IATA as an Opportunity for Next-Generation Risk Assessment: The Propylparaben Case Study Next Generation Read Across

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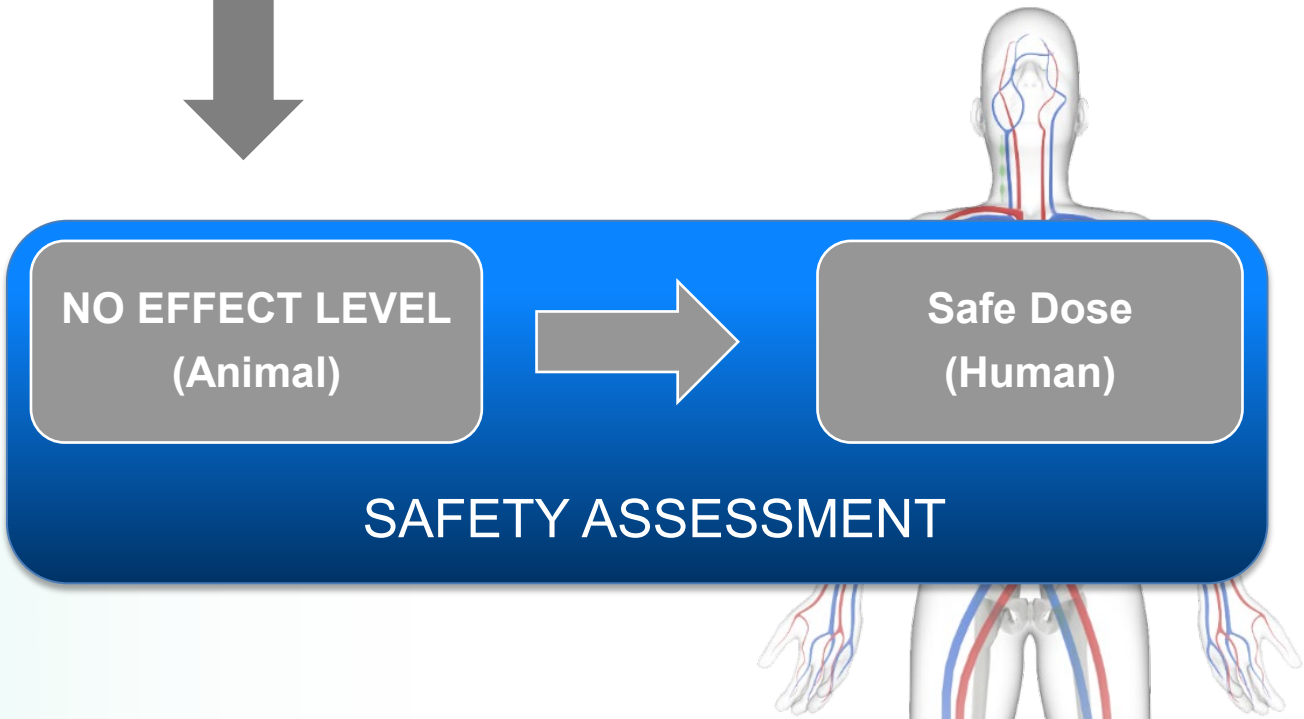
Outline

- Past, today, future
- What is next generation read across (RAX)?
- The challenges
- The propylparaben case study
- Assessing confidence in RAX
- Wrap up

Past, Today, Future

PAST

Animal Testing



NO EFFECT LEVEL (Animal)

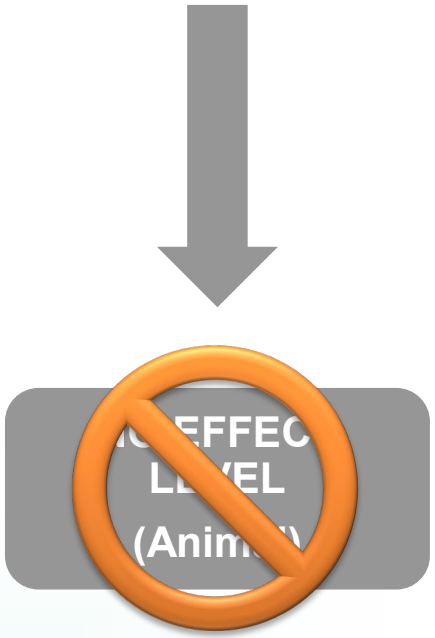
Safe Dose (Human)

SAFETY ASSESSMENT

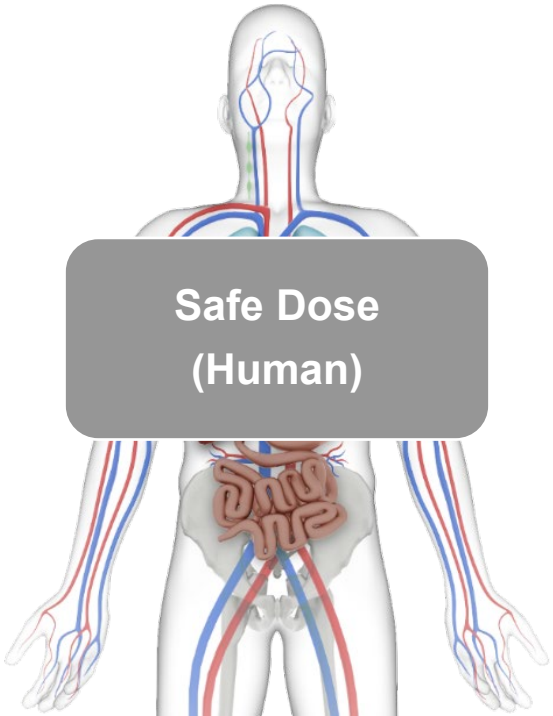
NEW INGREDIENT

TODAY

Animal Testing



Safe Dose
(Human)



NEW INGREDIENT

TODAY

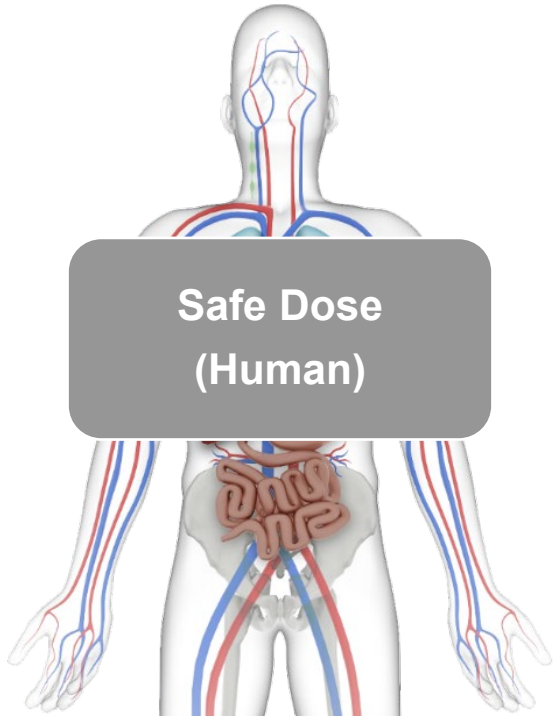
Animal Testing

TTC

Read-Across

Legacy animal data *

NO EFFECT LEVEL (Animal)

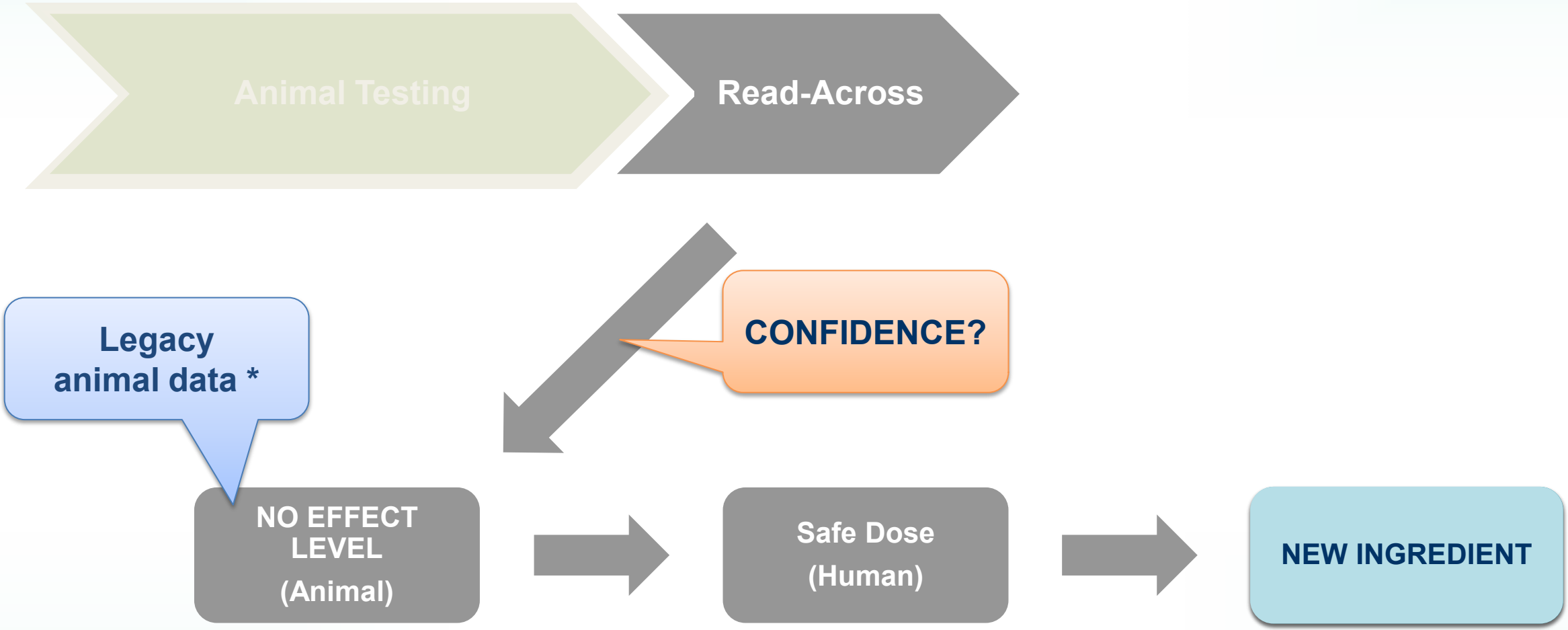


Safe Dose (Human)

NEW INGREDIENT

* test conducted before March 2013

TODAY



* test conducted before march 2013

TODAY

Animal Testing

Next Gen.
Read-Across

NEW APPROACH METHODS

CONFIDENCE !

Legacy animal data *

NO EFFECT LEVEL (Animal)

Safe Dose (Human)

NEW INGREDIENT

* Test conducted before March 2013

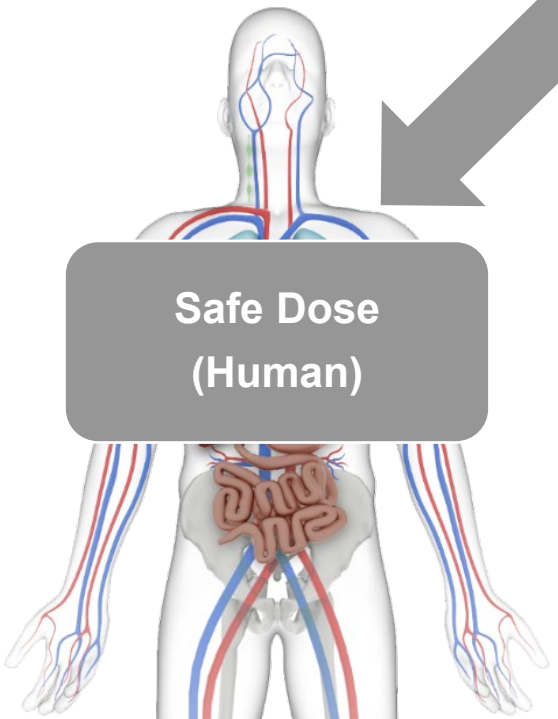
FUTURE

Animal Testing

Next Gen.
Read-Across

Next Generation
Risk Assessment

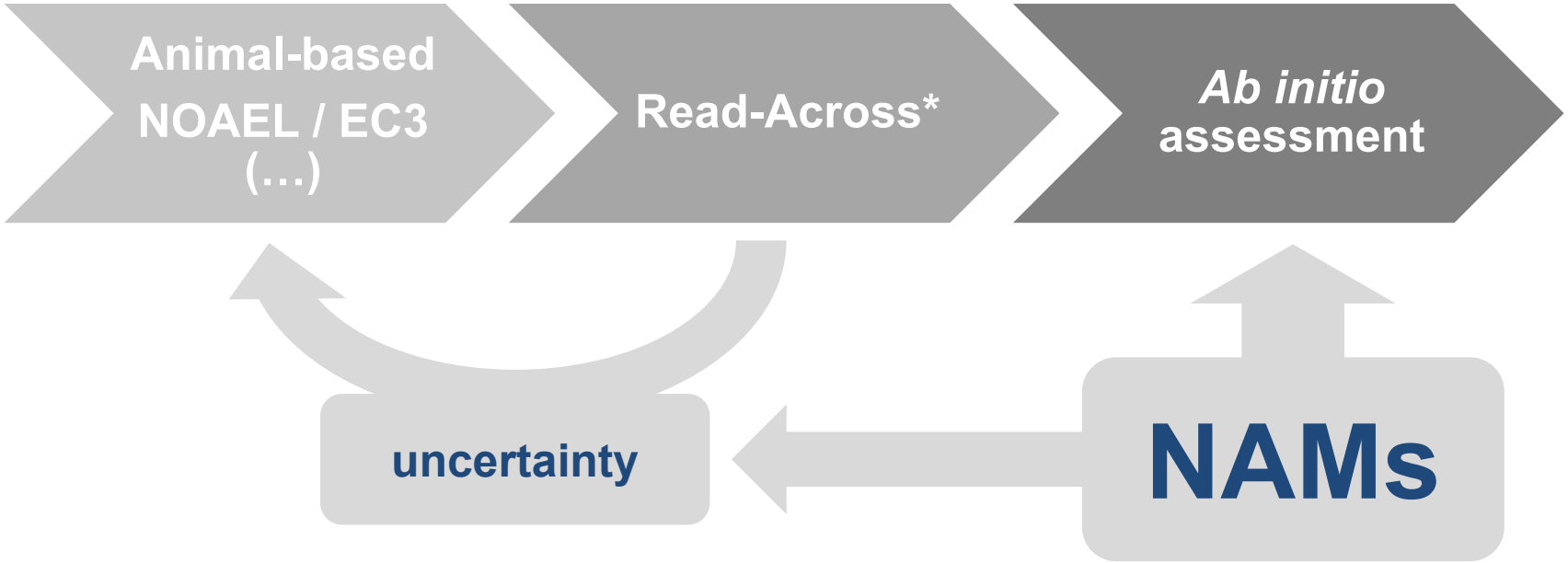
NEW
APPROACH
METHODS



Safe Dose
(Human)

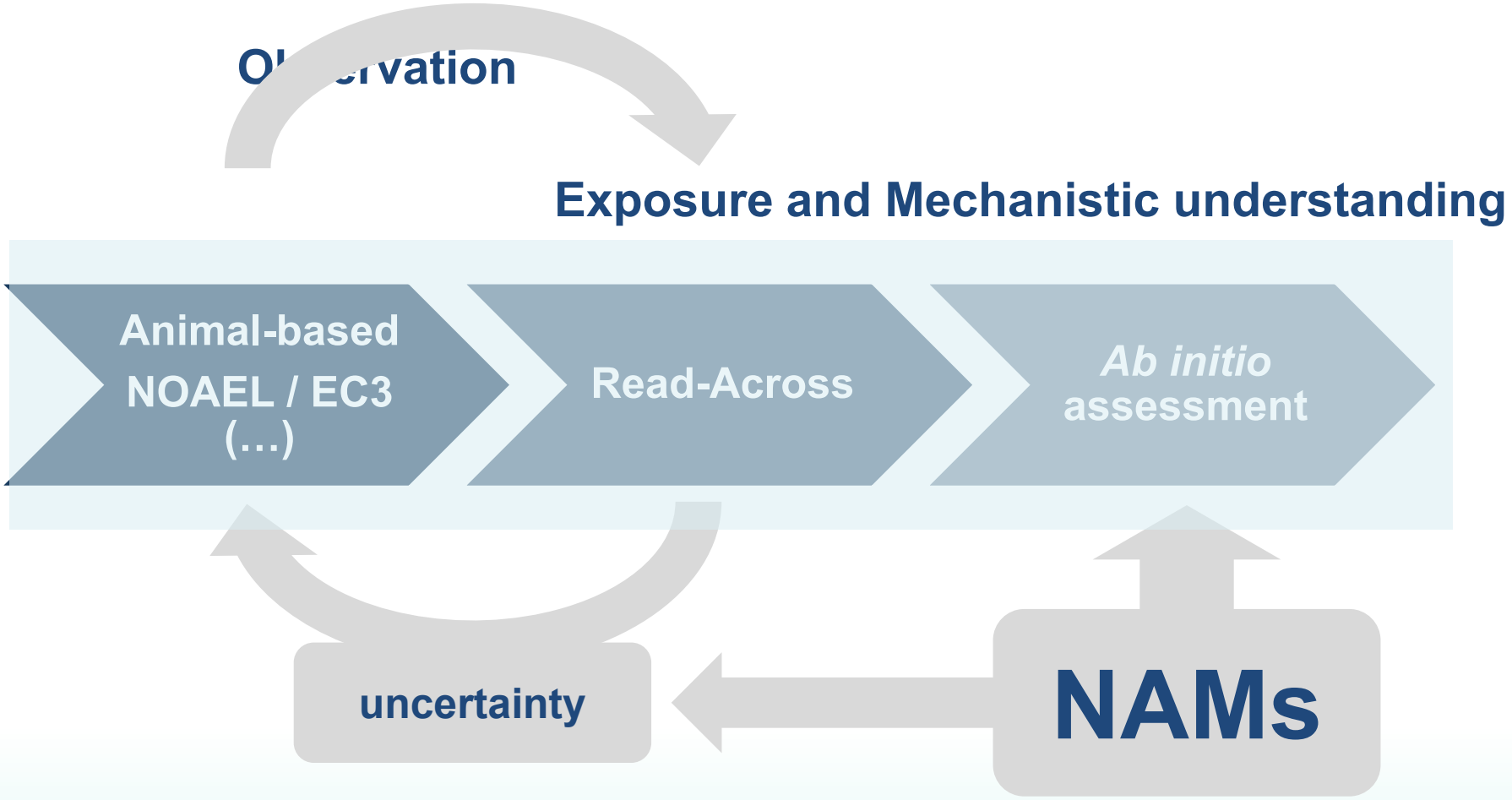
NEW INGREDIENT

Paradigm Change in Toxicology is NOW



*Applicable to multiple endpoints: genotoxicity, skin sensitization, systemic toxicity

Paradigm Change in Toxicology is NOW



What is Next Generation Read Across (RAX)?

What is Next Generation Read Across (RAX)

From THIS >>>>> **To THIS**

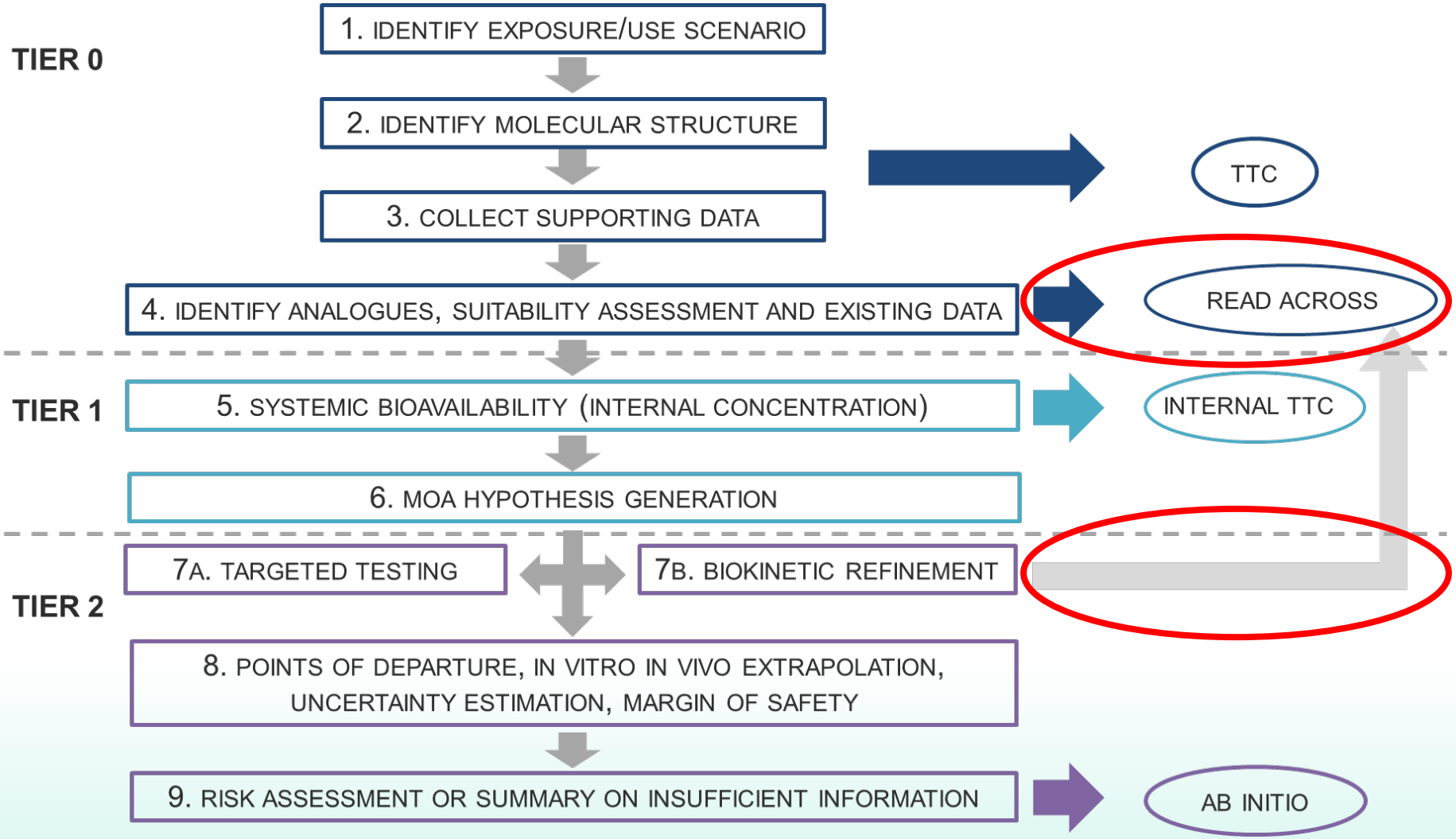
Traditional Read Across

- Taking account of physico-chemical properties → analogue ID and selection
- Data collection and hypothesis
- Predict a NOEL

Next Generation Read Across

- Taking account of physico-chemical properties → analogue ID and selection
- Data collection and chemical specific NAM to inform hypothesis
  - Strengthen analogue ID
  - Predict internal exposure
- Making a safety decision based on internal exposures of human versus animal study

Context in Seurat-1 Workflow



Working Towards a RAX Solution

Seurat-1 → **EuToxRisk** → **LRSS**

Grouping (read across)

- Data from New Approach Methodology(ies) may provide critical information to strengthen the toxicodynamic similarity rationale
- Toxicokinetic (i.e., ADME) similarity, especially metabolism, is often the driver in overall uncertainty

Similarity in chemistry is often not enough to justify fully a read-across prediction

Grouping (read across)

- Using NAMs to illustrate shared MoA/AOP: MIEs and KEs from AOPs (when known/available) can help anchor (dis)similarities in toxicokinetics and toxicodynamics properties
- Derive a PoD to use in risk assessment

NAMs allow integrating mechanistic knowledge in human hazard assessment

Next Generation RAX

- Incorporating chemical specific toxicokinetic and toxicodynamic NAM to strengthen hypothesis
- Using NAM to derive internal exposure in human and animal studies
- Make a human safety decision

NAMs can be used to support a human safety decision

Schultz T, and Cronin, M. 2017. Lessons learned from read-across case studies for repeated-dose Toxicity. Regulatory Toxicology and Pharmacology 88. 185-191

http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#Project

THE CHALLENGES

THE CHALLENGES

How to build confidence in the use of NAM data to support read-across?

- How can NAMs strengthen analog identification?
- How can NAMs inform similarities/differences in toxicokinetics and toxicodynamics – analog suitability assessment?
- What role can NAM play in the safety assessment – definition of a margin of safety?
- How to assess confidence in the read-across supported by NAM data?

Next Generation RAX
The Propylparaben Case Study

Propylparaben Case Submitted to OECD: Under Review

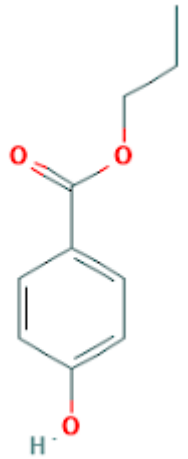
1. **Identify use scenario**

Decision context: *Safety assessment of propylparaben (PP) as preservative at 0.19% in cosmetics (dermal route)*

Information gap*: for demonstration purposes, reproductive toxicity study data on PP was excluded*

Case Study on the use of New Approach Methods to inform a theoretical Read-Across for propylparaben using an Integrated Approach to Testing and Assessment exploring the Endocrine Activity of Parabens

2. Identify molecular structure



Physical-Chemical Properties and Structure Activity Relationships

3. Collect existing data

4. Identify analogues, suitability assessment, and existing data

- Analogue ID according to *structure, reactivity, metabolism, and physical chemical properties* (Wu et al 2010)
- 58 analogues with structural similarity >70%
- 3 analogues with highly similar structure and metabolism
  - All 3 have *in vivo* data
  - Metabolite also has *in vivo* data
- Similar physico-chemical properties but increasing side chain → ↑LogP which can affect bioavailability and may inform on potency
- Source compound selected on basis of highest Tanimoto coefficient

Systemic Bioavailability

5. **Systemic bioavailability (Parent vs. metabolite, target organs, internal concentration)**

Propylparaben (and analogues)

- Rapid skin penetration with extensive first pass cutaneous metabolism
  - <0.5% parent paraben
- High clearance compound (liver > skin)
  - Extensively and rapidly metabolized to 4-HBA in skin and liver (other metabolites are minor)
  - Metabolism attributed to carboxylesterases
  - Stable in human plasma and binding to plasma protein extensive

Mode of Action

6. **MoA hypothesis generation (WoE based on available tools – *in silico, in chemico* and *in vitro*)**

Propylparaben (and other short chain parabens)

- Source chemicals supported by orthogonal data streams
- Alkyl chain length appears to result in a potency trend, i.e., MP<EP<PP<BP
  - In silico *alerts and docking simulations*
    - Weak estrogen receptor binding
    - Primary metabolite has no apparent alerts
  - *Toxcast data*
    - Estrogen receptor activity
    - Primary metabolite has no apparent bioactivity
  - *Transcriptomics data*
    - Significant overlap in affected pathways
      - Estrogen response genes upregulated
    - Activity associated most closely with BP
    - Fewer genes affected by primary metabolite

Targeted Effects

7a. Targeted testing

Propylparaben (estrogen and DART associations)

- Calux assays (EATS panel)
  - Estrogenic + anti-androgenic activity increases with increasing chain length
  - Activity of the parabens decreased significantly in the presence of rat liver S9
  - Primary metabolite devoid of activity
- Toxcast estrogen receptor model used as a proxy for biological reactivity

	AC10.median	Calculated Scaling (potency) Factor*
17beta-Estradiol	-3.07	-16.6
Butylparaben	0.18	1.0
Propylparaben	0.50	0.4
Ethylparaben	0.95	0.2
Methylparaben	1.41	0.1

* calculated as 1/(AC10 of individual paraben/AC10 of BP

External Exposure

7b. Biokinetic refinement (*in vivo* clearance, population, *in vitro* stability, partition)

- Cosmetic only
- Deterministic (theoretical)
  - Propylparaben used as preservative at maximum concentration 0.19% in all Cosmetics
  - Applied amount of 17.4 g/day = total amount of cosmetic product (SCCS Notes of Guidance)

External Exposure (mg/kg/day)

	Tier 1: presence in all cosmetics at maximum concentration
PP	0.48

Internal Exposure

7b. Biokinetic refinement (*in vivo* clearance, population, *in vitro* stability, partition)

PBPK modelling to estimate internal plasma concentration from human cosmetic exposure

- Using published model
  - Predictions verified by comparison to human data on analogue, underpinned by similar *in vitro* ADME behaviour
  - Model reported to be sensitive to fraction absorbed through skin and absorption rate
  - Population variability in internal dose not analysed but expected to be consistent with default UF of 3
    - Neonatal period not specifically considered

Addition of SC injection route to enable simulation of internal concentration from source chemical NOEL

- Lack of rat kinetic data to verify prediction

Internal Exposure (Cmax)

	Tier 1: presence in all cosmetics at maximum concentration
PP	0.020 uM

Margin of Internal Exposure

A MoIE differs from a traditional margin of exposure (MoE)

"calculated as the ratio of a measure of internal exposure, such as blood concentration or target-tissue dose, rather than comparing external exposure concentration or ingested doses" (Bessems et al. 2017)

No need to account for inter-species kinetic variability as using internal concentrations

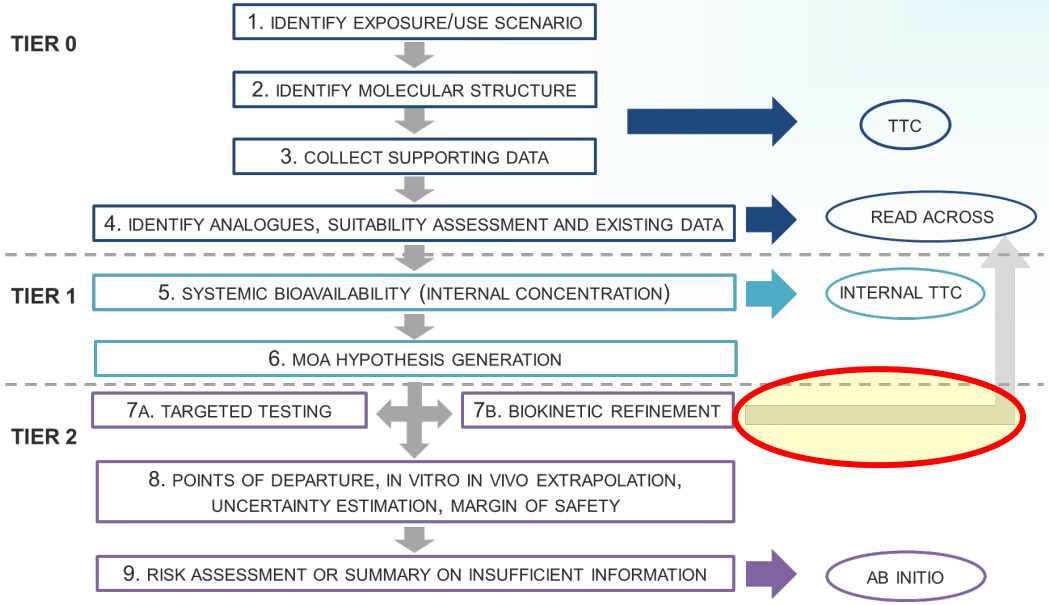
A "safe" MoE ≥ 100 $$MoE = \frac{PoD}{Exposure}$$

A "safe" MoIE is 25 $$MoIE = \frac{PoD\ translated\ in\ Systemic\ compartment}{Systemic\ Exposure}$$

J.G.M. Bessems et al Toxicology 392(2017)119–129

Risk Characterisation

8. **Point of Departure (PoD), IVIVE, Margin of Safety (MoS), Uncertainty Estimation**



Margin of internal exposure using

- Estimated Cmax at NOEL for source chemical
- Potency relative to source chemical

MoIE

Tier 1: presence in all cosmetics at maximum concentration

PP 292

Uncertainty Assessment

Data type/ Endpoint	How used[a]	Direction and Magnitude of Uncertainty[b]
***In vivo* data**	WOE, RAX, RA	++
Exposure data	RA	++
NAM		
Molecular Docking/ER activity	WOE	+/-
ToxCast/ Potency	WOE, RA	+/-
ADME Properties/pHBA activity	WOE, RAX	+/-
CALUX assays/ER activity	WOE	+/-
Toxicogenomics	WOE	+/-
PBBK	RA	+/-

[a]How data was used in the case: RAX=read-across; RA=risk assessment; WOE=weight of evidence for biological similarity

[b]Key to direction and magnitude:

+, ++ = uncertainty results minor or major conservatism in the safety assessment (i.e., overestimation of risk).

-, - - = uncertainty results in minor or major concerns in the safety assessment (i.e., underestimation of risk).

Assessing Confidence in RAX

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Based on: Schultz, T., Richarz, A. and Cronin, M. Computational Toxicology 9 (2019) 1–11

Evolved based on learning from CosEU_SCCS RAX workshop November 2018 and case study experiences

1. What type of category formation was attempted and was it suitable for the context of the read-across?
2. How well made was the premise or hypothesis of the read-across argument?
3. What rationale was used to select the NAMs used and how did they support the decision making?
4. How was mechanism of action considered supported and assessed?
5. How was similarity defined and assessed?
6. What were the uncertainties in the toxicological data for read-across data and how did they allow for an assessment of robustness of these data?
7. How were NAMs applied and did they assist in the reduction of uncertainty?
8. What is the overall certainty and is it acceptable as part of an exposure led risk assessment? If not acceptable, what information is required to increase confidence?
9. What are the key strengths and limitations of the case study?

Assessing Confidence in RAX

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Evolved based on learning from CosEU_SCCS RAX workshop November 2018 and case study experiences

1. What type of category formation was attempted and was it suitable for the context of the read-across?

   Several source substances to one target chemical – low uncertainty

2. How well made was the premise or hypothesis of the read-across argument?

   Target chemical with bioavailability and bioactivity properties similar to source compounds, same metabolite, potency trend with side chain length – low uncertainty

3. What rationale was used to select the NAMs used and how did they support the decision making?

   Weak endocrine activity informed NAM selection, NAM informed internal data gap – low/medium uncertainty

4. How was mechanism of action considered supported and assessed?

   Estrogen receptor as common mechanism for the esters - low/medium uncertainty

5. How was similarity defined and assessed?

   In silico *(phyChem properties, alerts), in vitro TK and TD properties with quantitative adjustments - low/medium uncertainty*

Assessing Confidence in RAX

Based on: Schultz, T., Richarz, A. and Cronin, M. Computational Toxicology 9 (2019) 1–11

Evolved based on learning from CosEU_SCCS RAX workshop November 2018 and case study experiences

6. What were the uncertainties in the toxicological data for read-across data and how did they allow for an assessment of robustness of these data?

   Source chemical PoD has Klimish score 3 and very conservative - medium uncertainty

7. How were NAMs applied and did they assist in the reduction of uncertainty?

   Support similarity of bioavailability and bioactivity, inform MoA and derive potency relative to source chemical; estimate Cmax from legacy data and fom human exposure – low uncertainty

8. What is the overall certainty and is it acceptable as part of an exposure led risk assessment? If not acceptable, what information is required to increase confidence?

   low/medium uncertainty

9. What are the key strengths and limitations of the case study?

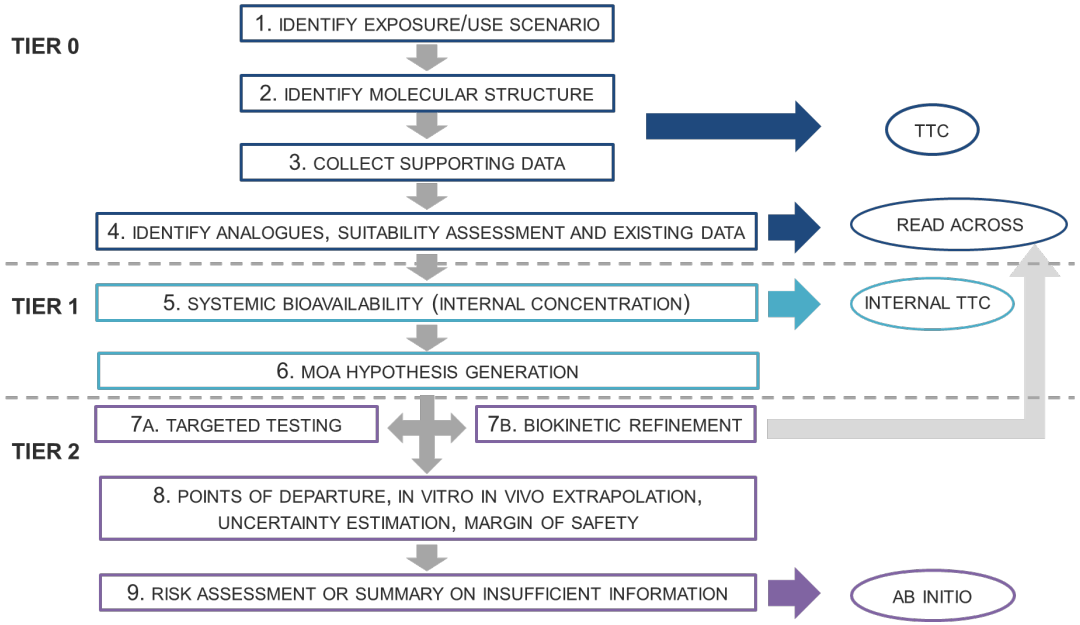
   Strengths: workflow, consideration of realistic exposure, value added by NAMs, safety decision based on internal exposure

   Limitations: cross species extrapolation not well defined, Verification of internal exposure estimates, No consideration of in vitro biokinetics in potency ranking, Data summary and organization a challenge

Wrap Up

Summary of Considerations for Next Generation RAX

- **Exposure**
  - External
  - Internal
  - *In vitro*
- **Mode of Action**
  - Physical-chemical properties and Structure Activity Relationships
  - Relevant kinetics and bioavailability
  - Untargeted testing and analogies
  - Targeted effects and variability (incl. relative potency)
- **Risk characterisation**
  - Margin of Internal Exposure
  - Confidence/Uncertainty assessment



Berggren et al. *Computational Toxicology. 4. P31-44. (2017)*
OECD IATA Case Studies Project, Series on Testing & Assessment No. 275. ***ENV/JM/MONO(2017)27***

Summary

- RAX based on chemical similarity alone has limitations – hypothesis generation
- NAM data can make RAX more robust – testing of hypothesis
- Similarities/differences in toxicokinetics and toxicodynamics can be informed by NAM
  - Used to qualitatively or quantitatively strengthen the analogue ID and predict internal exposures
  - Inputs to safety assessment can be based on internal exposures
- This is an incremental step change in RAX → Overarching framework on next-generation read-across being drafted, could be aligned with guidance(s)

Acknowledgements

Propylparaben

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Read-across WG

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- Lester, Cathy (P&G)
- Mahony, Catherine (P&G)
- Naciff, Jorge (P&G)
- Vandenbossche, Evita (Unilever)
- Yang, Chihae (AM-MN)

References

- *Ab initio* chemical safety assessment: A workflow based on exposure considerations and non-animal methods **Berggren et al.** Computational Toxicology. 4. P31-44. (2017)
- Chemical safety assessment workflow based on exposure considerations and non-animal methods - OECD IATA Case Studies Project, Series on Testing & Assessment No. 275. **ENV/JM/MONO(2017)27**
- Wu, S.; Blackburn, K.; Amburgey, J.; Jaworska, J.; Federle, T. A., A framework for using structural, reactivity, metabolic and physicochemical similarity to evaluate the suitability of analogs for SAR-based toxicological assessments. *Regulatory Toxicology and Pharmacology* **2010,** *56*, 67-81.
- https://www.ce-toxgps.eu/
- Case Study on the use of New Approach Methods to inform a theoretical Read-Across for Propylparaben using an Integrated Approach to Testing and Assessment exploring the Endocrine Activity of Parabens – **submitted to OECD IATA case studies' project, under review**
- Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to Caffeine - **submitted to OECD IATA case studies' project, under review**
- The margin of internal exposure (MOIE) concept for dermal risk assessment based on oral toxicity data - A case study with caffeine. **Bessems et al.**, Toxicology, 392:119-129 (2017)
- Assessing uncertainty in read-across: Questions to evaluate toxicity predictions based on knowledge gained from case studies. Schultz TW, Richarz A-N, Cronin MTD (2019). Computational Toxicology 9, 1–11
- An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs) Avila et al., Regulatory Toxicology and Pharmacology 104662 vol 114 (2020)

Back-up slides

1. What type of category formation was attempted and was it suitable for the context of the read-across?

Several source substances to one target chemical

- Target and source chemicals are identified as short linear chain parabens
  - Source chemicals flank the target chemical
- Category members share same primary metabolite 4-hydroxybenzoic acid and similar linear aliphatic alcohols

Low uncertainty. Source chemicals were clearly identified and chemistry and properties were described

2. How well made was the premise or hypothesis of the read-across argument?

- Target chemical will have similar bioavailability and bioactivity as the source chemicals MP, EP, and BP
- Chain length differences in the parent esters will result in a predictable potency trend in observed effects across category members with increasing alkyl chain length.
- Common metabolite pHBA does not contribute significantly to toxicity

Low uncertainty. Case study with clearly stated hypothesis.

3. What rationale was used to select the NAMs used and how did they support the decision making?

Data gap for reproductive toxicity:

- Established weak endocrine activity used to select NAM
  - Weak estrogenic activity related to parent supported by *in silico*, high content and high throughput testing and modelling
- Internal exposure data gaps informed by NAM
  - ADME information used twice, i) to inform similarity across analogues ii) to inform relevant kinetics
  - PBPK modelling supported predictions of human exposure and *in vivo* POD

Low – medium uncertainty. Case study based around a mechanistic hypothesis and took account of exposure. Multiple data informing mode of action and bioavailability. No *in vivo* TK data exists for parabens. Human exposure estimates were verified through existing human data, either on the chemical itself or a suitable analogue. The modelling did not account for metabolites.

4. How was mechanism of action considered, supported and assessed?

Estrogen receptor activity was considered to be the common mode of action for the parent esters

- Supported by way of
  - *in silico* alerts and molecular docking
  - Toxcast data (emphasis on EPA ER model)
  - Toxicogenomics assays (emphasis on MCF-7 cells, rich in Nuclear Receptors)
  - Calux EATS assays (+/- metabolism)

Low – medium uncertainty. Within the context of mechanistic plausibility, reported toxicodynamic properties were sufficiently well to establish similarity in hazard.

5. How was similarity defined and assessed?

- Structural/physicochemical similarity
- Targeted *in silico* tools
- Untargeted and targeted *in vitro* dynamics
- Targeted *in vitro* kinetics
- Quantitative adjustments

Low – medium uncertainty. Similarity based on structure, physical-chemical and *in vitro* data demonstrated.

6. What were the uncertainties in the toxicological data for read-across data and how did they allow for an assessment of robustness of these data?

- Source chemical POD has Klimish score of 3 (non-guideline, no dose-response)
  - No measurement of internal plasma levels
- Highly conservative compared to other *in vivo* studies

Medium uncertainty. Associated with conservative endpoint data used to derive the POD. Medium levels of uncertainty due to the lack of measurement of internal plasma levels.

7. How were NAMs applied and did they assist in the reduction of uncertainty?

NAM applied to

- Support similarity of bioavailability and bioactivity
- Estimate Cmax at NOEL for source chemical
- Estimate Cmax from human cosmetic exposures
- Inform mode of action and derive potency relative to source chemical

Low uncertainty. Case study well supported by New Approach Methodologies (NAMs) data that allowed for better understanding of kinetics and mechanism of action.

8. What is the overall certainty and is it acceptable as part of an exposure led risk assessment? If not acceptable, what information is required to increase confidence?

- Overall uncertainty defined as low/medium
- Margin of internal exposure is acceptable (>290)

High confidence. Case study showed a strong Weight of Evidence, combining multiple lines of evidence that supported the read-across hypothesis for the given exposure scenario.

9a. What are the key limitations of the case study?

- Cross species extrapolation not well defined
- Verification of internal exposure estimates
  - Use of immature analog approach
- No consideration of *in vitro* biokinetics in potency ranking
- Data summary and organisation a challenge

Gaps in knowledge requiring further research. Documenting and reporting data requiring review and dialogue.

9b. What are the key strengths of the case study?

- The workflow
- Consideration of realistic cosmetic exposure
- NAM data used to:
  - Strengthen analogue ID
  - PBPK model for internal human and animal exposures
- Make a safety decision based on internal exposures

Value of workflow and value added by NAMs acknowledged.