide *preliminary* information on a) the predictive capacity of the test method and b) its potential use for contributing to sub categorisation of sensitizing chemicals.

The ESAC considered the scientific work presented of good quality. Overall, the conclusions made by the working group (WG) correspond well with the conclusions formulated in the report by the Validation Management Group (VMG).

- The within laboratory reproducibility (WLR) of the test method with respect to concordance of classification (S/NS) was considered acceptable.
- The data were considered strong enough to support transferability of the test to properly equipped, trained and staffed laboratories with the appropriate analytical capabilities.
- The between laboratory reproducibility (BLR) of the test method with respect to concordance of classification was considered sufficient when compounds outside the applicability domain were excluded.
- The preliminary predictive capacity as evaluated in this study is consistent with the published data (references 6, 7).
- The potential for use of DPRA reactivity information in potency sub-categorisation requires further examination with a larger dataset.

The ESAC had some questions about the statistical calculations underlying the determination of an adequate sample size to analyse reproducibility as a primary study goal. The ESAC was concerned that possible limitations of the assay were not described in sufficient detail in the validation study report.

The predictive capacity, applicability domain and limitations of the test are not defined yet, but the available data suggest that the test is a useful tool for early decision making during product development (screening) and a component in a weight-of-evidence (WoE) approach or integrated testing strategy (ITS).

The ESAC recommends that the possible limitations of the DPRA should be further investigated specifically in relation to pre-/pro-haptens, either by additional prospective testing or through analysis of existing information as there may be a risk of false negative results associated with these chemicals.

1. Mandate of the ESAC

The opinion of ESAC should support ECVAM with respect to the development of recommendations regarding the reliability (transferability, within and between laboratory reproducibility) of the DPRA and the potential regulatory use of the test method.

(1) Study design – transferability, reliability and relevance

- The ESAC was requested to review whether the prevalidation study was conducted appropriately in view of the objective of the study:
  - Reproducibility of the DPRA method within one laboratory (WLR);
  - Transferability to other laboratories;
  - Reproducibility in other laboratories (BLR);
  - Predictive capacity of the test method.

- With respect to the design and conduct of the study, the following issues were to be addressed:
  - Clarity of the test definition (module 1)
  - Clarity of the definition of the study objective
  - Appropriateness of the study design in view of study objective
  - Appropriateness of the study execution:
  - Appropriateness of the statistical analysis used for analysing WLR, transferability, BLR and (preliminary) predictive capacity.

(2) Conclusions of the study

The ESAC was requested to assess the justification and plausibility of

- Reproducibility (WLR and BLR) and transferability;
- Preliminary predictive capacity;
- Possible gaps between study design and study conclusions which remain to be addressed in view of the suggested conclusions/use;
- Applicability and possible limitations of the test method, in particular in view of its potential use within an ITS for sensitisation.

(3) Possible contribution of test method to integrated approach

The ESAC is requested (a) to evaluate, on the basis of the data submitted in the validation study, the possible use of the test method (also within a strategy) to identify skin sensitisers, (b) to make additional recommendations (as required) on the proper scientific use of the test method within such a strategy taking specific aspects of this method into account (e.g. applicability, limitations etc.) and (c) to identify possible further information required (i.e. are there gaps) to be able to conclude on the plausibility of the suggested use (including within an ITS).

2. Detailed opinion of the ESAC

The ESAC was asked to provide an opinion on an EURL-ECVAM led study assessing the transferability and reproducibility (within- and between-laboratories) of the DPRA (primary objective of the study) in view of its possible future use as part of a non-animal testing strategy for skin sensitization. The study had also been designed to provide *preliminary* information on a) the predictive capacity of the test method and b) its potential use for contributing to sub-categorisation of sensitizing chemicals.

(1) Study design – transferability, reliability and relevance.

The WLR was assessed at the level of concordance in prediction (S/NS). WLR for the three laboratories was in the range from 73% to 100% . The lowest value derived from the lead laboratory. The ESAC concluded that, in the context of the study, and in view of the fact that both naive laboratories exceeded the target of 85% as chosen by the VMG, the WLR was sufficient.

- The definition of the reproducibility target (85%) was based upon i) the background and specific objectives of the validation study; ii) the standards of performance that can realistically be expected from an *in vitro* test and standards of performance which have been considered acceptable in previous validation studies; iii) the proposed used of the *in vitro* tests (i.e. as a partial replacement method to become part of a toolbox of tests to be used in combination); and iv) the power of the design of the validation study.
- Transferability activities were divided into Training, Transferability and Qualification Runs The WLR was formulated for each partner to include 1) concordance in prediction, 2) depletion values for cysteine and lysine, as well as 3) control values. The data were considered strong enough to support transferability of the test to properly equipped, trained and staffed laboratories with the appropriate analytical capabilities.
- During the transfer and blind testing phase one laboratory had difficulties in meeting the acceptance criteria defined in the SOP, due to the Reference Control C being marginally outside the acceptance criteria. The cause of the problem could not be identified. The ESAC recommends that these acceptance criteria should be re-examined.
- The BLR was assessed in terms of 1) concordance in prediction and 2) depletion values for cysteine and lysine. Eighteen of the 24 chemicals were consistently classified (S/NS) by the three laboratories resulting in a BLR reproducibility of 75%, which is below the target (80%). The reproducibility assessment included 3 chemicals (beryllium sulphate, nickel chloride and dihydroeugenol) that were considered by the VMG as outside the applicability domain of the test. The exclusion of these three substances considered to fall outside the applicability domain would lead to a BLR of 87.5%. For 15 out of the 24 chemicals the laboratories assigned the same reactivity class resulting in a BLR of 62.5%. Data variability was observed for results from chemicals with low or no reactivity.
- The secondary goals included a <u>preliminary</u> evaluation of the ability of the test to discriminate skin sensitizers from non-sensitizers, and a preliminary consideration of the ability to contribute to sub-categorization of skin sensitising chemicals (GHS sub-category 1A and 1B). The validation study report (VSR) did not present a summary of the predictive capacity based on all 24 chemicals tested, since the VMG

judged three of them (beryllium sulphate, nickel chloride, dihydroeugenol) to fall outside the applicability domain. The two pre-haptens (4-phenylendeiamine and R(+)-Limonene) were included in the analysis as the VMG felt that there was insufficient evidence to exclude them from the evaluation of the predictive performance. The predictive capacity for all 24 substances was 77.8% (sensitivity: 70.8%; specificity: 91,7%) while 82.4% (sensitivity: 73.5%; specificity: 91,7%) for the 19 substances (since the WG felt that other two substances would fall outside the applicability domain, PPD and limonene).

- The project was described and designed in clearly recognizable and well described phases including Test Definition (Module 1), Transferability (Module 3), Within Laboratory Reproducibility (WLR) (Module 2), Between Laboratory Reproducibility (BLR) (Module 4) and Predictive Capacity (Module 5).
- Overall, the chosen statistical approach was considered appropriate. In the calculations of suitable sample size it was not clear for the ESAC why a power of 75% was chosen for the BLR, especially as a more conventional 80% power was used for the WLR. However, since more chemicals than the minimum number were tested in both cases the actual power of the study was considered sufficient.

(2) Conclusions of the study

- Overall, the study design and the quality of the selected chemicals (N=24) were considered appropriate for the purpose of addressing the first objective of the study: Assessing the WLR and BLR of the DPRA.
- Overall, the conclusions made by the WG correspond well with the conclusions drawn by the VMG as described in the VSR, indicating that these conclusions are supported by the results shown in the report.
  - The WLR of the test method with respect to concordance of classification (S/NS) met the target of 85% and was considered sufficient for the purpose of this study.
  - The data were considered strong enough to support transferability of the test to properly equipped, trained and staffed laboratories with the appropriate analytical capabilities.
- In spite of a BLR (75%) below the target of 80%, the BLR of the test method with respect to concordance of classification was considered sufficient after the removal of the compounds outside the applicability domain. The potential for use of DPRA reactivity information in potency sub-categorisation requires further examination with a larger dataset.
  - The number of chemicals (N=24) did not provide support for a firm conclusion about the predictive capacity of the test method. The preliminary data were, however, considered promising.
  - The number of chemicals did not allow drawing a conclusion about the applicability domain of the test. Empirically the applicability domain seems to exclude pre-/pro-haptens and metal salts.

- Chemicals that preferably react with amino acids other then cysteine and lysine may fall outside the applicability domain. In addition, some pre-/pro-haptens were reported as correctly identified. Finally, the data seem to indicate that the test method has problems identifying weak sensitizers. The uncertainty about the applicability domain may result in an unacceptable level of false negative results.

(3) Possible use of the test method within an integrated approach

As outlined in the VSR and the ECVAM request for ESAC advice, the DPRA cannot be used as a stand-alone test method in a regulatory context but should be considered for use in an Integrated Testing Strategy (ITS). On the basis of the present report, especially negative outcomes have to be considered with care.

- As pre-haptens are not consistently correctly predicted by the DPRA, there remains uncertainty about whether to consider pre-haptens as part of the applicability domain of the method or not.
- Unless there are sufficiently accurate assays available identifying chemicals as pre-/pro-haptens in view of excluding them from routine testing using the DPRA, such compounds will be tested in the DPRA and may cause false negative results.
- The selection of cysteine and lysine-containing peptides selects for the majority, but not all, reactive chemicals.

Regarding reactivity class, the data obtained did not support the possibility to use DPRA as a stand-alone test method for potency classification. This is in agreement with the statement of the VMG that the assay should be further evaluated for its capacity to "contribute" to a potency classification (VSR page 8).

Information generated by the DPRA can be used to support regulatory decision making when used in the context of a weight-of-evidence approach or ITS. It is important to use the test in a context that allows confident conclusions about the protein-reactivity of the chemical, especially when the chemical in question is negative in the DPRA. As such the method may be helpful to address testing requirements of e.g. the REACH legislation and the 7th Amendment of the Cosmetic Directive.

Its inclusion into future ITSs can be considered for the purpose of an eventual full replacement of current *in vivo* hazard identification assays.

Recommendations:

The DPRA addresses a key mechanism (haptenation) in the development of skin sensitization/allergic contact dermatitis. Overall the provided data support transferability and reproducibility of the test to qualified laboratories. The predictive capacity of the test is not defined yet, but the preliminary data profiles the test as a useful tool for early decision making during product development (screening) and a component in a weight-of-evidence approach or ITS for safety/hazard assessment.

The ESAC recommends that the limitations of the DPRA (risk of false negative results) are further investigated specifically in relation to pre-/pro-haptens either by additional prospective testing or through analysis of existing information.

3. Informative background to the Mandate and Opinion

Skin sensitizsation is the toxicological endpoint associated with substances that have the intrinsic ability to cause Allergic Contact Dermatitis, ACD in humans. ACD represents the most common manifestation of immunotoxicity in humans, i.e. adverse effects of xenobiotics involving the immune system. The identification of the ***skin sensitization potential*** represents an important component of the safety assessment of any new substance and especially for those intended for topical application (e.g. cosmetics). Current regulatory predictive tests for skin sensitization rely on the use of animals, these include:

a) the traditional guinea pig tests: *Buehler Test* and *Guinea-pig Maximisation Test* (OECD TG 406, Ref.1),

b) the *Local Lymph Node Assay* (LLNA, OECD TG 429, Ref.2) and its recently OECD adopted non-radioactive variants (OECD TG 422A, Ref.3 and OECD TG 422B, Ref.4).

Despite the progress that has been made in the development of alternative methods for skin sensitisation hazard identification, there are currently no validated methods available. In addition none of the tests currently under development/evaluation is able to fully characterise the relative potency of sensitising substances and therefore, none of these assays is considered a stand-alone method, capable of fully replacing current animal procedures, in particular as regards to cosmetics.

The current view therefore is to combine different test methods in order to address different key mechanisms of skin sensitisation: skin bioavailability, haptenation (the protein binding of chemicals which triggers immunological responses), epidermal inflammation, dendritic cell activation and migration, T cell proliferation. Test methods are currently under development which have been specifically designed to address these key mechanistic steps involved in skin sensitisation. Before these test methods can be routinely used, e.g. in ITSs, their capacity to produce reproducible results needs to be demonstrated as a first step.

The *Direct Peptide Reactivity Assay* DPRA is addressing one of the key upstream events in the cascade of mechanisms leading to the induction of skin sensitisation. It measures the ability of chemicals to react with proteins (haptenation). There is good evidence that haptenation is a determinant step in the induction of skin sensitisation. Chemical allergens are usually low molecular weight chemicals which are not immunogenic per se. However, chemical allergens (or their metabolites, oxidation products) have electrophilic properties that allow them to bind covalently with the nucleophilic side chains of amino acids of skin proteins to form an immunogenic conjugate. Already in 1936 this correlation between the reactivity of chemicals with proteins and their skin sensitisation potential was described (Landsteiner and Jacobs, Ref.5) and has in the meantime been extensively described in the literature. This knowledge is being exploited for the development of several *in chemico* reactivity assays with relevance for the testing of sensitisation potential, amongst these the DPRA assay.

The DPRA is designed to screen the ***sensitisation potential*** of chemicals by measuring peptide depletion with UV-HPLC, following incubation of the test chemicals with synthetic heptapeptides containing either cysteine (peptide/chemical ratio in the reaction mixture 1:10) or lysine residues (peptide/chemical ratio in the reaction mixture 1:50) (Gerberick 2004, Ref.6). The average of peptide depletion values for cysteine and lysine are used to classify chemicals into four reactivity categories: minimal, low, moderate and high reactivity (Gerberick 2007, Ref.7). Based on the known correlation between haptenation/chemical reactivity and sensitisation potential, it is assumed that these reactivity classes as predicted by the DPRA may contribute to the characterisation of sensitiser potency.

The possible predictive capacity of the DPRA is supported by the data of the original DPRA submission. On the basis of 133 chemicals, the DPRA classified chemicals as sensitisers or non-sensitisers (in relation to LLNA data) with an accuracy of 86% (87% sensitivity, 83% specificity).

4. References

1. OECD, Organisation for Economic Cooperation and Development (1992) Skin Sensitisation Guidelines for Testing of Chemicals No. 406, Paris
2. OECD, Organisation for Economic Cooperation and Development (2002) The Local Lymph Node Assay. Guidelines for Testing of Chemicals No. 429, Paris
3. OECD, Organisation for Economic Cooperation and Development (2010a) Skin Sensitization: Local Lymph Node Assay: DA, Guidelines for Testing of Chemicals No. 442A, Paris
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6. Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrall SW, Lepoittevin JP, (2004) Development of a peptide reactivity assay for screening contact allergens. Toxicol Sci. 81; 332-43.
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- Dr. Alexandre ANGERS *(specific support)*

Annex 2 EURL ECVAM request for ESAC advice

EURL ECVAM request for ESAC advice on an ECVAM-coordinated study concerning the transferability and reliability of the Direct Peptide Reactivity Assay (DPRA) for skin sensitisation testing

1. TYPE OF REQUEST

Request Type		**Identify request ("YES")**
1) ESAC Peer Review of a Prevalidation Study or Validation Study		**YES**
If R1)applies please specify further:		
►Prevalidation Study	**YES** At present (January 2012) ECVAM is conducting a study of three test methods for skin sensitisation testing: 1) the *Direct Peptide Reactivity Assay* (DPRA), 2) the *human Cell Line Activation Test* (h-CLAT) and 3) the *Myeloid U937 Skin Sensitisation Test* (MUSST). The study assesses transferability and reproducibility of these test methods in view of their possible future use (e.g. as partial replacement methods) within an integrated approach for skin sensitisation hazard identification aiming at the full replacement of the currently used regulatory *in vivo* assays for this purpose. In addition the data generated in this study will inform possible future evaluations on the predictive capacity of these assays. While assessment of the h-CLAT and MUSST test methods are foreseen to be completed in 2012, the evaluation of the **DPRA test method** was finalised in 2011 and the adopted Validation Study Report is foreseen to be available by January 2012. ESAC review will commence in February 2012 employing the same ESAC WG which is currently (January 2012) peer reviewing the KeratioSens submission.	
Prospective Validation Study	**No**	
Retrospective Validation Study	**No**	
Validation Study based on Performance Standards	**No**	
2) Scientific Advice on a test method submitted to ECVAM for validation (e.g. the test method's biological relevance etc.)		**No**
3) Other Scientific Advice		**No**

(e.g. on test methods, their use; on technical issues such as cell culturing, stem cells etc.)	

2. TITLE OF STUDY OR PROJECT FOR WHICH SCIENTIFIC ADVICE OF THE ESAC IS REQUESTED

Prevalidation of the Direct Peptide Reactivity Assay (DPRA) for skin sensitisation testing

3. BRIEF DESCRIPTION OF THE STUDY OR PROJECT

1) Background to skin sensitization and current predictive tests

Skin sensitisation is the toxicological endpoint associated with substances that have the intrinsic ability to cause Allergic Contact Dermatitis, ACD in humans. ACD represents the most common manifestation of immunotoxicity in humans, i.e. adverse effects of xenobiotics involving the immune system. The identification of the ***skin sensitization potential*** represents an important component of the safety assessment of any new substance and especially for those intended for topical application (e.g. cosmetics). Current regulatory predictive tests for skin sensitization rely on the use of animals, these include:

a) the traditional guinea pig tests: *Buehler Test* and *Guinea-pig Maximisation Test* (OECD TG 406, Ref.1),

b) the *Local Lymph Node Assay* (LLNA, OECD TG 429, Ref.2) and its recently OECD adopted non-radioactive variants (OECD TG 422A, Ref.3 and OECD TG 422B, Ref.4).

Despite the progress that has been made in the development of alternative methods for skin sensitisation hazard identification, there are currently no validated methods available. In addition none of the tests currently under development/evaluation is able to fully characterise the relative potency of sensitising substances and therefore, none of these assays is considered a stand-alone method, capable of fully replacing current animal procedures, in particular as regards to cosmetics.

The current view therefore is to combine different test methods in order to address different key mechanisms of skin sensitisation: skin bioavailability, haptenation (the protein binding of chemicals which triggers immunological responses), epidermal inflammation, dendritic cell activation and migration, T cell proliferation. Test methods are currently under development which have been specifically designed to address these key mechanistic steps involved in skin sensitisation. Before these test methods can be routinely used, e.g. in integrated testing strategies, their capacity to produce reproducible results needs to be demonstrated as a first step.

2) Background to the DPRA, h-CLAT, MUSST

DPRA:

The *Direct Peptide Reactivity Assay* DPRA is addressing one of the key upstream events in the cascade of mechanisms leading to the induction of skin sensitisation. It measures the ability of chemicals to react with proteins (haptenation). There is good evidence that haptenation is a determinant step in the induction of skin sensitisation. Chemical allergens are usually low molecular weight chemicals which are not immunogenic per se. However, chemical allergens (or their metabolites, oxidation products) have electrophilic properties that allow them to bind covalently with the nucleophilic side chains of amino acids of skin proteins to form an immunogenic conjugate. Already in 1936 this correlation between the reactivity of chemicals with proteins and their skin sensitisation potential was described (Landsteiner and Jacobs,

Ref.5) and has in the meantime been extensively described in the literature. This knowledge is being exploited for the development of several *in chemico* reactivity assays with relevance for the testing of sensitisation potential, amongst these the DPRA assay.

The DPRA is designed to screen the ***sensitisation potential*** of chemicals by measuring peptide depletion with UV-HPLC, following incubation of the test chemicals with synthetic heptapeptides containing either cysteine (peptide/chemical ratio in the reaction mixture 1:10) or lysine residues (peptide/chemical ratio in the reaction mixture 1:50) (Gerberick 2004, Ref.6). The average of peptide depletion values for cysteine and lysine are used to classify chemicals into four reactivity categories: minimal, low, moderate and high reactivity (Gerberick 2007, Ref.7). Based on the known correlation between haptenation/chemical reactivity and sensitisation potential, it is assumed that these reactivity classes as predicted by the DPRA may contribute to the characterisation of sensitiser potency.

The possible predictive capacity of the DPRA is supported by the data of the original DPRA submission. On the basis of 133 chemicals, the DPRA classified chemicals as sensitisers or non-sensitisers (in relation to LLNA data) with an accuracy of 86% (87% sensitivity, 83% specificity).

h-CLAT & MUSST

The h-CLAT and MUSST are based on the use of Dendritic Cell (DC)-like cell lines. Using flow cytometry, these test methods monitor the induction of cell surface markers associated with DC activation, following exposure to the chemical. In the MUSST, changes in CD86 expression in the U937 cell line are detected; in the h-CLAT modulation of both CD86 and CD54 expression are recorded in THP-1 cells.

3) Study goals and design

In the first quarter of 2009 the DPRA was formally submitted to ECVAM together with other two test methods namely the human Cell Line Activation Test (h-CLAT) and the Myeloid U937 Skin Sensitisation Test (MUSST), developed by companies associated with the European Cosmetics Association (Colipa) and optimized within Colipa ring trials. Following detailed scientific assessment of the information submitted, ECVAM judged the three methods to be mature enough to enter the ECVAM validation process.

In September 2009 a formal study on the three above mentioned test methods was launched, with the main overall objective to evaluate their transferability and reliability (reproducibility within and between laboratories) when challenged with 24 coded chemicals.

As a secondary goal of the study, the experimental data will be used to perform:

a) A preliminary evaluation of the ability of each of the three tests to reliably discriminate skin sensitising (S) from non-sensitising (NS) chemicals as defined by the Globally Harmonised System (GHS, Ref. 6) for the classification and labelling of substances for skin sensitisation (category 1; no category) and as implemented in the European Commission Regulation (EC) No 1272/2008 (Ref.8) on classification, labelling and packaging (CLP) of substances and mixtures.

b) Where possible, a preliminary consideration of the ability of each of the three tests to contribute to sub-categorisation of skin sensitising chemicals, e.g. into Sub-category 1A (strong sensitisers) and Sub-category 1B (other sensitisers) as adopted in the 3rd revised version of the GHS.

The study experimental design foresees the testing of the 24 coded test items in each of the three participating laboratories for the assessment of the between-laboratory reproducibility. A subset of 15 of these chemicals are being tested two additional times in each laboratory for the

evaluation of within-laboratory reproducibility.

With respect to ECVAM's modular approach of validation (Hartung et al., 2004, Ref.10) the study will provide information on module 1) test definition, module 2) within laboratory reproducibility, module 3) transferability and module 4) between laboratory reproducibility. In addition, the data generated will provide preliminary information on module 5) predictive capacity; however, the number of chemicals tested is based on statistical considerations related to the evaluation of the reproducibility, and a larger sample size would be required for module 5 to be considered fulfilled.

References

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4. OBJECTIVES, QUESTIONS, TIMELINES

4.1 OBJECTIVE

Objective *Why does ECVAM require advice on the current issue?*	The opinion of ESAC should support ECVAM with respect to the development of recommendations regarding the reliability (transferability, within and between laboratory reproducibility) of the DPRA and the potential regulatory use of the test method.

	In addition the ESAC should advice with regard to the possible necessary further work required (in relation to predictive capacity, applicability, limitations of the test method) to assess the potential contribution of the DPRA to a future (not yet designed) testing strategy or test battery that would aim to achieve full replacement of the currently used animal tests for skin sensitisation hazard assessment. Moreover, based on the evaluation of the data submitted, the ESAC should provide advice on the potential usefulness of the DPRA test method within a testing strategy for skin sensitisation testing and the proper scientific use of the test method within such a testing strategy (e.g. with respect to its specific applicability and limitations). It is explicitly noted that the ESAC is <u>not</u> requested to suggest the precise placing of the submitted method in a hypothetical ITS, but rather to provide advice on the characteristics of the method relevant for its subsequent integration into an ITS at a later point in time (i.e. when other buildings blocks of such an ITS are known).

4.2 QUESTION(S) TO BE ADDRESSED

Questions *What are the questions and issues that should be addressed in view of achieving the objective of the advice?*	1) **DESIGN & CONDUCT OF STUDY:** The ESAC is requested to review whether the prevalidation study was conducted appropriately in view of the objective of the study. The study objective was to assess (1) the reproducibility of the DPRA method within one laboratory (2) its transferability to other laboratories (3) its reproducibility in other laboratories (BLR). (4) Furthermore, the study aimed at assessing, in a preliminary manner, the predictive capacity of the test method When reviewing the design and conduct of the study, the following issues should be addressed in particular: Clarity of the test definition (module 1) Clarity of the definition of the study objective Appropriateness of the study design in view of study objective, *inter alia:* Is the **number of tested chemicals (24)** sufficient for the purposes of the study? Are the **reference data** used for assessing in particular the predictive capacity appropriate and of good quality? Was the identification of **chemicals** conducted in an appropriate manner (i.e. presence or absence of selection criteria, justification etc.)? Is the **adverse effect range of the selected chemicals** appropriate for the purpose of the study In case of **gaps** (chemical class etc.) – are these justified? Is the **number of laboratories** sufficient? Appropriateness of the **study execution** (e.g. were there pre-defined acceptance criteria, were these respected? How were exceptions / deviations handled? Were provisions specified for retesting? Was the number of repetitions sufficient? etc.) Appropriateness of the **statistical analysis** used for analysing WLR, transferability, BLR and (preliminary) predictive capacity. 2) **CONCLUSIONS OF STUDY:** The ESAC is requested to assess whether the conclusions, as presented in the Validation Study Report, are substantiated by the information generated in the study and are plausible with respect to existing information and current views (e.g. literature). In particular:

	Are the conclusions on **reproducibility** (WLR and BLR) as well as transferability justified and plausible? Are the conclusions on preliminary **predictive capacity** justified and plausible with respect to existing information Are there **possible gaps between study design and study conclusions** which remain to be addressed in view of the suggested conclusions / use (see also point 3)? Do the data generated with this defined set of chemicals and available existing evidence provide sufficient information on **applicability and possible limitations** of the test method, in particular in view of its potential use within an ITS for sensitisation? 3) **SUGGESTED USE OF THE TEST METHOD:** The ESAC is requested (a) to evaluate, on the basis of the data submitted in the validation study, the possible use of the test method (also within a strategy) to identify skin sensitisers, (b) to make additional recommendations (as required) on the proper scientific use of the test method within such a strategy taking specific aspects of this method into account (e.g. applicability, limitations etc.) and (c) to identify possible further information required (i.e. are there gaps) to be able to conclude on the plausibility of the suggested use (including within an ITS).

4.3 TIMELINES

Timelines concerning this request *When does ECVAM require the advice?*	**Timeline**	**Indication**
	Finalised ESAC Opinion required by:	ESAC 37, 6/7 October 2012 or before through written procedure
	Request to be presented to ESAC by written procedure (e.g. due to urgency) prior to the next ESAC	YES, **before** ESAC 36 (20/21 March 2012)
	Request to be presented to ESAC at ESAC plenary meeting	NO

5. ECVAM PROPOSALS ON HOW TO ADDRESS THE REQUEST WITHIN ESAC

5.1 ECVAM PROPOSAL REGARDING REQUEST-RELATED STRUCTURES REQUIRED

Specific structures required within ESAC to address the request *Does the advice require an ESAC working group, an ESAC rapporteur etc.?*	**Structure(s) required**	**Required according to ECVAM? (YES/NO)**
	1) ESAC Rapporteur	NO
	2) ESAC Working Group	**YES**
	3) Invited Experts	YES
	Ad 3): If yes – list names and affiliations of suggested experts to be invited and specify whether these are member of the EEP	
	If other than above (1-3):	

5.2 DELIVERABLES AS PROPOSED BY ECVAM

Deliverables *What deliverables (other than the ESAC opinion) are required for addressing the request?*	**Title of deliverable other than ESAC opinion**	**Required? (YES/NO)**
	D1 ESAC Rapporteur Report and draft opinion	NO
	D2 ESAC Peer Review Report and draft opinion	**YES**
	If other than above (D1-D2):	NO

6. LIST OF DOCUMENTS TO BE MADE AVAILABLE TO THE ESAC

Count	Description of document	Available (YES/NO)	File name
1	OECD, Organisation for Economic Cooperation and Development (1992) Skin Sensitisation Guidelines for Testing of Chemicals No. 406, Paris	YES	ER2011-03_TG 406
2	OECD, Organisation for Economic Cooperation and Development (2002) The Local Lymph Node Assay. Guidelines for Testing of Chemicals No. 429, Paris	YES	ER2011-03_TG 429
3	OECD, Organisation for Economic Cooperation and Development (2010a) Skin Sensitization: Local Lymph Node Assay: DA, Guidelines for Testing of Chemicals No. 442A, Paris	YES	ER2011-03_TG 442A
4	OECD, Organisation for Economic Cooperation and Development (2010b) Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA, Guidelines for Testing of Chemicals No. 442B, Paris	YES	ER2011-03_TG 442B
5	Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrall SW, Lepoittevin JP, (2004) Development of a peptide reactivity assay for screening contact allergens. Toxicol Sci. 81; 332-43.	YES	ER2011-03_scientific paper on DPRA
6	Gerberick GF, Vassallo JD, Foertsch LM, Price BB, Chaney JG, Lepoittevin JP, (2007). Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. Toxicol Sci. 97, 417-27.	YES	ER201-03_DPRA Classification Tree Model

7. TERMS OF REFERENCE OF THE ESAC WORKING GROUP

7.1 ESTABLISHMENT OF THE ESAC WORKING GROUP

During its 34th meeting on March 22-23 the ESAC plenary unanimously decided to establish an ESAC Working Group Sensitisation charged with the detailed scientific review of four test methods for skin sensitisation.

7.2 TITLE OF THE ESAC WORKING GROUP

Full title:
ESAC Working Group on Skin Sensitisation Test Methods
Abbreviated title:
ESAC WG Sensitisation

7.3 MANDATE OF THE ESAC WG

The EWG is requested to conduct a scientific review of the relevant studies concerning four skin sensitisation test methods (DPRA, MUSST, h-CLAT, Keratinosens). The review needs to address the questions put forward to ESAC by ECVAM.
The review should focus on the appropriateness of design and conduct of the study in view of the study objective and should provide an appraisal to which extent the conclusions of the Validation Management Team (VMT) / test method submitter are substantiated by the information generated during the study and how the information generated relates to the scientific background available.

7.4 DELIVERABLE OF THE ESAC WG

The ESAC WG is requested to deliver to the chair of the ESAC and the ESAC Secretariat a detailed **ESAC Working Group Report** outlining its analyses and conclusions**.** A reporting template has been appended (Appendix 1) intended to facilitate the drafting of the report.
The conclusions drawn in the report should be based preferably on consensus. If no consensus can be achieved, the report should clearly outline the differences in the appraisals and provide appropriate scientific justifications.
The WG is further asked to prepare a draft ESAC opinion as basis for the discussions by the entire ESAC, which shall adopt its opinion to the extent possible by consensus and on the basis of the ESAC WG report as well as all documents that were made available to the WG as well as to all ESAC members.

7.5 PROPOSED TIMELINES OF THE ESAC WG

The following timelines have been proposed by ECVAM. These should be agreed upon during the face-to-face meeting (Item 1 in the table):

Item	Proposed date/time	Action	Deliverable
1	1-3 February	Face to face meeting	1)Input on draft mandate 2) Initial drafting of report
2	20. / 21. March	Progress report of WG Chair at ESAC36	Presentation / oral summary
3	14 May	Final report to be delivered to ESAC Coordinator/Secretariat.	Final draft report
4	8 June	Feedback from ESAC to WG on draft report (written procedure)	Feedback on final draft report
5	29 June	Final draft report available	Final report

7.6 QUESTIONS WHICH SHOULD BE ADDRESSED BY THE ESAC WG

The ESAC WG is requested to address the **three questions posed to the ESAC** which have been broken down further in more **specific questions** by Secretariat (see section 4.2) and were discussed with the ESAC WG and approved by the ESAC.

When preparing the final ESAC WG report to address these questions, the ESAC WG is requested to use a pre-defined reporting template. This template (see appendix 1) follows ECVAM's modular approach and addresses to which extent the standard information requirements have been addressed by the study. In addition, the template allows for addressing the specific questions outlined in section 4.2. The Secretariat will provide guidance if necessary.

APPENDIX 1 REPORTING STRUCTURE FOR THE *ESAC WG REPORT*

The following suggested structure follows the ECVAM information requirements ("modules") for scientific review following validation and allows at the same time for the description of the analysis and conclusions concerning more specific questions. A template has been created on the basis of the structure below and this template will be made available to the ESAC.

The template can be used for various types of validation studies (e.g. prospective full studies, retrospective studies, performance-based studies and prevalidation studies). Depending on the study type and the objective of the study, not all sections may be applicable. However, for reasons of consistency and to clearly identify which information requirements have not been sufficiently addressed by a specific study, this template is uniformly used for the evaluation of validation studies. The current template is version 5.

END OF EURL ECVAM RECOMMENDATION

European Commission
EUR 26383– Joint Research Centre – Institute for Health and Consumer Protection

Title: EUR 26383 - EURL ECVAM Recommendation on the **Direct Peptide Reactivity Assay (DPRA) for Skin Sensitisation Testing**

Luxembourg: Publications Office of the European Union

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Abstract

Identification of the skin sensitisation hazard of chemicals has traditionally relied on the use of animals. Progress in the development of alternative methods has been prompted by the increasing knowledge of the key biological mechanisms underlying this human health effect, as documented by the OECD's recent report summarising the key biological events leading to skin sensitisation ("Adverse Outcome Pathway" (AOP) for skin sensitisation). The molecular initiating event defined within this AOP is the covalent binding of chemicals with skin proteins. Thus peptide reactivity assays may provide valuable information in the context of integrated approaches such as Weight of Evidence (WoE) or Integrated Testing Strategies (ITS) for skin sensitisation hazard and safety assessment. Based on these considerations, EURL ECVAM coordinated a validation study on the Direct Peptide Reactivity Assay (DPRA) addressing mainly the test method's transferability and within- and between-laboratory reproducibility. Following independent scientific peer review by the EURL ECVAM's Scientific Advisory Committee (ESAC) and having considered the input from regulators, stakeholders, international partners and the general public, EURL ECVAM concluded that the DPRA may prove a valuable component of a WoE or ITS for skin sensitisation hazard assessment. In addition to this, the DPRA may also be able to contribute to the assessment of sensitising potency, e.g. by supporting sub-categorisation of sensitisers according to UN GHS. However it is recognised that further efforts are required to explore how DPRA data may contribute to potency assessment.

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