

Independent Peer-Review Panel Report

on the scientific validity of the

kinetic Direct Peptide Reactivity Assay (kDPRA)

as a modified version of the DPRA assay according to
OECD TG 442C, to extend its regulatory applicability to identify
UN GHS Subcategory 1A

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I. Summary

The kinetic Direct Peptide Reactivity Assay (kDPRA) is a modified version of the DPRA assay from OECD TG 442C, with the aim to identify potency Subcategory 1A (Subcat. 1A) chemicals based on the obtained kinetic reactivity rates. An industry-coordinated validation study involving seven participating laboratories was conducted between spring 2018 and summer 2019. The method and its validation study then underwent an independent peer-review between August 2019 and February 2020 by an international panel that included nominations from validation bodies. A total of 12 criteria were evaluated by the Peer Review Panel (PRP) to assess the scientific validity of the kDPRA.

In summary, the PRP agreed that the description of the test method and its Standard Operating Procedure were sufficient and adequate and that the kDPRA adequately adhered to the essential test method components required. The kDPRA within- (96%) and between- (88%) laboratory reproducibility (n= 24 tested in 3 (12) to 4 (12) laboratories) were considered to be adequate for the identification of UN GHS Subcat. 1A vs. non-Subcat 1A, i.e. the intended use. Furthermore, the predictive capacity of the kDPRA refined cut-off was considered to be appropriate for predicting UN GHS Subcat. 1A chemicals versus non-Subcat. 1A, with the caveat that the kDPRA performance needs to be reconsidered when combining kDPRA with other assays such as DPRA. In particular, kDPRA showed a sensitivity of 84.4% (38/45), a specificity of 85.9% (116/135), an accuracy of 85.6% (154/180) and a balanced accuracy of 85.2% for the intended purpose as compared to the LLNA *in vivo* reference data for 180 chemicals, of which 24 were tested blind. The applicability domain of kDPRA was considered well characterized and adequate based on the chemistries of tested chemicals.

Based on their evaluation, the PRP concluded that the data provided by the test method developers is sufficient and adequately supports the scientific validity of the kDPRA for the identification of UN GHS Subcat. 1A. Furthermore, additional investigation, outside of the scope of the present peer-review, is suggested in order to further characterize the performances of the kDPRA when used in combination with other test methods in the context of testing strategies.

II. Background and peer-review process

The kinetic Direct Peptide Reactivity Assay (kDPRA) is based on the same principle as the DPRA assay from OECD TG 442C, with the modification that in the kDPRA peptide depletion is measured at multiple time points and multiple concentrations to quantify peptide reactivity with a dynamic range of several orders of magnitude (Roberts and Natsch, 2019; Natsch et al., 2011).

If the current OECD TG 442C supports the discrimination between skin sensitizers and non-sensitizers for the purpose of hazard classification and labelling, the kDPRA aims at identifying potency Subcategory 1A (Subcat. 1A) versus non-Subcat. 1A as a stand-alone assay (Wareing et al., 2017) based on the derived kinetic reactivity rates. A standard operating procedure for the kDPRA was developed and an industry-coordinated validation study took place involving seven participating laboratories between spring 2018 and summer 2019.

The scientific validity of the kDPRA was assessed by an international Peer Review Panel between August 2019 and February 2020. The peer-review was organized by the National Coordinators for the OECD TG Program from Germany and Switzerland, with the help of Chantra Eskes as an independent consultant. The international PRP comprised nominations from international validation bodies and by the PRP organizers as listed below. Each PRP member provided a declaration of interest as to ensure no conflicts of interest existed (note: the declaration of interests can be made available upon request).

- Emanuela Corsini (Università degli Studi di Milano, Italy), nominated by the PRP organisers.
- Steven Enoch (Liverpool John Moores University, UK), nominated by EURL-ECVAM.
- Sebastian Hoffmann (seh consulting + services, Germany), nominated by the PRP organisers.
- Tsuyoshi Kawakami (NIHS, Japan), nominated by JaCVAM.
- Judy Strickland (ILS-NICEATM, US), nominated by ICCVAM.

A draft validation study report was made available to the PRP in August 2019 which was revised based on the comments from the PRP (Anon., 2020). Overall, and a total of four teleconferences took place during the peer-review of kDPRA as described below.

- 29 Aug. 2019 PRP + test developers: Presentation by the test method developers and preliminary questions from the PRP.
- 24 Sept. 2019 PRP only: PRP draft evaluation in the absence of the test method developers.
- 15 Nov. 2019 PRP + test developers: Responses from test method developers to additional questions raised by the PRP.
- 13 Jan. 2020 PRP only: Finalization of the PRP evaluation of the kDPRA test method.

III. Peer-review panel evaluation

The criteria for peer-review evaluation were proposed by the independent consultant and were revised by the PRP members. In particular, kDPRA was considered to be a modified version of the DPRA. However, the PRP was of the opinion that not all elements of the kDPRA can be compared to the performance standards established for DPRA within the OECD GD 303 adopted in 2019 (OECD, 2019b). For example, the kDPRA essential test method components could be compared to DPRA, but the kDPRA predictive capacity cannot be compared to DPRA since kDPRA does not provide the same predictions as DPRA (Subcat. 1A vs. non-Subcat. 1A for kDPRA, whereas DPRA supports the discrimination between sensitizers vs. non-sensitizers). Furthermore, GD 303 was published after the testing phase of the kDPRA validation study took place. As consequence, the reference chemicals recommended within GD 303 were not available when planning the kDPRA validation study, and were therefore not necessarily tested during the kDPRA validation study.

1. Rationale for the test method

Rationale for the kDPRA test method, including a description of the advantages of the test method in terms of i) mechanistic advantages, applicability, predictive capacity, technical advances, reduction in hazardous

reagents, ii) IP rights, geographical availability and animal welfare, iii) costs, analysis time, sample amount, competitiveness, iv) others.

The PRP agreed that the description of the test method provided in the validation report was sufficient and adequate. The main difference of kDPRA as compared to DPRA is that it can identify UN GHS Subcat. 1A chemicals, providing therefore an added value. However, the use of kDPRA within the context of testing strategies deserves further investigation. Regarding the technical advantages, the use of fluorescence detection in the kDPRA represents a simple and more broadly applicable method as compared to the use of HPLC for DPRA, which requires specialized equipment. Finally, according to the test developers, the costs of kDPRA are similar to the costs of DPRA.

2. Test method protocol

A detailed protocol for the kDPRA test method should be available.

A Standard Operating Procedure (SOP) is available for the kDPRA and was considered sufficient and adequate by the PRP.

3. Adherence to the essential test method components

Adherence to the essential test method components as described in TG 442C and GD 303 should be demonstrated regarding i.e., the procedural conditions.

The PRP agreed that the kDPRA adequately adheres to the essential test method components as described by OECD TG 442C and GD 303 (OECD, 2019a, 2019b).

4. Biological / mechanistic relevance

The toxicological mechanisms, the relationship between the test method endpoint(s) with the biological effect, the toxicity of interest and the limitations of the test method should be adequately addressed and described.

The PRP agreed this criterion was adequately addressed and described.

5. Selection of test chemicals

The test method's performance should be based on testing of representative, preferably coded reference chemicals, for which data on the validated reference method exists.

The PRP agreed that the total number of tested chemicals was appropriate (n=24 chemicals for assessing within- and between- laboratory reproducibility, and n = 176 for assessing predictive capacity). Albeit there were few UN GHS No Category (No Cat.) chemicals tested for evaluating within- and between- laboratory reproducibility, the distribution of chemicals used to assess within- and between-laboratory reproducibility (i.e., 12 Subcat. 1A, 10 Subcat. 1B and 2 No Cat.) was considered appropriate for the intended purpose of the kDPRA , i.e. distinguishing UN GHS Subcat. 1A vs. the non-Subcat. 1A. The PRP also acknowledged the fact that these chemicals are different from the reference chemicals recommended within OECD GD 30 due to the fact that GD 303 was adopted only after completion of the kDPRA validation study.

6. Within- and between-laboratory reproducibility

The within- and between-laboratory reproducibility of the kDPRA test method should be demonstrated and be comparable or higher than the one of the validated reference method.

The PRP noted that 2 of the 7 participant laboratories were fully naïve to the kDPRA assay and also DPRA, and that good results were obtained in these two laboratories demonstrating a good transferability of the test method. Furthermore, the obtained within- (96%) and between- (88%) laboratory reproducibility (n= 24 tested in 3 (12) to 4 (12) laboratories) were considered to be adequate for the identification of UN GHS Subcat. 1A vs. non-Subcat. 1A. Although this criterion cannot be compared directly to the validated reference method, since DPRA is used to support the discrimination between sensitizers vs. non-sensitizers, the values obtained with kDPRA were in line if not higher than the values obtained with the DPRA validation dataset (87% for within- and 88% for between- laboratory reproducibility). The PRP however, recommends that further investigations are conducted on the use of kDPRA to predict non-sensitizers as a possible replacement to DPRA.

7. Predictive capacity

The predictive capacity obtained with the kDPRA test method should be appropriate and demonstrated using representative chemicals. The performance of the kDPRA test method should have been evaluated in relation to existing relevant toxicity data as well as information from the relevant target species.

The predictive capacity of the kDPRA refined cut-off was considered to be appropriate for predicting UN GHS Subcat. 1A chemicals versus non-Subcat. 1A, with the caveat that the predictive capacity values need to be reconsidered when kDPRA is used in combination with other test methods that support the identification of non-sensitizers (i.e., UN GHS No Category) like DPRA. As compared to the LLNA *in vivo* data (i.e., the reference method which kDPRA aims to partially replace), kDPRA showed a sensitivity of 84.4%, a specificity of 85.9%, an accuracy of 85.6% and a balanced accuracy of 85.2% for 180 chemicals, of which 24 were tested blind (see table below). These values were derived from an extended database of 186 chemicals tested out of which 6 could not be evaluated due to quenching, autofluorescence or reaction with the fluorescent dye. The final database of 180 chemicals contained 45 UN GHS Subcat. 1A, 96 UN GHS Subcat. 1B, 37 UN GHS No Cat. and 2 UN GHS Subcat. 1B/No Cat. according to the LLNA reference data, and 33 UN GHS Subcat. 1A, 52 UN GHS Subcat. 1B, 36 UN GHS No Cat. and 2 UN GHS Subcat. 1B/No Cat. according to the human reference data. Furthermore, the predictive capacity of kDPRA as compared to human data was assessed and compared to the predictive capacity of the LLNA vs. human data as shown in the table below. The PRP was of the opinion that the above performances of kDPRA cannot be compared to the performance standards required for me-too DPRA assays described within OECD GD 303, since the kDPRA is used to predict UN GHS Subcat. 1A vs. the non-Subcat. 1A, whereas DPRA is used to support discrimination between sensitizers vs. non-sensitizers.

Predictive capacity of the kDPRA assay to identify UN GHS Subcat. 1A chemicals versus non-Subcat. 1A

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Balanced accuracy (%)
kDPRA vs. LLNA	84.4 (38/45)	85.9 (116/135)	85.6 (154/180)	85.2
kDPRA vs. Human	63.6 (21/33)	88.9 (80/90)	82.1 (101/123)	76.3
LLNA vs. Human	54.5 (18/33)	91.1 (82/90)	81.3 (100/123)	72.8

8. Applicability Domain

The applicability domain of the kDPRA test method should be clearly defined.

The PRP agreed that the applicability domain of kDPRA was well characterized and adequate based on the chemistries of tested chemicals. Furthermore, the PRP considered that this criterion could not be compared to the validated reference method DPRA, since the kDPRA is used to predict UN GHS Subcat. 1A vs. the non-Subcat. 1A, whereas DPRA is used to support the discrimination between sensitizers vs. non-sensitizers.

9. Accordance with the principles of Good Laboratory Practices

All data from the validation study supporting the validity of the kDPRA test method should be obtained in accordance with the principles of Good Laboratory Practice (GLP).

The PRP agreed that the GLP-like conditions used within the validation study were sufficient and adequate.

10. Completeness of data and documents

Completeness of all data and documents supporting the assessment of the validity of the kDPRA test method.

The PRP agreed that the completeness of data and documents was sufficient and adequate. The test developer provided all data required by the PRP during the peer review process, and even more.

11. Validation study management and conduct

The PRP agreed that the validation study management and conduct was adequate. It is noted that the PRP had transparent and full access to the conducted data analyses.

12. Conclusions

All data should adequately support the peer review assessment that the kDPRA test method is structurally and mechanistically similar to the validated reference method, and demonstrate adequate performances (reliability and relevance) for the proposed specific testing purpose i.e., that the kDPRA test method is scientifically valid.

The PRP concluded that the data provided by the test method developers support the scientific validity of the kDPRA for its intended purpose, i.e., the identification of UN GHS Subcat. 1A vs. non-Subcat. 1A. Furthermore, additional investigation, outside of the scope of the present peer-review, is suggested in order to further characterize the predictive capacity of the kDPRA when used in combination with other test methods in the context of testing strategies.

IV. References

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