An Integrated Approach to Testing and Assessment (IATA) to Inform a Theoretical Read-Across for Dermal Exposure to Propylparaben from Cosmetics: a joint case study with Cosmetics Europe

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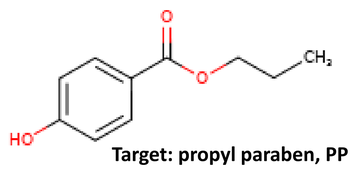
In Read Across, untargeted and targeted NAMs can help to strengthen the identification of suitable substances for read across, increasing confidence in the analogue identification and NOEL used as a POD for the risk assessment. NAMs have also been used to inform on relative potency of analogue mode of action and to predict internal exposure in both human and animal studies allowing for a risk assessment approach based on internal exposures of the human versus the animal study. This 'next generation Read Across' approach is presented here, and illustrated by the joint Cosmetics Europe-EuToxRisk parabens case study.

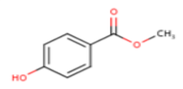
1. **Identify use scenario**
2. **Identify molecular structure**
3. **Collect existing data**
4. **Identify analogues, suitability assessment and existing data**
5. **Systemic bioavailability (Parent vs metabolite, target organs, internal concentration).**
6. **MoA hypothesis generation (WoE based on available tools – *in silico, in chemico* and *in vitro*)**
7a. **Targeted testing**
7b. **Biokinetic refinement (*in vivo* clearance, population, *in vitro* stability, partition)**
8. **Point of Departure (PoD), IVIVE, Margin of Safety (MoS), Uncertainty Estimation**
9. **Final risk assessment or summary on insufficient information approach**

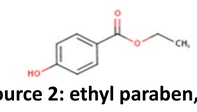
Decision context: Safety assessment of Propyl paraben (PP) as preservative at 0.19% in cosmetics (dermal route)
Information gap: for demonstration purposes, reproductive toxicity study data on PP was excluded

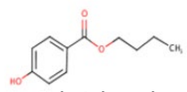


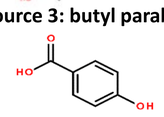
Target: propyl paraben, PP

Analogue identification: 3 suitable analogues identified according to *structure, reactivity, metabolism and physical chemical properties* (Wu et al 2010). Source compound selected on basis of highest Tanimoto coefficient and *in silico/vitro* alerts


Source 1: methyl paraben, MP


Source 2: ethyl paraben, EP


Source 3: butyl paraben, BP


Metabolite: p-hydroxy benzoic acid, pHBA

Tier 0 Data	Results: evidence gathered substantiates category suitability (form common metabolite)
TTC	0.275 mg/Kg/day > 0.03 mg/kg/day: **Not applicable, exposure** exceeds TTC
Analogue identification	
Physicochemical properties	Literature & in silico (Epi suite, OECD QSAR Toolbox): **similar physico-chemical properties but increasing side chain → ↑LogP** which can affect bioavailability and may inform on potency
In silico alerts and docking simulations	OECD QSAR toolbox, Endocrine disruptome: docking simulations indicate a homogenous profile of **weak binding activity for receptors considered by the Endocrine Disruptome tool**.
In vivo data	Literature: **No specific target organ toxicity** at very high doses in repeated dose toxicity studies. Source compound has highly conservative NOEL (non-guideline study with single dose studied)
In vitro data	ToxCast: **Low activity in *in vitro* assays** relevant for endocrine activity many orders of magnitude lower than natural estrogens/androgens.

Tier 0 conclusion: The data gathered is not sufficient to substantiate the read across hypothesis, therefore the assessment progressed to Tier 1

Which NAMs (existing and/or newly generated) for toxicodynamics and -kinetics are needed for comparisons across the chemicals in the assessment?

Tier 1 data	Results: ADME and biological properties support the read-across hypothesis
***In vitro* skin penetration in non-viable human skin**	Systemic exposure after topical application rapid and equivalent across all category members.
In vitro metabolism in viable human skin	Extensive first-pass cutaneous metabolism - less than 0.5% of the systemically bioavailable amount in the form of the parent paraben.
Primary human hepatocytes	Primary metabolic pathway for all four parabens in hepatic and skin models is hydrolysis to pHBA. Further metabolism of pHBA is minimal.
Human liver S9 and EpiSkin S9	Rate of ester hydrolysis significantly higher in the liver than in skin. Any parent paraben systemically bioavailable after topical application is rapidly hydrolyzed once it reaches the liver.
Human plasma protein binding	All 4 parabens stable in human plasma, with minimal hydrolysis by esterases. Extensive binding to plasma proteins: MP < EP < PP < BP.
ToxCast data	Bioactivity: MP < EP < PP < BP. No significant activity for pHBA. Rank order of potency in estrogen receptor activity: MP < EP < PP < BP. Relative potency scaling factors for estrogen receptor activity from EPA model AC10 median values for PP, EP, and MP: 0.37, 0.2 and 0.13, respectively, BP (value of 1).

Tier 1 conclusion: NAMs support the similarity hypothesis of the category. Proceed to tier 2 for quantitative safety assessment

Tier 2 data	Results: NAMs inform on several aspects of read-across based safety assessment of low toxicity chemicals
CALUX	For all parabens: weak estrogenic activity << Estradiol (no activity for pHBA), estrogenic potency increased with chain length. Weak anti-androgenic activity << Flutamide (no activity for pHBA) Presence of rat liver S9 decreased bioactivity in the EATS panel and pHBA was essentially devoid of significant biological activity.
Transcriptomics	Changes in expression of a large number of genes in MFC7 cells by all parabens: up-regulated estrogen response genes, indicating they share potential MoAs related to endocrine effects. Activity generally increasing with increasing chain length: PP was most similar to that of BP. Fewer genes were affected by pHBA; which were mostly different from those affected by the parabens. Overall biological similarity.
Exposure Assessment: deterministic and refined probabilistic consumer exposure	A probabilistic model was used to estimate the exposure of PP across worst case and realistic exposure scenarios, taking account of consumer habits and practices)
PBBK modeling	Legacy data used: PoD dose of 2 mg/kg/day BP administered by SC injection to rats. The C_{max} was 2.1 µM *(Fisher et al., 1999)* Internal exposure estimates for PP were: C_{max} = 0.022 µM from the SCCS deterministic consumer exposure estimates; C_{max} = 0.018 µM from the Crème deterministic (worst case) consumer exposure estimates; and C_{max} = 0.0006 µM from the Crème probabilistic (realistic) consumer exposure estimates.
Margin of Safety (internal exposure and relative potency)	SCCS deterministic consumer exposure scenario: **MoIE = 2.1 / (2.0E-2 * 0.37) = 284** Crème deterministic (worst case) consumer exposure: **MoIE = 2.1 / (6.4E-3 * 0.37) = 887** Crème probabilistic (realistic) consumer exposure: **2.1 / (5.8E-4 * 0.37) = 9786**

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).

Questions to consider, to assess confidence in read-across assessment

- Type of category formation attempted? Suitability for the context of the read-across?
- How well made was the premise or hypothesis of the read-across argument?
- Rationale was used to select the NAMs used and how did they support the decision making?
- How was mechanism of action considered supported and assessed?
- How was similarity in TD/effects defined and assessed?
- How was similarity in TK/potency defined and assessed?
- 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?
- How were NAMs applied and did they assist in the reduction of uncertainty?
- Overall certainty, is it acceptable as part of an exposure led risk assessment? If not acceptable, what information is required to increase confidence?
- Key strengths and limitations of the case study?

Conclusions and perspectives

- Read-across based on chemical similarity alone has limitations – hypothesis generation
- NAMs data can make read-across more robust – testing of hypothesis
- Similarities/differences in toxicokinetics and toxicodynamics can be informed by NAMs
- Used to qualitatively and/or quantitatively strengthen the analogue ID and predict internal exposures
- Inputs to safety assessment can be based on internal exposures

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www.eu-toxrisk.eu/