How to resolve inconclusive predictions from defined approaches for skin sensitisation in OECD Guideline No. 497

8th December 2022
Agenda

• Introduction to speakers
• The Animal-Free Safety Assessment Collaboration
• Background of defined approaches for skin sensitisation
• Case studies
• Conclusions
• Q&A
Today’s speakers

Dr. Donna Macmillan
Dr. Yuan Gao
Dr. Martyn Chilton
Dr. Petra Kern
The HSI-coordinated **Animal-Free Safety Assessment (AFSA) Collaboration** works to accelerate global adoption of a modern, species-relevant approach to safety assessment that will better protect people and our planet, and hasten the replacement of animal testing.
Current members

*Listed organizations are members of at least 1 AFSA workstream, listing does not imply participation in or endorsement of other work areas.
Background

Dr. Donna Macmillan
Humane Society International
Skin Sensitisation

• Until recently, the murine local lymph node assay (LLNA) was considered the ‘gold-standard’ method to predict skin sensitisation.

• However, the publication of three mechanistically-based test guidelines led to the development of defined approaches (DAs) which cover several key events in the AOP, and predict skin sensitisation as well, or better than the LLNA.

The adverse outcome pathway (AOP) for skin sensitisation initiated by covalent binding to proteins (OECD 2014).

DA: uses a defined set of in chemico, in vitro, in silico, physchem information sources to predict an endpoint using a fixed data interpretation procedure (DIP) e.g. without using expert judgement.
Defined Approaches for Skin Sensitisation

• In summer 2021, after several years of work, a groundbreaking guideline was published by the OECD - Defined Approaches for Skin Sensitisation (DASS).

• This guideline contains two defined approaches
  → 2o3
  → ITS
    • v1 (Derek Nexus)
    • v2 (OECD QSAR Toolbox)
2o3 defined approach

START

Conduct any two of the assays addressing the three KE of the 2o3 DA

Assays that can be used:
Molecular Initiating Event: DPRA
Key Event 2: KeratinoSens™
Key Event 3: h-CLAT
ITS defined approach

**Flowchart Diagram**

START

- Both assays are applicable
- Applicable in in vitro outcome?
  - Yes
    - In silico prediction in domain?
      - Yes
        - Sum scores from DPRA, h-CLAT, and Derek/OECD QSAR toolbox
          - Combined score
            - 6-7: GHS 1A
            - 2-5: GHS 1B
            - 0-1: NC
          - ITS prediction
            - 6: GHS 1A
            - 5: GHS 1B
            - 2-4: Inconclusive
            - 1: NC
          - Partial information sources:
            - Two in chemico/in vitro outcomes
        - Combined score
          - 3-4: GHS 1A
          - 2: GHS 1B
          - 0-1: Inconclusive
    - No
      - In silico prediction in domain?
        - Yes
          - Sum scores from DPRA and h-CLAT
            - Combined score
              - 6: GHS 1A
              - 5: GHS 1B
              - 2-4: Inconclusive
              - 1: NC
            - ITS prediction
              - 6: GHS 1A
              - 5: GHS 1B
              - 2-4: Inconclusive
              - 1: NC
          - Partial information sources:
            - Two in chemico/in vitro outcome and one in silico prediction
        - No
          - Neither assay is applicable
          - STOP — ITS prediction cannot be made

Score | h-CLAT MIT µg/ml | DPRA mean Cys and Lys depletion (%) | DPRA Cys depletion (%) | In silico
---|---|---|---|---
3 | ≤10 | ≥42.47 | ≥98.24 | TSv1: Derek Nexus
2 | >10, ≤150 | ≥22.62, <42.47 | ≥23.09, ≥98.24 | TSv2: QSAR Toolbox
1 | >150, ≤5000 | ≥6.38, <22.62 | ≥13.89, <23.09 | Positive
0 | not calculated | <6.38 | <13.89 | Negative

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*Conclusive for hazard, inconclusive for potency

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8th December 2022

10
Inconclusive predictions

- Limitations/applicability domain restrictions are carried through to the DA. Results can’t be used (considered inconclusive) if they are:

  - **In vitro**
    - Negative in the h-CLAT assay and have a high logP (>3.5)
    - Considered borderline in DPRA, KeratinoSens™, h-CLAT (only for 2o3)

  - **In silico**
    - Outside the (Q)SAR applicability domain (only for ITS)

- This can lead to inconclusive predictions from the DAs
  - In practice, these inconclusive predictions only occur rarely
    - ~5-20% depending on DA
  - They can be resolved using a weight-of-evidence approach using additional lines of evidence
A weight-of-evidence approach

- No animal data was used or read-across employed for the case studies in our publication
Case study 1

Dr. Yuan Gao
Procter & Gamble
Benzyl benzoate

• Chemical information
  → CAS: 120-51-4
  → Preservative/disinfectant
  → MW = 212.24 g/mol
  → Log P = 3.97

• Prediction using defined approaches in OECD GL 497
  → Inconclusive hazard prediction in 2o3 DA due to negative DPRA, positive KeratinoSens™ and inconclusive h-CLAT results
  → Inconclusive hazard and potency predictions in both ITS DA (score = 1) due to negative DPRA and positive in silico results in combination with an inconclusive h-CLAT result
Benzyl benzoate – gather and assess data

• Key event 1 (KE1) assays:
  → ADRA – negative (1.8% mean peptide depletion)
  → Cor1-C420 – negative (<1% mean peptide depletion)
  → DPRA – negative (1.6% mean peptide depletion)
  → kDPRA – 1B/NC (no log $K_{\text{max}}$ calculated, not reactive)
  → PPRA – negative (Cys $D_{\text{Pmax}} = 0\%$ (direct), 15.9% (HRP/H$_2$O$_2$), Lys $D_{\text{Pmax}} = 8.8\%$)

• Key event 2 (KE2) assays:
  → KeratinoSens™ – positive ($I_{\text{max}} = 5.75$, EC1.5 = 72.5 μM)
  → EpiSensA – positive (2/4 marker genes > cut-off)
  → SENS-IS – positive (weak sensitizer; 3/21 SENS genes and 9/17 ARE genes activated at a concentration of 50%)
Benzyl benzoate – gather and assess data

• Key event 3 (KE3) assays:
  → h-CLAT – negative (inconclusive as log P > 3.5)
  → U-SENS™ – positive (EC150 > 200 µg/ml - based on EC150 it would usually mean NS but based on method rules including other factors it is classified as a sensitiser)
  → GARDskin – positive (cDV = 2.3)

• (Quantitative) Structure Activity Relationships ((Q)SAR):
  → Derek Nexus – positive (benzyl ester alert, S_N2 mechanism, predicted EC3 = 6.2%)
  → OECD QSAR Toolbox – positive (alkyl ester and thioester alert, S_N2 mechanism, positive by read-across)
  → TIMES-SS – positive (parent weak sensitiser, metabolite non-sensitiser)
  → Toxtree – positive (acyl transfer agent domain alert)
Benzyl benzoate – weight of evidence

- **Our assessment:** GHS 1B – weak sensitising potential
  - KE1 negative predictions, KE2 and KE3 all positive predictions
  - Weakly positive results from assays and models that predict potency (SENS-IS, Derek and TIMES-SS)
Benzyl benzoate – compare to human potency

- Human: **non-sensitiser** (Human potency class 5, HDSG non-sensitiser)
- LLNA: **weak sensitiser** (EC3 = 17%) (OECD DASS dataset)
- LLNA: **non-sensitiser** (EC3 > 50%) (ECHA)
Case study 2

Dr. Martyn Chilton
Lhasa Limited
N,N-Dibutylaniline

• Chemical information
  → CAS: 613-29-6
  → Used in the dye industry
  → MW = 205.34 g/mol
  → Log P = 3.9

• Prediction using defined approaches in OECD GL 497
  → Inconclusive hazard prediction in 2o3 DA due to negative DPRA, borderline negative KeratinoSens™ and inconclusive h-CLAT results
  → Inconclusive hazard and potency predictions in both ITS DA due to negative DPRA and variable in silico results in combination with an inconclusive h-CLAT result
N,N-Dibutylaniline – gather and assess data

• Key event 1 (KE1) assays:
  ➔ ADRA – negative (0.1% mean peptide depletion)
  ➔ Cor1-C420 – negative (3% mean peptide depletion)
  ➔ DPRA – negative (0% mean peptide depletion)
  ➔ kDPRA – 1B/NC (no log $K_{\text{max}}$ calculated, not reactive)

• Key event 2 (KE2) assays:
  ➔ EpiSensA – positive (2/4 marker genes > cut-off)
  ➔ KeratinoSens™ – negative (borderline as $I_{\text{max}} = 1.4$)
N,N-Dibutylaniline – gather and assess data

• Key event 3 (KE3) assays:
  → h-CLAT – negative (inconclusive as log P > 3.5)
  → IL-8 Luc – positive (no raw data available)

• (Quantitative) Structure Activity Relationships ((Q)SAR):
  → Derek Nexus – negative (no alerts fired, no misclassified or unclassified features)
  → OECD QSAR Toolbox – positive (no alert fired for parent, predicted metabolite butanal fires Schiff base aldehyde alert, positive by profiling)
  → TIMES-SS – negative (no alerts fired for parent or metabolites, parent out of domain)
  → Toxtree – negative (no alerts fired)
**Our assessment: Non-sensitiser – not classified under GHS**

→ QSAR Toolbox is positive due to a metabolite that actually has negative *in vivo* data

→ Is there an active metabolite picked up by the two IL-8 based assays?
  * Additional studies could be undertaken?
N,N-Dibutylaniline – compare to human potency

- Human: no data available
- LLNA: weak sensitiser (EC3 = 20%) (OECD DASS dataset)
α-Tocopherol

• Chemical information
  → CAS: 59-02-9
  → Common cosmetic ingredient (Vitamin E)
  → MW = 430.71 g/mol
  → Log P = 9.4

• Prediction using defined approaches in OECD GL 497
  → Inconclusive hazard prediction in 2o3 DA due to negative DPRA, positive KeratinoSens™ and inconclusive h-CLAT results
  → Inconclusive hazard and potency predictions in both ITS DA (score = 0-1) due to negative DPRA and variable in silico results in combination with an inconclusive h-CLAT result
α-Tocopherol – gather and assess data

• Key event 1 (KE1) assays:
  → ADRA – negative (0% mean peptide depletion)
  → DPRA – negative (3.6% mean peptide depletion)
  → kDPRA – 1B/NC (no log $K_{\text{max}}$ calculated, not reactive)
  → PPRA – negative (Cys $D_P_{\text{max}} = 0.6\%$ (direct), 8.5% (HRP/H$_2$O$_2$), Lys $D_P_{\text{max}} = 6.7\%$

• Key event 2 (KE2) assays:
  → KeratinoSens™ – positive ($I_{\text{max}} = 2.09$, EC1.5 = 115 μM)
  → EpiSensA – negative (0/4 marker genes > cut-off)
  → SENS-IS – positive (moderate sensitizer; 4/21 SENS genes and 4/17 ARE genes activated at a concentration of 10%)
α-Tocopherol – gather and assess data

• Key event 3 (KE3) assays:
  → h-CLAT – negative (inconclusive as log P > 3.5)
  → U-SENS™ – negative (EC150 > 200 µg/ml)
  → GARDskin – positive (cDV = 0.7)

• (Quantitative) Structure Activity Relationships ((Q)SAR):
  → Derek Nexus – negative (no alerts fired, no misclassified or unclassified features)
  → OECD QSAR Toolbox – positive (no alert fired for parent or metabolites, positive by read across but out of mechanistic domain)
  → TIMES-SS – negative (no alerts fired for parent or metabolites, parent out of domain)
  → Toxtree – negative (no alerts fired)
α-Tocopherol – audience poll

KE1
- PPRA
- ADRA
- KoPRA
- DPRA

KE2
- EpiSens A
- KeratinoSens™
- SENS-15

KE3
- GARDskin
- U-SENS™
- h-CLAT

(Q)SAR
- Toxtree
- Derek Nexus
- TIMES-SS
- QSAR Toolbox

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How to resolve inconclusive predictions from defined approaches for skin sensitisation in OECD Guideline No. 497
8th December 2022 29
α-Tocopherol – weight of evidence

- **Our assessment: Mixed data – some sensitising potential cannot be excluded**
  - Additional NAM studies could be undertaken, combined in other DA to set Point of Departure
  - Weight of individual NAMs for decision making?
  - Read-across: data from related materials could lead to final decision?
\(\alpha\)-Tocopherol – compare to human potency

- **Human**: non-sensitiser (Human potency class 6)
- **LLNA**: moderate sensitiser (EC3 = 7.4%) (OECD DASS dataset)
- **GPMT**: non-sensitiser (ECHA)
Concluding remarks
Conclusions

- The publication of OECD GL No. 497, Defined Approaches to Skin Sensitisation, is a significant milestone in the paradigm shift away from reliance on animal testing.
- Three case studies have been described today, benzyl benzoate, N,N-dibutyl aniline and α-tocopherol.
- Our publication did not consider animal data as novel substances would lack this data.
- However, to benchmark our approach we assessed against human/animal data today.
- A weight of evidence approach is typically protective of human health.
Future work

• More examples are needed including those using additional NAMs/read-across

• Open discussion around acceptable uncertainty
  → *In vivo* results typically taken at face value whereas *in vitro* results are scrutinised
    • *In vivo* uncertainty
      • Cross-species extrapolation
      • Cut-off criteria
      • Animal variability
      • Etc.
    • *In vitro* uncertainty
      • Use of multiple assays
      • Cut-off criteria
      • Etc.

• Expert review
  → Clear and concise expert review will increase confidence in the weight of evidence approach

• Hazard ➔ Risk/Point of Departure
  → Approach is conservative, some of the case studies appear to be sensitisers but human data suggests a lack of sensitisation – could be used safely at specific concentrations
Thank you for listening!

Q&A