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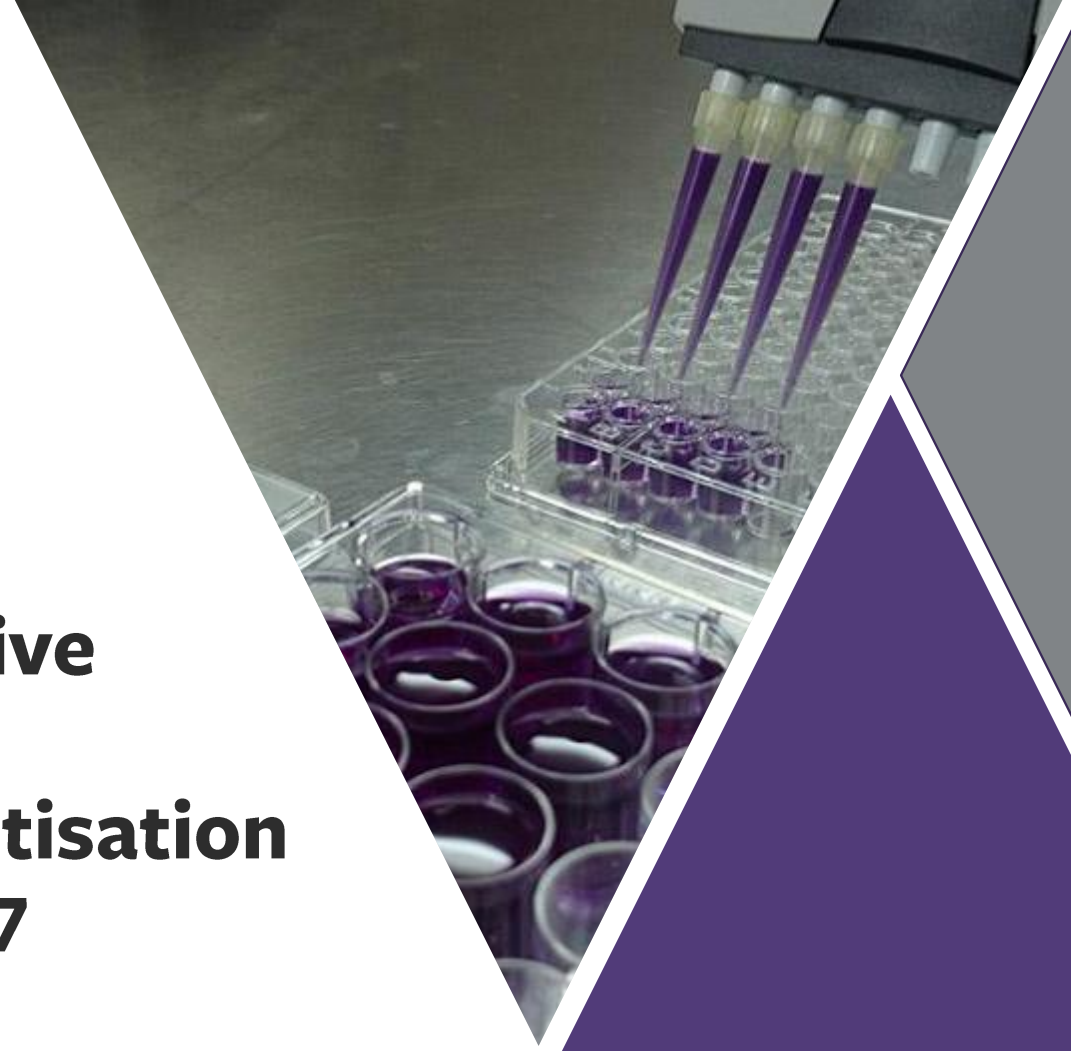

How to resolve inconclusive predictions from defined approaches for skin sensitisation in OECD Guideline No. 497

8th December 2022

HUMANE SOCIETY INTERNATIONAL

Lhasa Limited

P&G

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

AFSA COLLABORATION

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

COSMETICS

CHEMICALS

BIOLOGICALS

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

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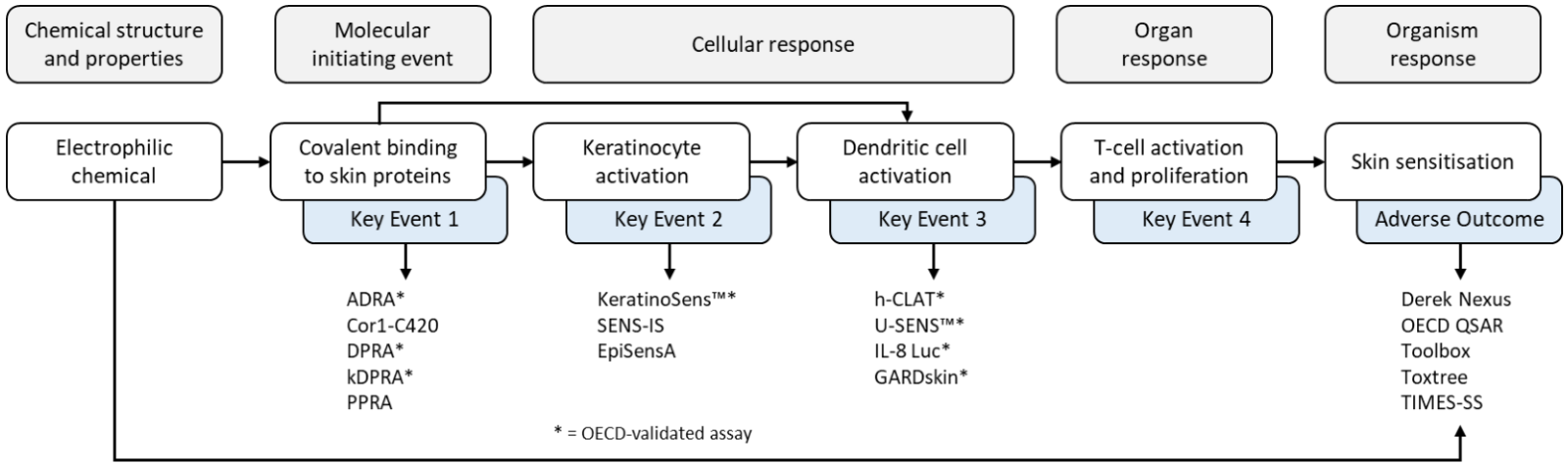
Skin Sensitisation

- 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).

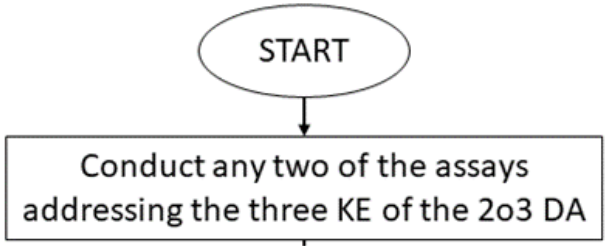
DA: uses a defined set of *in chemico, in vitro, in silico,* physchem information sources to predict an endpoint using a fixed data interpretation procedure (DIP) e.g. without using expert judgement

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).

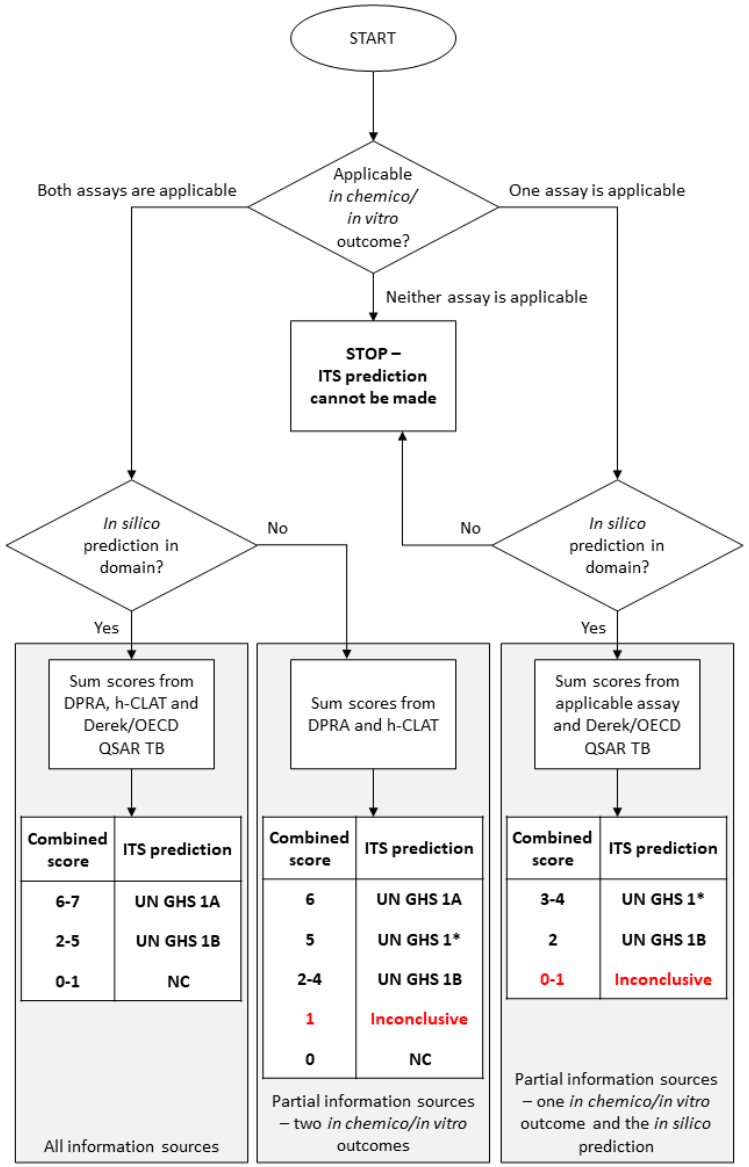
- This guideline contains two defined approaches
  - → 2o3
  - → ITS
    - v1 (Derek Nexus)
    - v2 (OECD QSAR Toolbox)

2o3 defined approach



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

ITS defined approach



Score	h-CLAT MIT µg/ml	DPRA mean Cys and Lys depletion (%)	DPRA Cys depletion (%)	*In silico* ITSv1: Derek Nexus ITSv2: QSAR Toolbox
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

Derek nexus

ITSv1

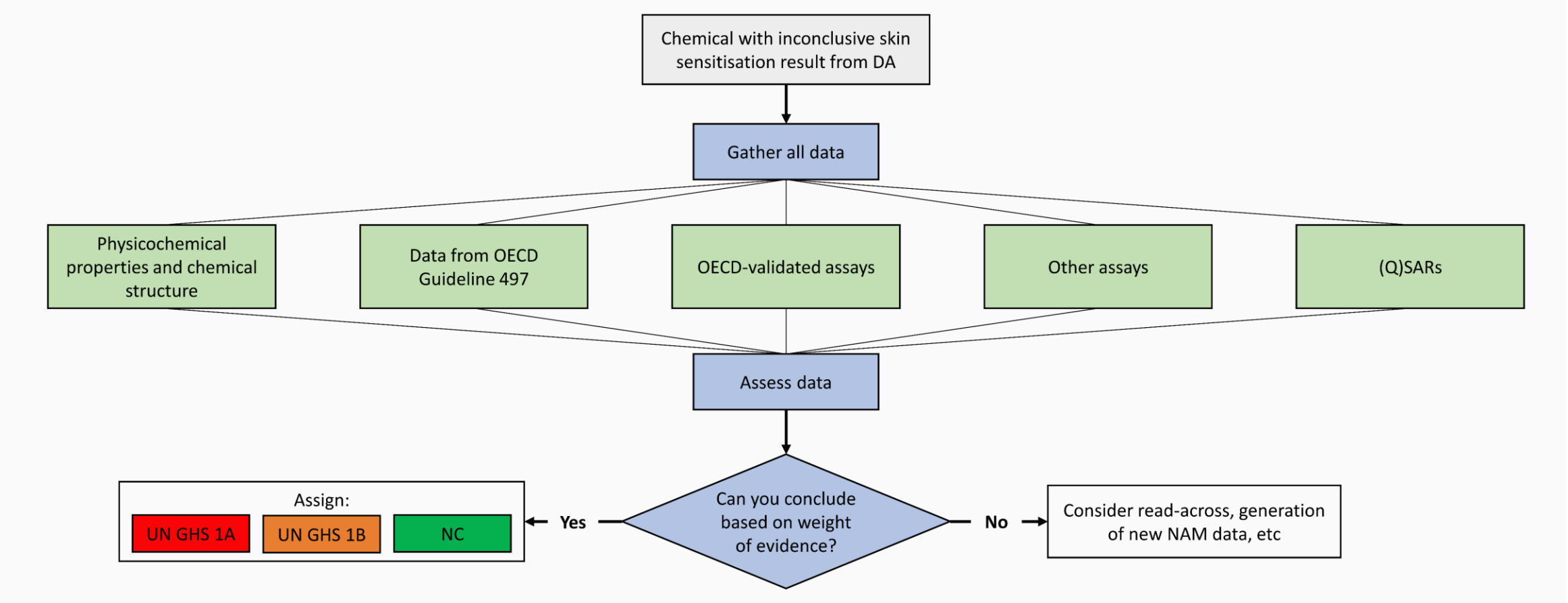
ITSv2

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

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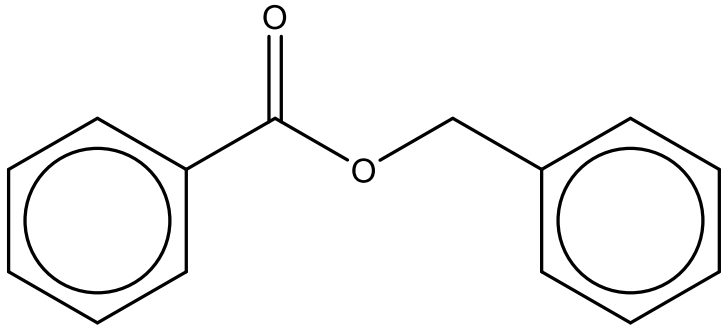
Benzyl benzoate



- 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_{max} calculated, not reactive)
  - → PPRA – negative (Cys DP_{max} = 0% (direct), 15.9% (HRP/H_2O_2), Lys DP_{max} = 8.8%)

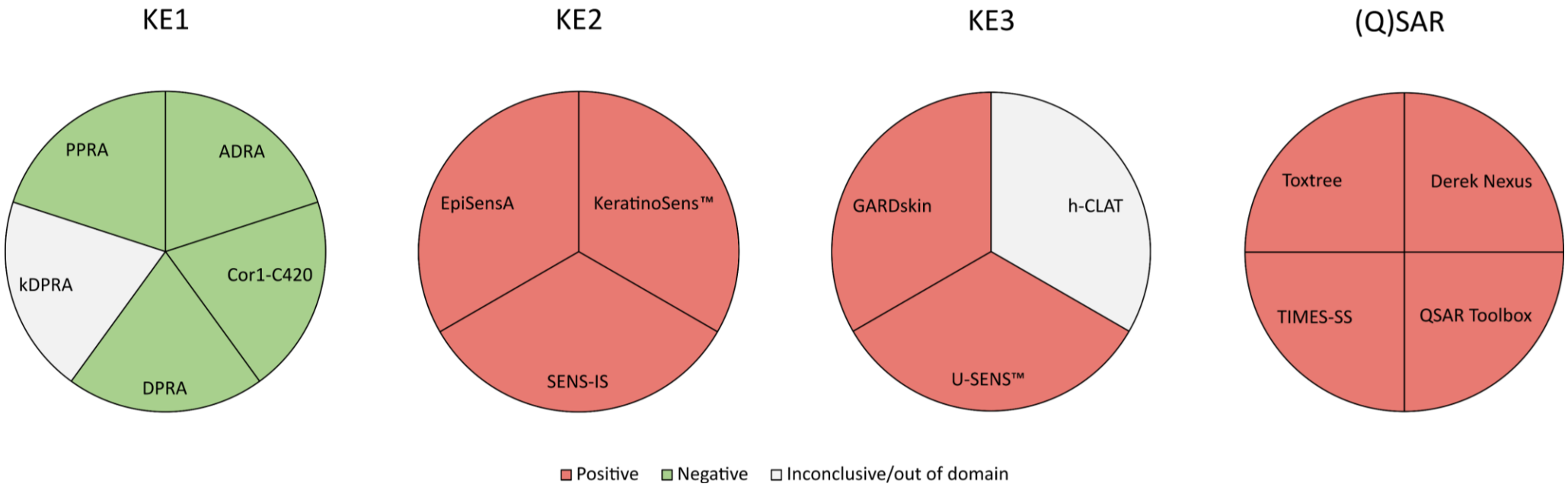
- Key event 2 (KE2) assays:
  - → KeratinoSens™ – positive (I_{max} = 5.75, EC1.5 = 72.5 µM)
  - → EpiSensA – positive (2/4 marker genes > cut-off)
  - → SENS-IS – positive (weak sensitizer; 3/21 SENS genes and 9/17 ARE genes activated at a concentration of 50%)

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)

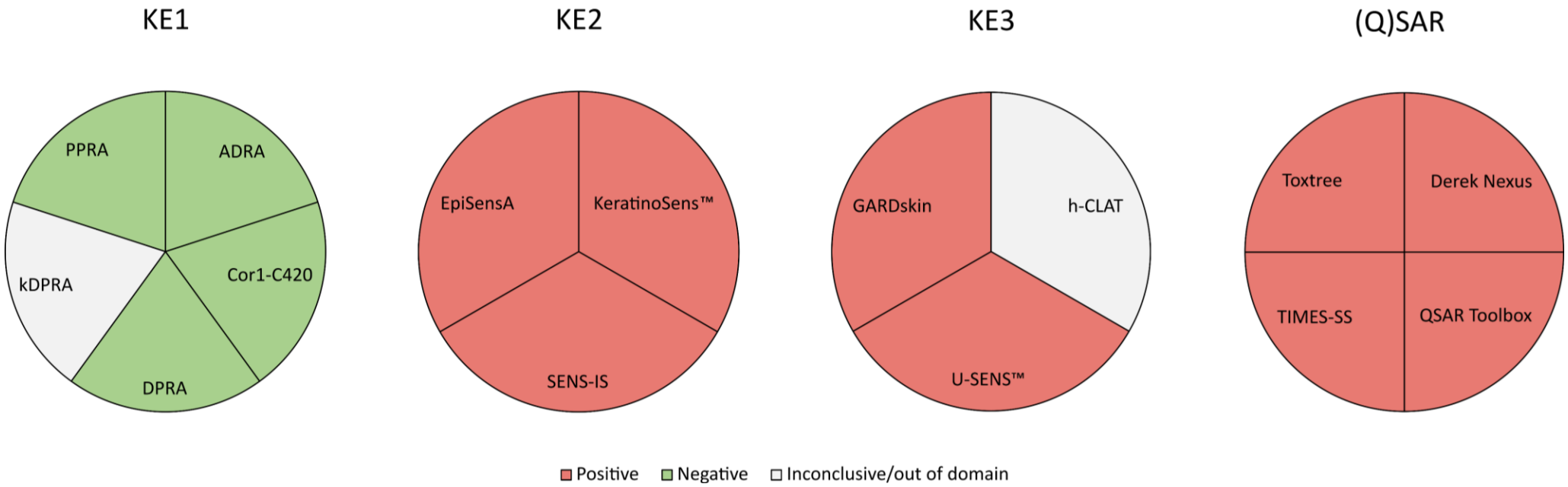
- (Quantitative) Structure Activity Relationships ((Q)SAR):
  - → Derek Nexus – positive (benzyl ester alert, S_N2 mechanism, predicted EC3 = 6.2%)
  - → OECD QSAR Toolbox – positive (alkyl ester and thioester alert, S_N2 mechanism, positive by read-across)
  - → TIMES-SS – positive (parent weak sensitiser, 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

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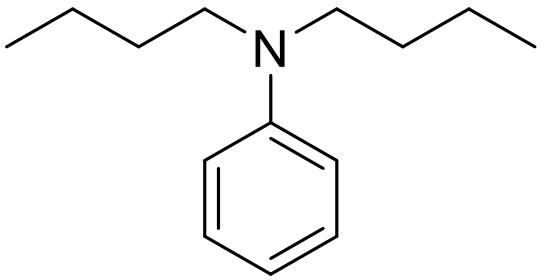
N,N-Dibutylaniline

- 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_{max} calculated, not reactive)

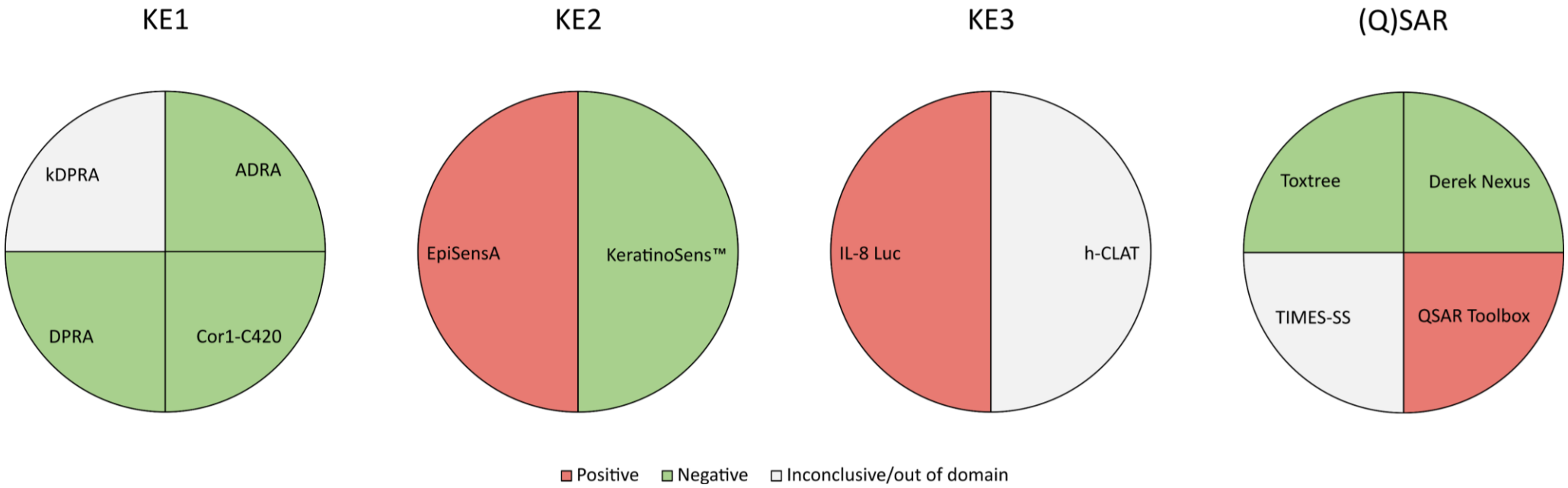
- Key event 2 (KE2) assays:
  - → EpiSensA – positive (2/4 marker genes > cut-off)
  - → KeratinoSens™ – negative (borderline as I_{max} = 1.4)

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)

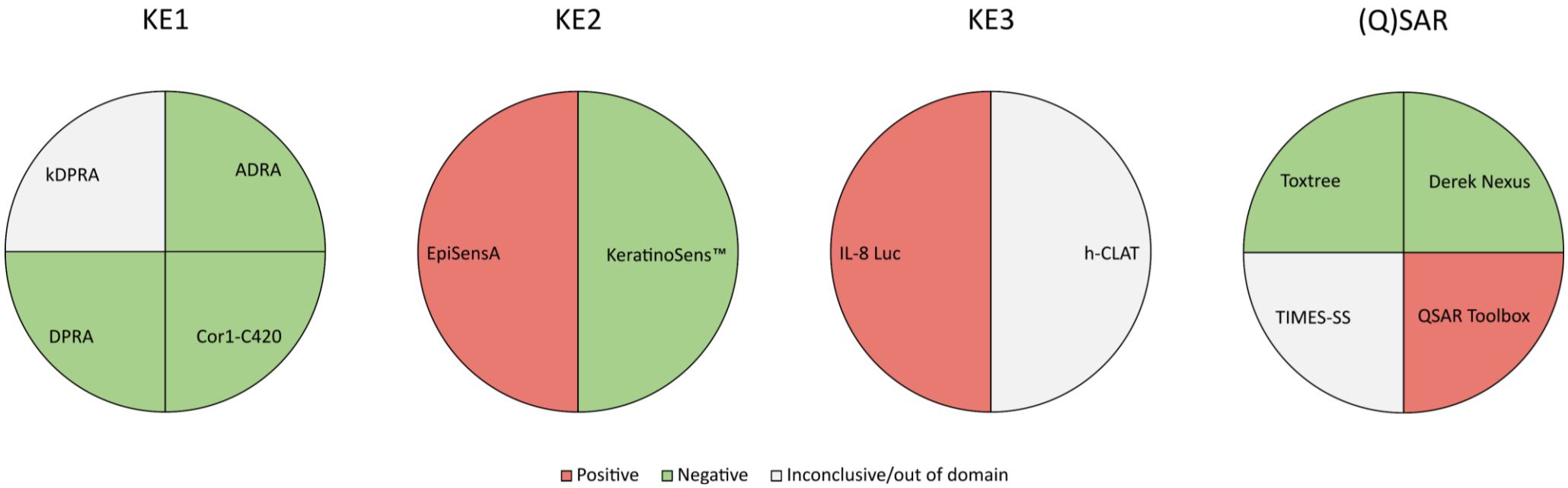
- (Quantitative) Structure Activity Relationships ((Q)SAR):
  - → Derek Nexus – negative (no alerts fired, no misclassified or unclassified features)
  - → OECD QSAR Toolbox – positive (no alert fired for parent, predicted metabolite butanal fires Schiff base aldehyde alert, positive by profiling)
  - → TIMES-SS – negative (no alerts fired for parent or metabolites, parent out of domain)
  - → Toxtree – negative (no alerts fired)

N,N-Dibutylaniline – weight of evidence



- **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)

Case study 3

Dr. Petra Kern
Procter & Gamble

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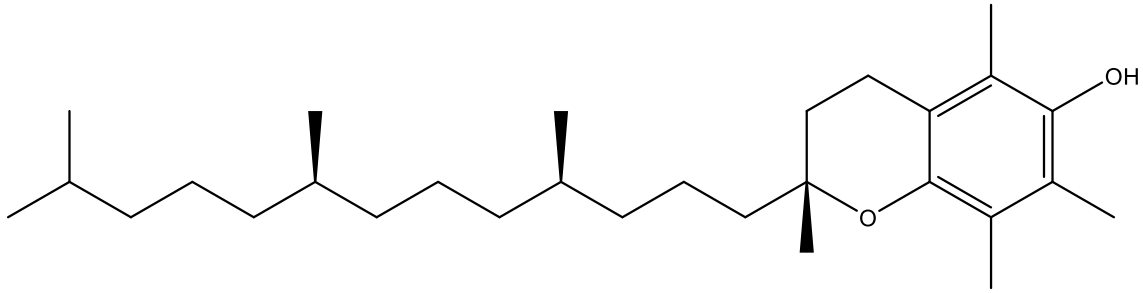
α-Tocopherol



- 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_{max} calculated, not reactive)
  - → PPRA – negative (Cys DP_{max} = 0.6% (direct), 8.5% (HRP/H_2O_2), Lys DP_{max} = 6.7%)

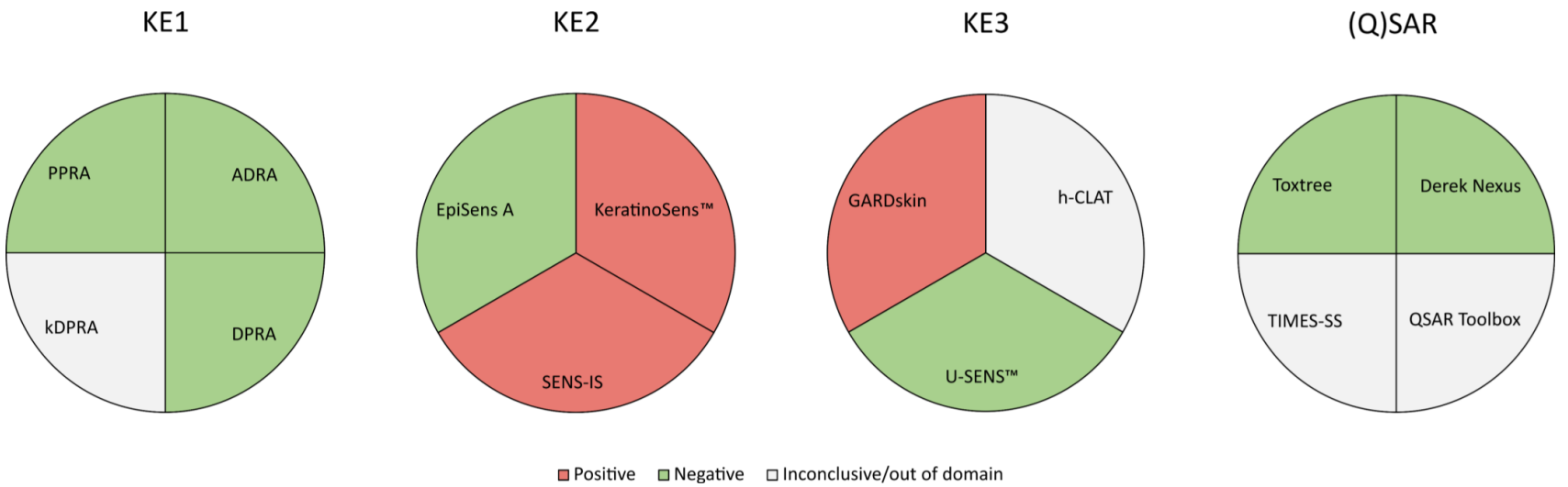
- Key event 2 (KE2) assays:
  - → KeratinoSens™ – positive (I_{max} = 2.09, EC1.5 = 115 µM)
  - → EpiSensA – negative (0/4 marker genes > cut-off)
  - → SENS-IS – positive (moderate sensitizer; 4/21 SENS genes and 4/17 ARE genes activated at a concentration of 10%)

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



Join at

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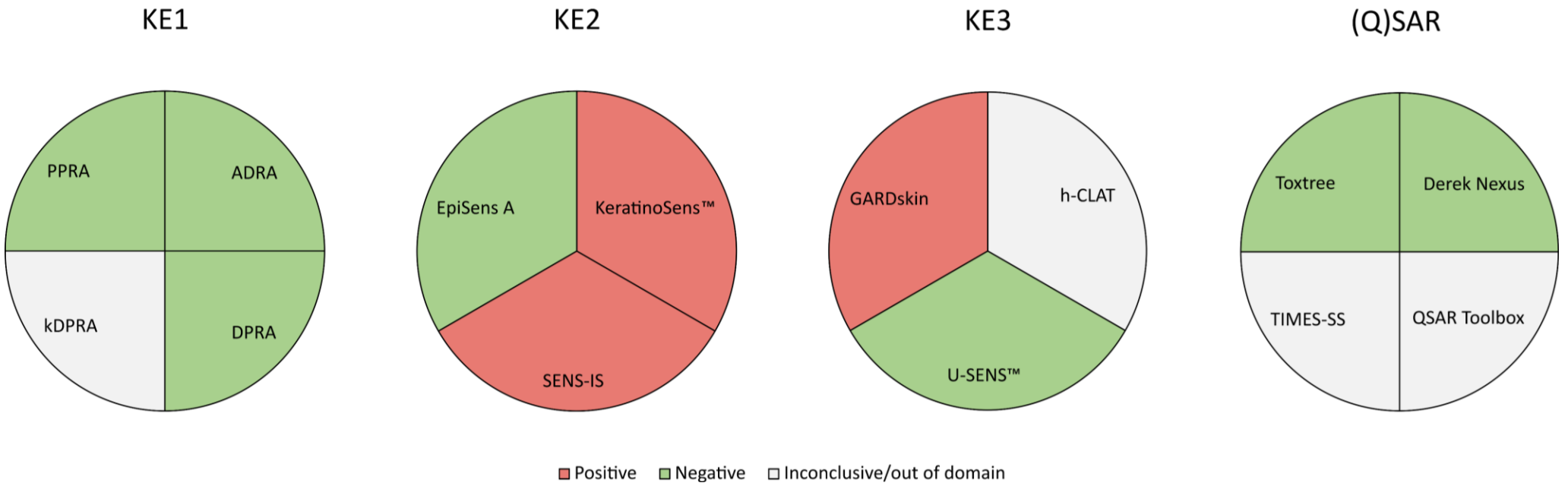
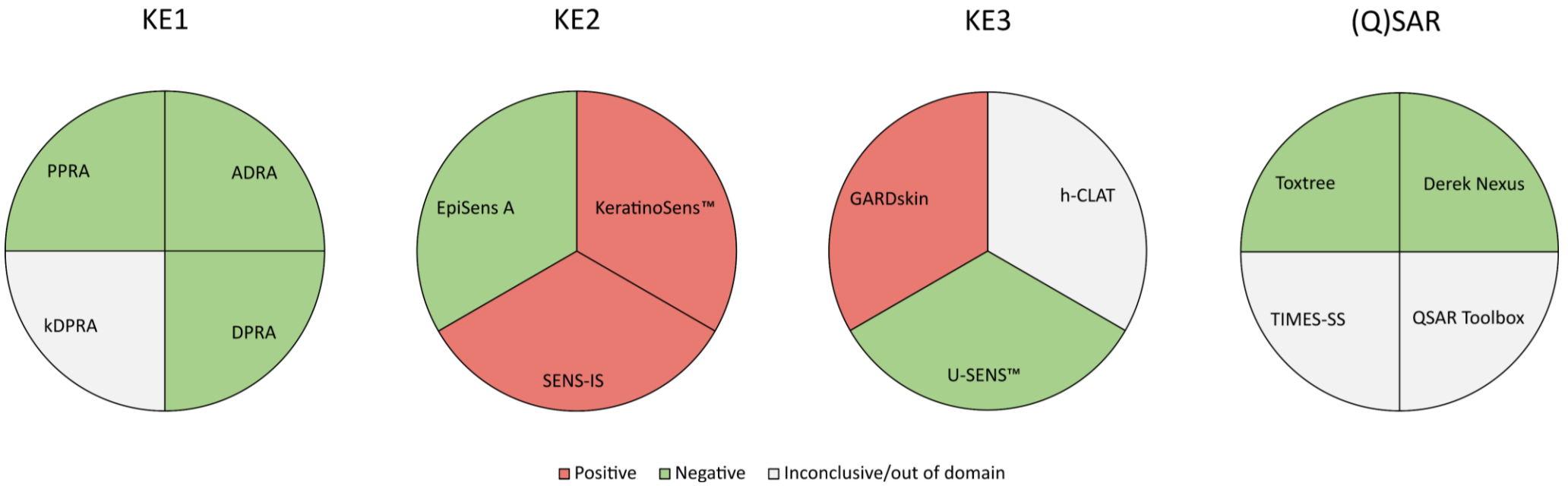
α-Tocopherol – weight of evidence



- **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?

α-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

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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

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