Defined Approaches to Skin Sensitisation and the integration of *in silico* models in an OECD Guideline

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BTS-IGHRC 'PHE virtual workshop 1: Current status of NAMs for regulatory purposes'

22-23 November 2021

HUMANE SOCIETY INTERNATIONAL

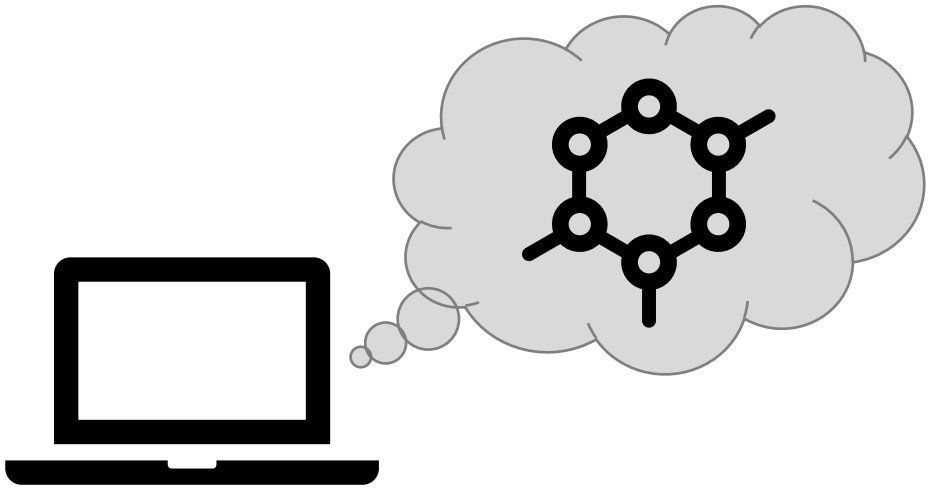
Public Health England

What is a defined approach?

- A Defined Approach (DA) consists of a selection of information sources (e.g *in chemico*, *in vitro* data, *in silico* predictions) used in a specific combination, interpreted using a fixed data interpretation procedure (DIP) (e.g. a mathematical, rule-based model).
- The DAs for skin sensitisation included in the groundbreaking Guideline No. 497 provide a qualitative output (sensitiser/non-sensitiser) or a categorical output (UN GHS 1A/1B/NC).

OECD (2021), Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

What is an *in silico* model?

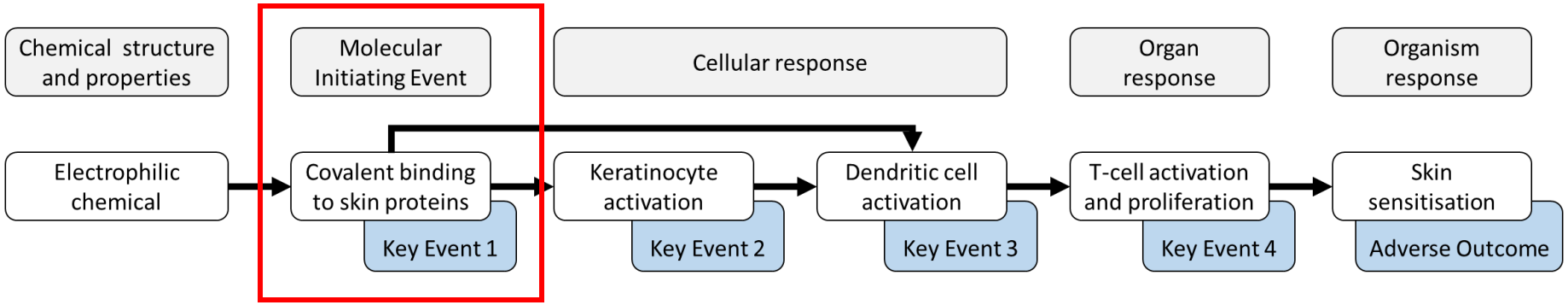


- *In silico* model = a model built using a computer.
- *In silico* models can:
  - Predict physicochemical properties (e.g. lipophilicity (logP), pKa).
  - Predict biodegradation.
  - Predict metabolism.
  - Predict where in the body the chemical will go.
  - Use similar chemicals to extrapolate information (e.g. for grouping, read-across).
  - Predict biological activity (e.g. reactivity, protein binding, toxicity).

- Toxicity is often predicted using SAR and/or QSAR models.
- The chemical structure is used to identify trends/patterns between structural features and toxicity (toxicophore).
  - SAR use the chemical structure itself to identify a structure-activity relationship.
  - QSAR convert the chemical structure to a molecular descriptor which is used to identify a quantitative structure-activity relationship.
- Examples:
  - Derek Nexus.
  - OECD QSAR Toolbox.
  - Danish QSAR database.
  - TIME-SS
  - MIE Atlas.
  - CATMoS.

Skin sensitisation and *in silico* models

- Skin sensitisation has a well-known Adverse Outcome Pathway (AOP).
- The Molecular Initiating Event (MIE) occurs when an electrophilic chemical can react with nucleophilic skin proteins.



- This interaction is relatively easy to model, and there are many *in silico* models that predict the skin sensitisation potential of chemicals with high performance.
- The majority of defined approaches to skin sensitisation have included *in silico* models.

OECD (2014), The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris.
Golden E, et al., Evaluation of the global performance of eight in silico skin sensitization models using human data. ALTEX. 2021;38(1):33-48.

Defined approaches using *in silico* models

OECD DA title (submitter)	Data inputs
An AOP-based "2 out of 3" Integrated Testing Strategy Approach to Skin Hazard Identification (BASF)	DPRA, h-CLAT, KeratinoSens™, U-SENS™
Sequential Testing Strategy (STS) for Hazard Identification of Skin Sensitisers (RIVM)	DPRA, h-CLAT, KeratinoSens™, HaCaT gene signature, MultiCASE, CAESAR, DEREK, OECD QSAR toolbox
A non-testing pipeline approach for skin sensitisation (DuPont/G. Patlewicz)	Existing data, protein binding profile, physicochemical properties, TIMESSS, expert judgment
Stacking Meta-model for Skin Sensitisation Hazard Identification (L'Oreal)	DPRA, KeratinoSens™, U-SENS™, TIMES-SS, ToxTree, volatility, pH
Integrated decision strategy for skin sensitisation hazard (ICCVAM)	DPRA, h-CLAT, KeratinoSens™, OECD QSAR Toolbox, physchem properties
Consensus of Classification Trees for Skin Sensitisation Hazard Prediction (EC-JRC)	TIMES-SS, DRAGON descriptors
Sensitizer Potency Prediction Based on Key Event 1 þ 2: Combination of Kinetic Peptide Reactivity Data and KeratinoSens™ Data (Givaudan)	Cor1C420 (kinetic peptide reactivity), KeratinoSens™, TIMES-SS
The Artificial Neural Network Model for Predicting LLNA EC3 (Shiseido)	DPRA, h-CLAT, ARE (or KeratinoSens™)
Bayesian Network DIP (BN-ITS-3) for Hazard and Potency Identification of Skin Sensitizers (P&G)	DPRA, h-CLAT, KeratinoSens™, TIMES-SS, bioavailability (solubility, log D, plasma protein binding)
Sequential Testing Strategy (STS) for Sensitising Potency Classification Based on *in Chemico* and *In Vitro* Data (Kao)	DPRA, h-CLAT
ITS for Sensitising Potency Classification Based on *In Silico, In Chemico*, and *In Vitro* Data (Kao)	DPRA, h-CLAT, Derek Nexus
Data Interpretation Procedure for Skin Allergy Risk Assessment (SARA) (Unilever)	Bioavailability, skin protein kinetics, ordinary differential equation model
A defined approach for predicting skin sensitisation hazard and potency based on the guided integration of *in silico, in chemico* and *in vitro* data using exclusion criteria (Lhasa Limited)	DPRA, KeratinoSens™, LuSens, h-CLAT, U-SENS™, Derek Nexus

Legend Uses an *in silico* model.

*Adapted from Kleinstreuer et al., Non-animal methods to predict skin sensitization (II): an assessment of defined approaches *. Crit Rev Toxicol. 2018 May;48(5):359-374.*

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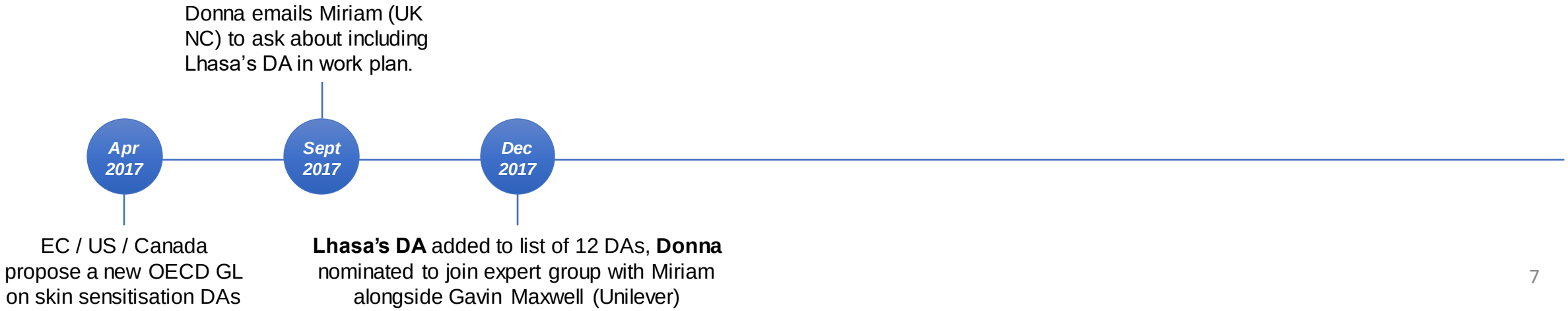
Back to the beginning…

- In 2017, there were two proposals (SPSFs) submitted to the OECD:
  - 12 previously published DAs (EC/US/Canada).
  - Lhasa Limited DA (UK).

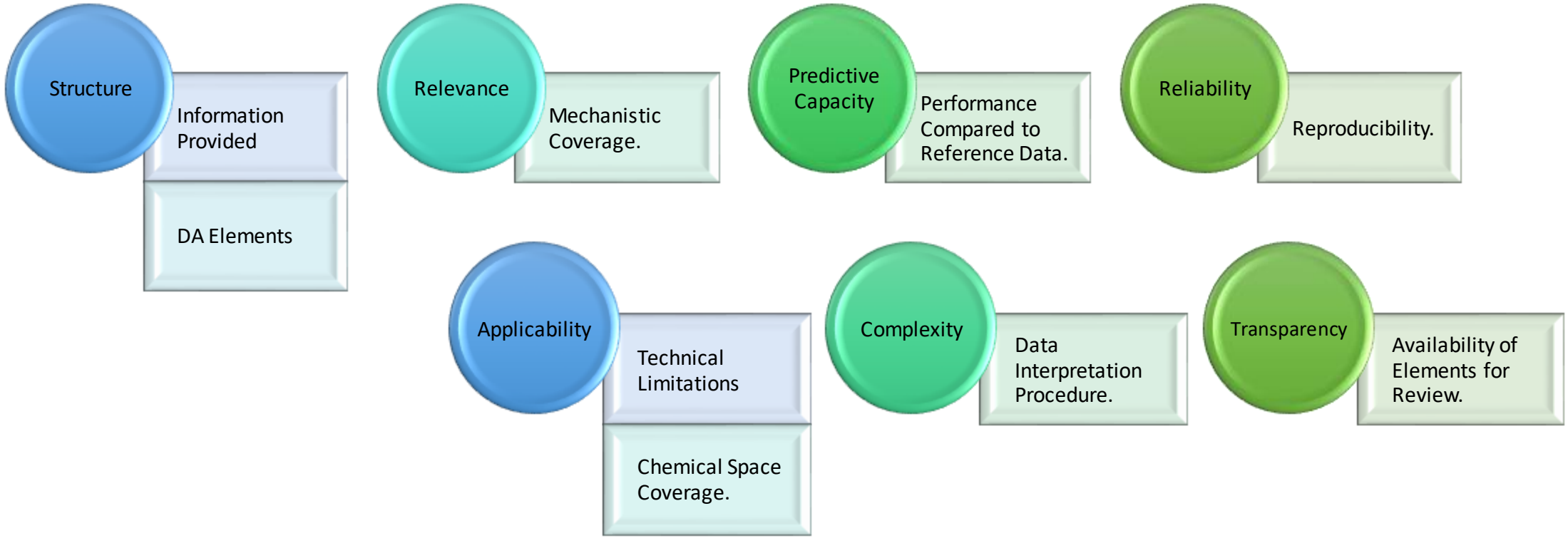
- It was agreed to merge them and have one project led by the EC/US/Canada which proposed to:
  - Organise an Expert Group for DASS.
  - Analyse animal (LLNA) and human data.
  - Develop an evaluation framework for DAs.
  - Draft a Guideline for the DAs.

- Miriam, Donna, and Gavin Maxwell (Unilever) then joined the expert group, to contribute to the work.



Evaluation Framework

- A robust evaluation framework was developed by the leads, agreed upon by the EG after discussion, to ensure each DA was assessed critically:



- Each DA element was already part of an OECD TG….except the *in silico* models.

Annex 1: Evaluation Framework – Supporting Information to the OECD Guideline No. 497 on Defined Approaches for Skin Sensitisation.

How to evaluate *in silico* tools?

General

- (Q)SARs should adhere to the OECD Validation Principles.

To facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information:

1) a defined endpoint[1]
2) an unambiguous algorithm[2]
3) a defined domain of applicability[3]
4) appropriate measures of goodness-of–fit, robustness and predictivity[4]
5) a mechanistic interpretation, if possible[5]

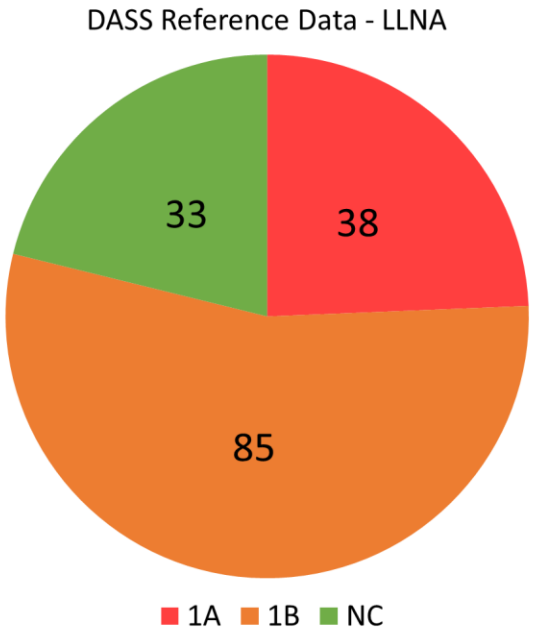
- QMRFs ((Q)SAR Model Reporting Format) should be available for each endpoint.
  - A harmonised template for summarising and reporting key information on QSAR models including the results of any validation studies.
  - Contained in the JRC QSAR Model Database, intended to help to identify valid QSARs, e.g. for the purposes of REACH.

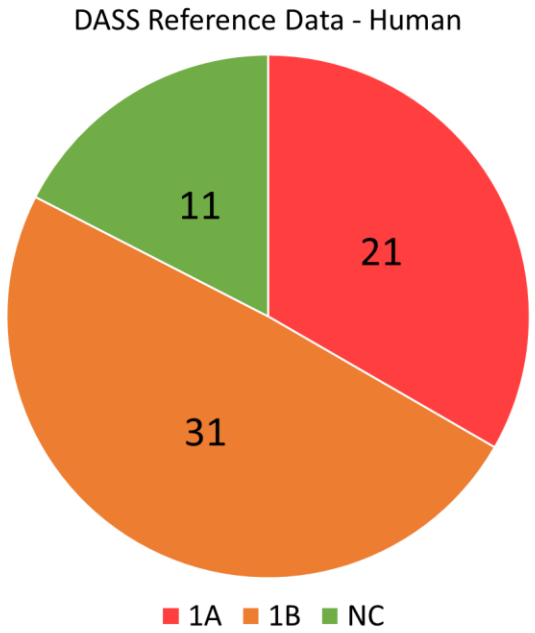
DASS-specific

- Transparency.
  - What data is used for the prediction?
  - What type of data is used?
  - Is the data visible?
- Applicability Domain.
  - Is the chemical of interest in or out of domain?
- Uncertainty/confidence.
  - How is uncertainty measured?
  - Is it quantified?
- Protocol.
  - How is a typical prediction run?
  - Are default settings used?
  - Are any default parameters changed?
- Version.
  - Is it the version information clear?

DASS Reference Dataset

- Significant time and effort was taken by the EG to ensure the reference dataset was high-quality.
  - Skin sensitisation is unique in that both human and animal data is available.
  - 2 sub-groups were created to curate and evaluate the data.
  - Their in-depth analysis looked at uncertainty, variability and reproducibility.
- Resulted in a highly-curated dataset.





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Legend Uses an *in silico* model. Simple, rule-based DAs evaluated by EG

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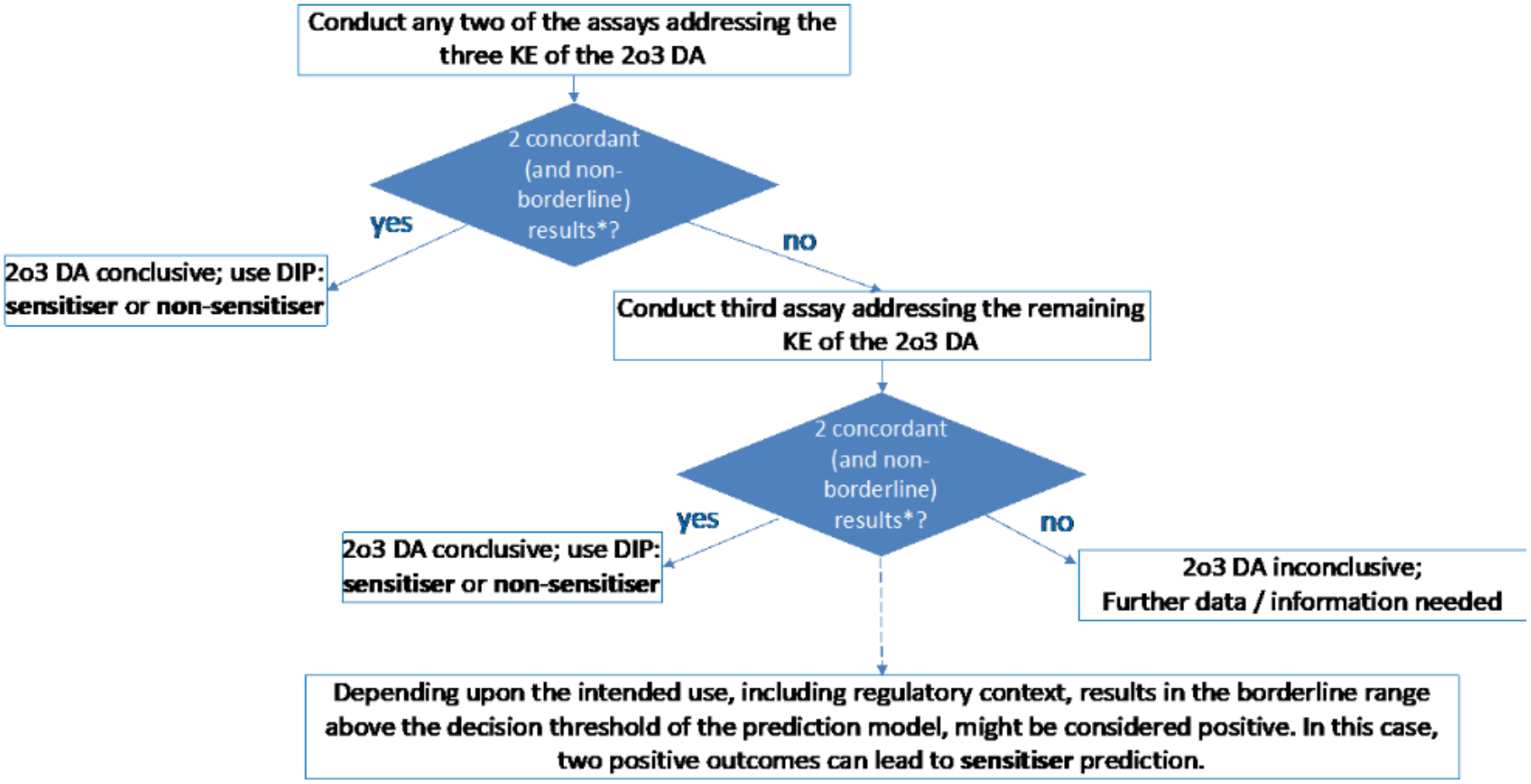
2o3 Defined Approach

Decision tree

Figure 2.1. Decision tree to be used for the 2o3 DA, taking into account borderline results



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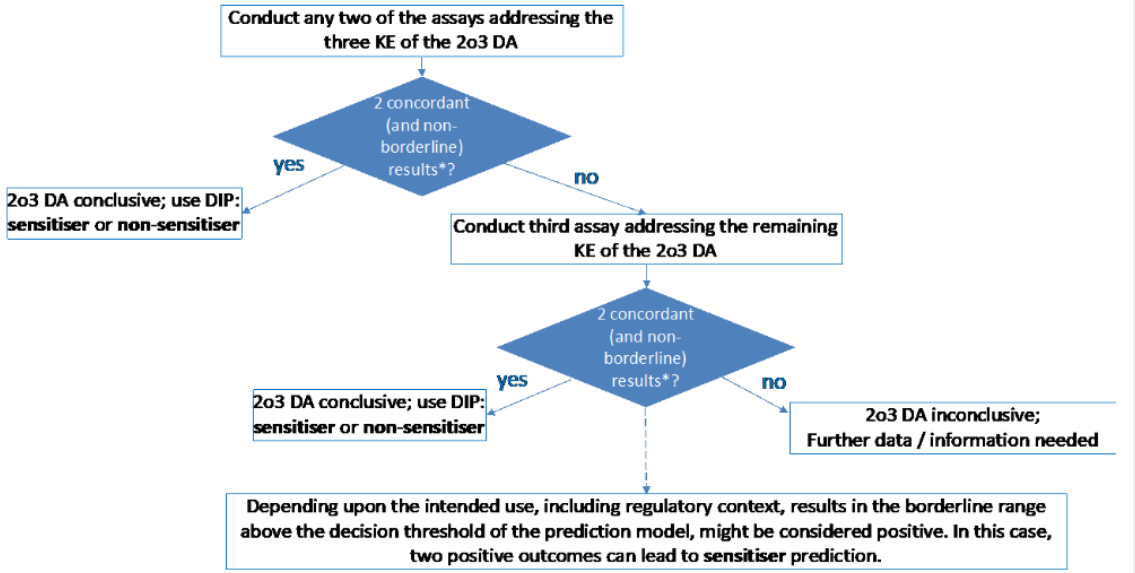


Strengths	Limitations
Simple decision tree	May provide an inconclusive prediction for: • Chemicals with borderline results • High logP chemicals
Takes borderline results into account	• Can only make hazard predictions

Performance

Table 2.1. Hazard identification performance of the "2o3" DA in comparison to LLNA reference data

	LLNA	
2o3 DA	Non	Sens
Non	22	19
Sens	4	89
Inconclusive	7	27

DA Performance vs. LLNA Data (N=134)	2o3
Accuracy (%)	83%
Sensitivity (%)	82%
Specificity (%)	85%
Balanced Accuracy (%)	84%

Table 2.2. Hazard identification performance of the "2o3" DA in comparison to human reference data

	Human	
2 of 3 DA	Non	Sens
Non	7	5
Sens	1	42
Inconclusive	3	7

DA Performance vs. Human Data (N=55)	2o3
Accuracy (%)	89%
Sensitivity (%)	89%
Specificity (%)	88%
Balanced Accuracy (%)	88%

OECD (2021), Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

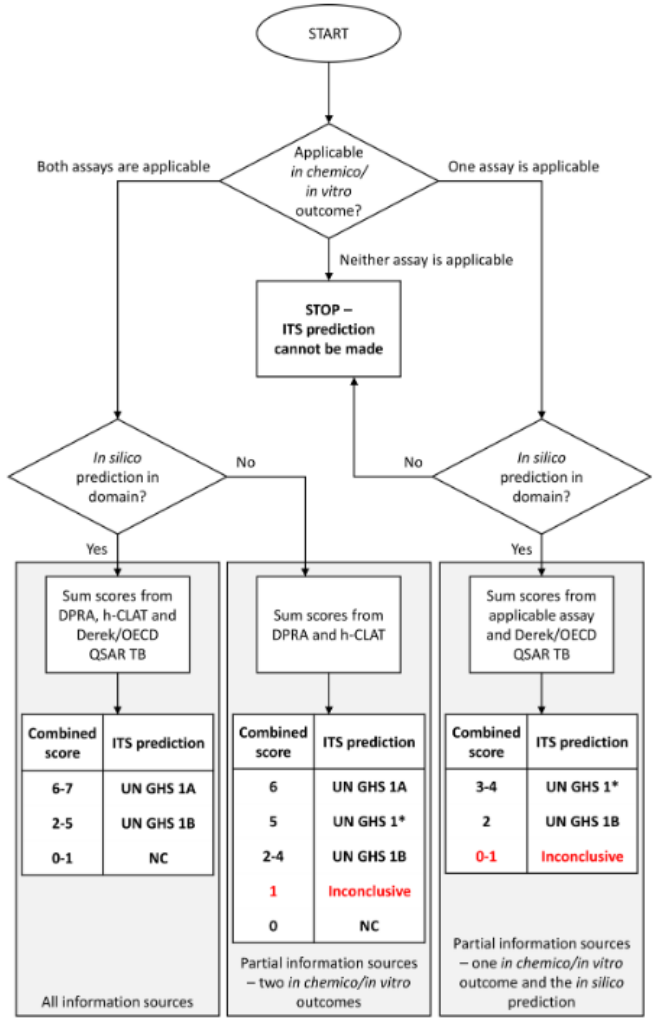
ITS Defined Approach

Decision tree

Table 3.1. Schematic of the ITS defined approach. The DA is a simple score-based system depending on assays from OECD TG 442E and 442C, and an *in silico* structure-based prediction, as shown.

Score	h-CLAT MIT µg/mL	DPRA mean Cysteine and Lysine% depletion	DPRA Cysteine % depletion*	*In silico* (ITSv1: DEREK; ITSv2: OECD TB)
3	≤10	≥42.47	≥98.24	
2	>10, ≤150	≥22.62, <42.47	≥23.09, <98.24	
1	>150, ≤5000	≥6.38, <22.62	≥13.89, <23.09	Positive
0	not calculated	<6.38	<13.89	Negative
	Potency	Total Battery Score		
	UN GHS 1A	6-7		
	UN GHS 1B	2-5		
	Not classified	0-1		

Figure 3.1. Decision tree for assigning confidence to the ITS DA predictions



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ITS Defined Approach

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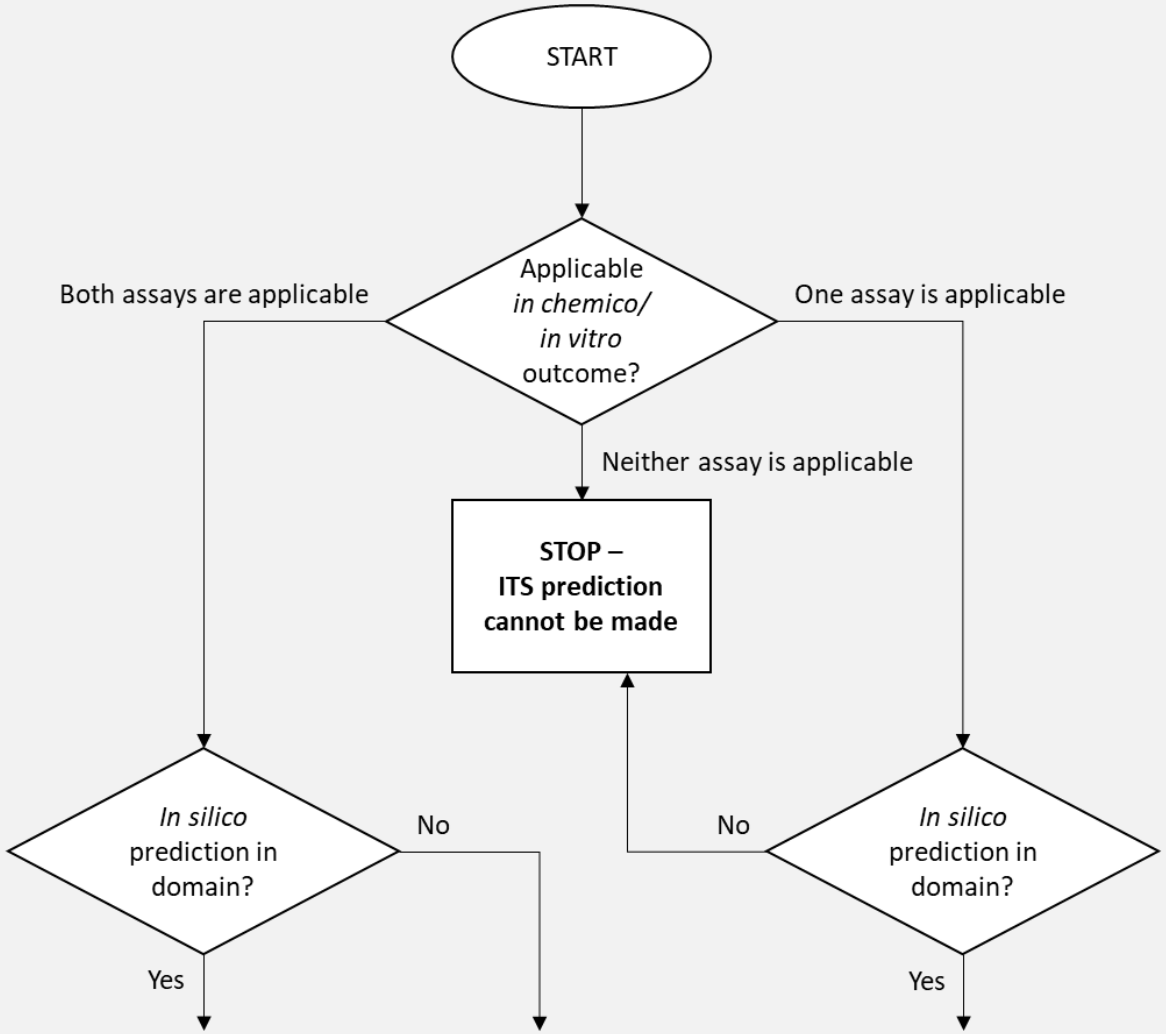

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ITS Defined Approach

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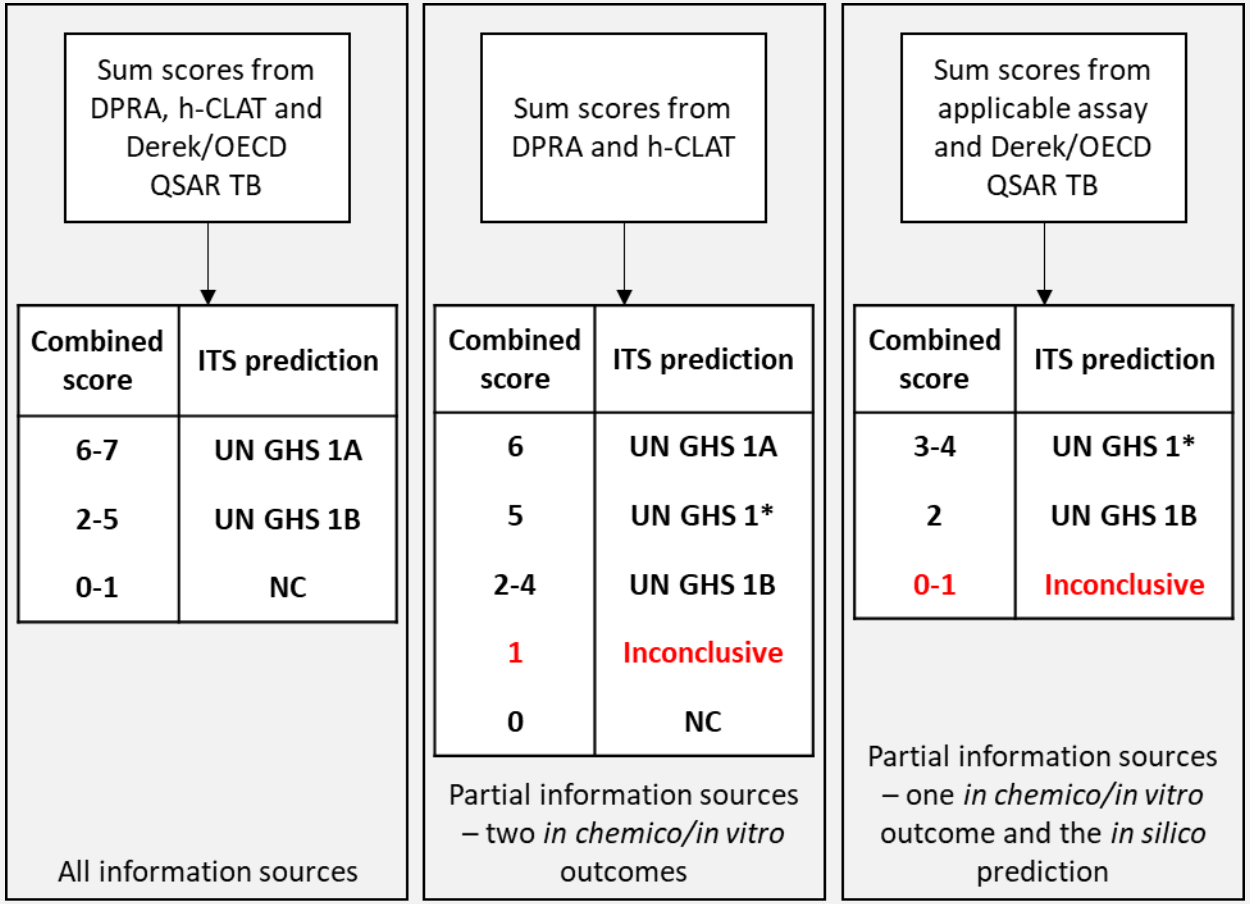


***Conclusive for hazard, inconclusive for potency**

OECD (2021), Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

ITS Defined Approach

Performance

Table 3.3. Potency categorisation performance of the ITSv1 DA in comparison to LLNA reference data, based on the UN GHS 1A/1B sub-categorisation

	LLNA		
ITSv1 DA	NC	1B	1A
NC	21	11	0
1B	9	55	10
1A	0	12	28
Inconclusive	3	7	0

71% correct classification overall

Table 3.7 Potency categorisation performance of the ITSv1 DA in comparison to Human reference data, based on the UN GHS 1A/1B sub-categorisation

	Human		
ITSv1 DA	NC	1B	1A
NC	4	4	0
1B	5	24	7
1A	0	3	13
Inconclusive	2	0	1

68% correct classification overall

Table 3.5. Potency categorisation performance of the ITSv2 DA in comparison to LLNA reference data, based on the UN GHS 1A/1B sub-categorisation

	LLNA		
ITSv2 DA	NC	1B	1A
NC	20	9	0
1B	10	54	10
1A	0	12	26
Inconclusive	3	10	2

71% correct classification overall

Table 3.9. Potency categorisation performance of the ITSv2 DA in comparison to Human reference data, based on the UN GHS 1A/1B sub-categorisation

	Human		
ITSv2 DA	NC	1B	1A
NC	4	3	0
1B	5	24	6
1A	0	3	12
Inconclusive	2	1	3

70% correct classification overall

Strengths
Predicts potency (UN GHS 1A/1B)
Can make a prediction with partial information sources
Limitations
May provide an inconclusive prediction for: • High logP chemicals • Chemicals with an out of domain *in silico* prediction
May provide a hazard prediction instead of potency when using partial information sources

OECD (2021), Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Inconclusive predictions

- Limitations of the individual *in chemico/in vitro* assays and *in silico* predictions are carried through to the DAs.
  - Borderline results from DPRA, KeratinoSens™, h-CLAT.
  - Negative results from h-CLAT for chemicals with a logP > 3.5 as OECD TG 442E states that these are not reliable.
  - Chemicals outside Derek Nexus or OECD Toolbox's applicability domain – these are not used in the DAs.
- This results in some **inconclusive predictions** from the DAs.
- However, the DASS Guideline states:

"DA predictions with high confidence for hazard identification and potency are considered conclusive. DA predictions with low confidence are considered inconclusive for hazard identification and/or potency. ***These 'inconclusive' predictions may nevertheless be considered in a weight-of-evidence approach and/or within the context of an IATA together with other information sources."***

How to use inconclusive results?

- ECHA have recently published some guidance for skin sensitisation which describes how to:
  - Use the *in chemico/in vitro* assays
  - Use the *in silico* tools
  - Use the defined approaches
  - Approach risk assessment
- ECHA's guidance re-iterates that:

For inconclusive predictions, no standalone conclusion on skin sensitisation potential, or the lack thereof, can be made. However, the information generated from the individual information sources can still be used in a weight of evidence approach to conclude on the skin sensitisation potential if adequate information is available. The weight of evidence assessment may, however, indicate the need to generate additional information e.g. through further experimental studies, from different in silico tools or by using a read-across approach."

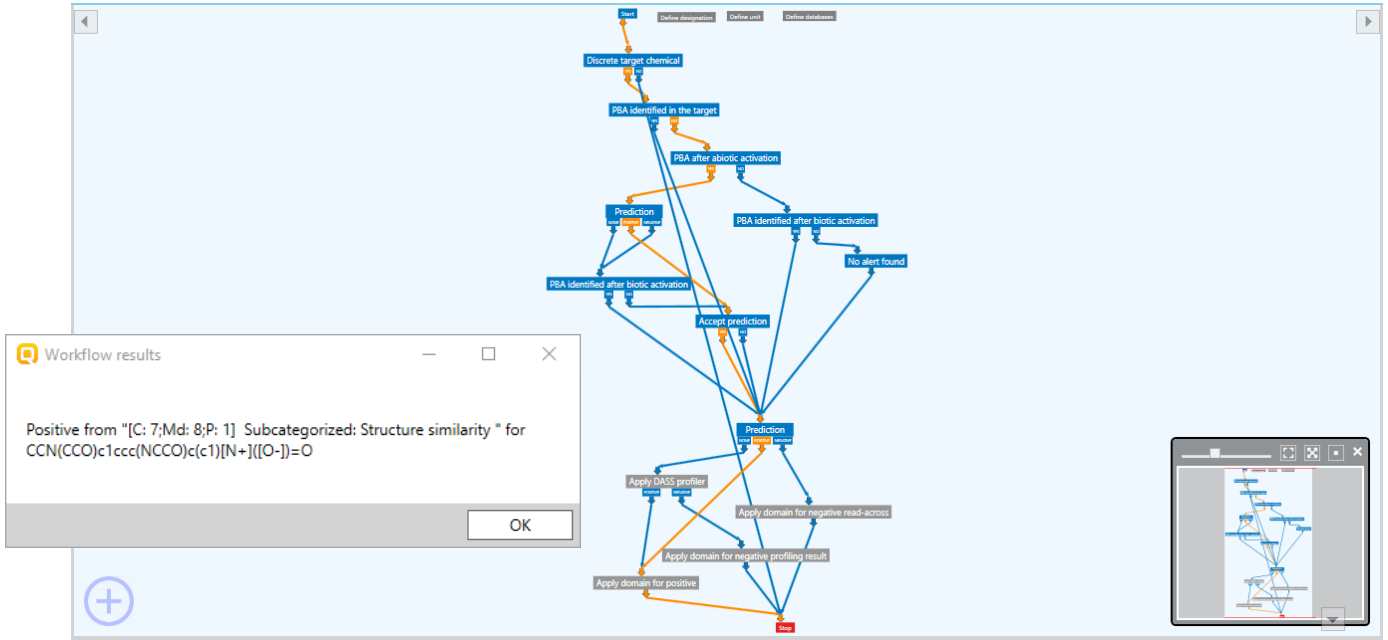
- HSI, Lhasa Limited, P&G and Firmenich are writing a publication which presents a scientific, logical approach to applying a weight of evidence to inconclusive DA results.
  - Expected publication date early next year.

https://echa.europa.eu/documents/10162/1128894/oecd_test_guidelines_skin_sensitisation_en.pdf/40baa98d-fc4b-4bae-a26a-49f2b0d0cf63?t=1633687729588

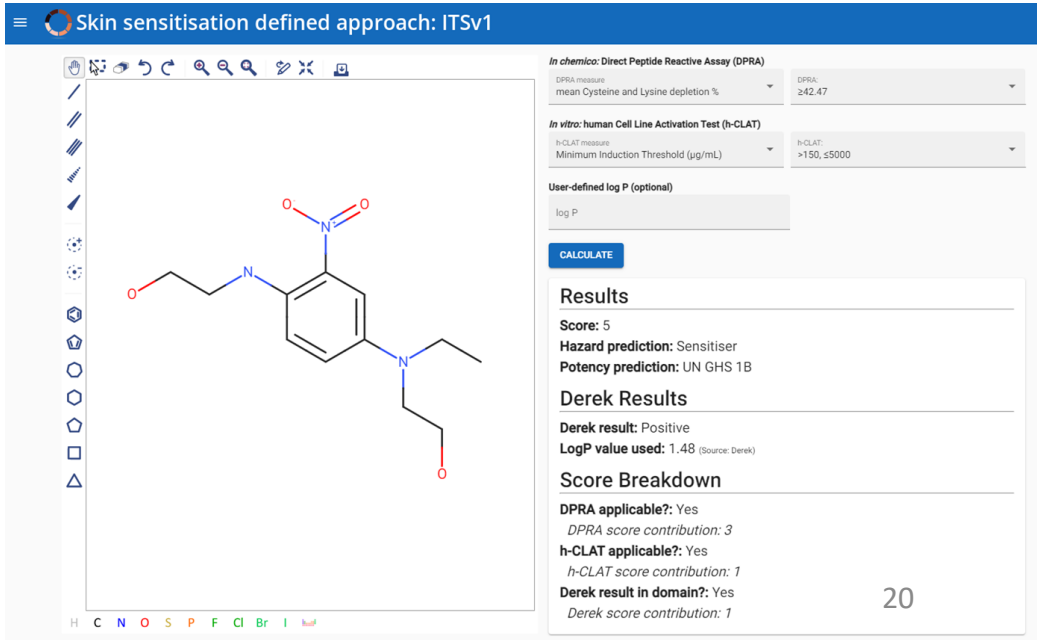
Helpful tools for the *in silico* models

- The DAs using *in silico* tools can seem complicated as protocol, applicability domain and uncertainty has to be taken into account prior to use of a prediction in ITSv1 or ITSv2.
- To assist with this, OECD QSAR Toolbox have developed the Automated Workflow for Skin Sensitization for Defined approaches (DASSAW) which generates the *in silico* prediction for ITSv2.
- Similarly, Lhasa Limited have developed an app which implements the protocol, generates the *in silico* prediction, and applies the decision tree for ITSv1.

OECD QSAR Toolbox DASSAW for ITSv2



Lhasa app for ITSv1



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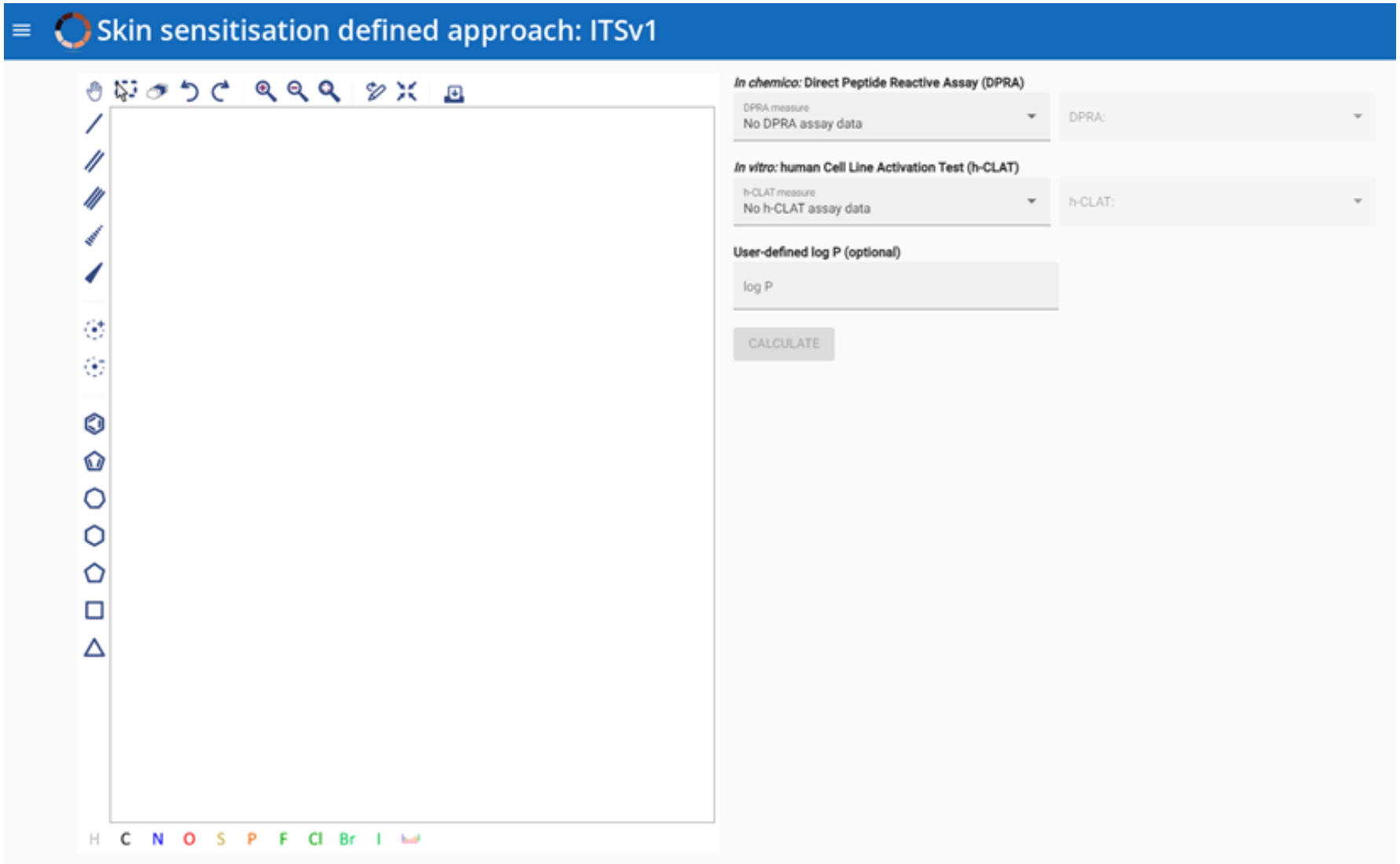
Skin sensitisation defined approach: ITSv1
In chemico: Direct Peptide Reactive Assay (DPRA)
DPRA measure
No DPRA assay data
DPRA:
In vitro: human Cell Line Activation Test (h-CLAT)
h-CLAT measure
No h-CLAT assay data
h-CLAT:
User-defined log P (optional)
log P
CALCULATE
H C N O S P F Cl Br I

Lhasa app – Summary

- Having a tool that implements this defined approach will make it more accessible.
  - Applies *in silico* protocol.
  - Calculates *in silico* applicability domain.
  - Handles assay limitations.
  - Applies Data Integration Procedure.
  - Handles partial information sources.
  - Calculates confidence in overall prediction.
  - Ensures DA is applied consistently.
- If you have any feedback for what would make the tool more useful, please get in touch.

Summary of DASS Guideline No. 497

OECD GUIDELINE FOR TESTING OF CHEMICALS

SUPPORTING DOCUMENT TO THE OECD GUIDELINE 497 ON DEFINED APPROACHES FOR SKIN SENSITISATION

7. List of Annexes to this Document[3]

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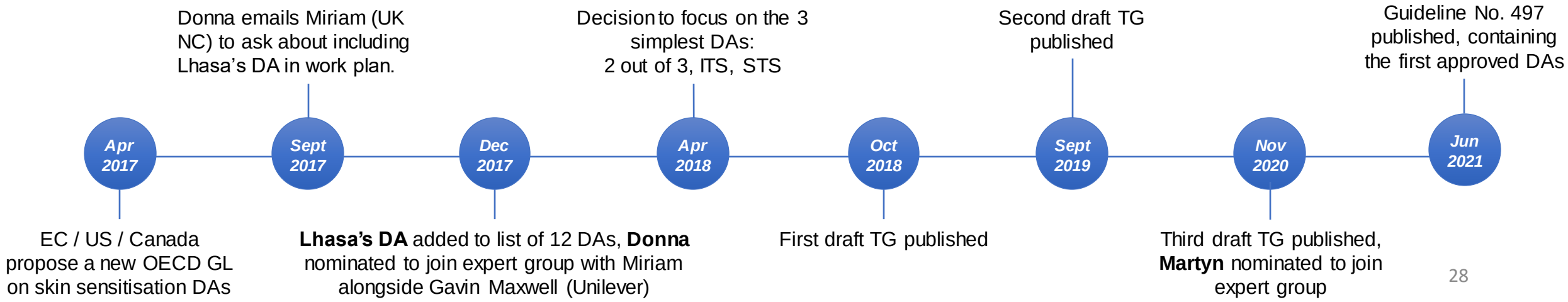
Conclusions

- 4 years to develop OECD Guideline No. 497
  - First Guideline to describe Defined Approaches.
  - First Guideline to include *in silico* results.
  - First Guideline to use such a highly-curated good quality dataset using both animal and human data.
- Predicts human skin sensitisation potential with more accuracy than the LLNA.
- Should be easy to add additional DAs to OECD Guideline No. 497 now we have robust evaluation framework and reference dataset.
- We hope the development of the DASS can promote the acceptance of other DAs and pave the way for more *in silico* tools to be incorporated in OECD TG.



Acknowledgements

- Miriam Jacobs (UK National Coordinator)
- The DASS project leads and the whole EG!!

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