

**ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE**

**Case Studies for the Integrated Approaches for Testing and Assessment of
propylparaben (PrP) from multiple sources using ASPA, Eleventh Review Cycle (2025)**

OECD Series on Testing and Assessment, No. 448

JT03594282

This work was approved and declassified by the Chemicals and Biotechnology Committee on 27/08/2026.

Please cite this publication as:

OECD (2026), *Case Studies for the Integrated Approaches for Testing and Assessment of propylparaben (PrP) from multiple sources using ASPA: Eleventh Review Cycle (2025)*, OECD Series on Testing and Assessment, No. 448, OECD Environment, Health and Safety, Paris, [https://one.oecd.org/document/ENV/CBC/MONO\(2026\)29/en/pdf](https://one.oecd.org/document/ENV/CBC/MONO(2026)29/en/pdf).

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Acknowledgements

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The authors are grateful to the Cosmetics Europe Long Range Science Strategy -LRSS- initiative who shared their data for this case study.

Foreword

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Q)SAR, chemical categories, and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA). There is a need for the investigation of the practical applicability of these methods/tools for different aspects of regulatory decision-making, and to build upon case studies and assessment experience across jurisdictions.

The objective of the IATA Case Studies Project is to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use. The aim is to create common understanding of using novel methodologies and the generation of considerations/guidance stemming from these case studies.

This case study was initially developed by Cosmetics Europe (BIAC) and data were reused in Riskhunt3R for illustrating practical use of IATA and submitted to the 2025 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team.

This case study is an illustrative example, and its publication as an OECD monograph does not translate into direct acceptance of the methodologies for regulatory purposes across OECD countries. In addition, this case study should not be interpreted as official regulatory decisions made by the authoring member countries.

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Executive Summary

Propylparaben is an ester of para hydroxybenzoic acid that is used in consumer goods like aqueous toys and personal care products as well as in pharmaceuticals as preservatives.

In Europe, propylparaben can be used as a preservative in cosmetics up to 0.18% as ester or 0.14% as acid. It can be found in body paints as well as in toys. Its use as food additive is not authorized, although it can be used as a food contact material. In the current work, the use in drugs was not addressed.

An IATA on the use of propylparaben in cosmetics exists and addressed exposure from cosmetics products based on read-across enhanced by NAMs.

Here, the exposure is expanded to encompass use of propylparaben in personal care products and aqueous toys (putty, slime and finger paint) and the assessment is made using the ASPIS Safety Assessment Algorithm (ASPA) as a framework for next-generation risk assessment (NGRA) with a focus on systemic effects.

The problem formulated is the following: is the combined use of propylparaben at maximum concentration from personal care products, consumer goods (putty, slime and finger paint) and toys safe for consumers? The working hypothesis is that propylparaben lacks systemic toxicity data and that no suitable analogue exists. The assumption is that all other effects (local, sensitization and genotoxicity...) were evaluated and there is no concern.

There are regulatory opinions on the safe use of propyl paraben (e.g., SCCS https://health.ec.europa.eu/system/files/2022-08/sccs_o_243.pdf%20 and CIR https://www.cir-safety.org/sites/default/files/Parabens_0.pdf). In the SCCS opinion (243), it is stated that “Propylparaben can maximally be used in any cosmetic product up to 0.14% (alone, as acid) or up to a maximum of 0.14% (as acid), as the sum of the individual concentrations of butyl paraben and propylparaben, when used together as a binary mixture in the same product. This would be equivalent to a maximum concentration of propylparaben of 0.183% in cosmetic products.”

Data from the literature and newly generated from RISK-HUNT3R were used through the exposure, the ADME/tk and toxicodynamics parts of ASPA were integrated to come to a safety decision.

Abbreviations

AR: androgen receptor

ASPA: ASPIS Safety Profiling Algorithm, an alternative safety profiling algorithm

ADME: absorption, distribution, metabolism, excretion

CIR: Cosmetic ingredient review

DART: developmental and reproductive toxicity

DTU: Danish technical University

EATS: estrogen, androgen, thyroid, steroidogenesis

EPA: Environmental Protection agency

EU: European Union

ER : estrogen receptor

HESS: Hazard Evaluation Support System Integrated Platform

<https://www.nite.go.jp/en/chem/qsar/hess-e.html>

IVIVE: in vitro in vivo extrapolation

MOA: mode of action

MOS: Margin of Safety

NAM: New Approach Methodologies

NGRA: Next Generation Risk Assessment

PBK: physiologically-based kinetic

pHBA: para-hydroxybenzoic acid

POD: point of departure

PR: Progesterone Receptor

PrP: Propylparaben

RAR : retinoic acid receptor

RXR : retinoid X receptor

SCCS: Scientific Committee on Consumer Safety

SED: Systemic exposure dosage

SMILES: Simplified Molecular Input Line Entry Specification

Tk: toxicokinetics

TTC: Threshold of Toxicological Concern

Introduction

The purpose of this case study is to test the applicability of the ASPA workflow developed in the EU-project RISK-HUNT3R for applying next-generation risk assessment (NGRA) to a consumer product.

Propylparaben is the test substance considered in this use case. An IATA on the use of propylparaben from cosmetics products already exist (ENV/JM/MONO(2020)16 - Series on Testing and Assessment No. 320: Case Study on the Use of an Integrated Approach to Testing and Assessment (IATA) and New Approach Methods to Inform a Theoretical Read-Across for Dermal Exposure to Propylparaben from Cosmetics). Only dermal exposure from cosmetics was considered in the existing IATA.

Here, combined exposure from personal care products as well as consumer goods (facial mask, clay mask and body paint) were considered. The working hypothesis is that propylparaben lacks systemic toxicity data and has no relevant analogues for read-across. ASPA is used to assess if the combined exposure to propylparaben as a preservative at maximum in these products is safe for adult consumers.

The main differences between this case study and the previous IATA case study (ref) are the following:

- Problem formulation: the exposure scenario was broadened to encompass cosmetics and other consumer goods. The working assumption was that propylparaben had no relevant analogues for read-across. The previous IATA was based on NAM-enhanced read-across and limited to exposure to cosmetics only.
- Exposure: aggregate exposure includes other consumer goods beyond cosmetics
- Dynamics: more in silico tools were used here (QSARs models from VEGA, Case Ultra, Leadscape and from the Danish Technical University covering alerts relevant for systemic toxicity like nuclear receptors and reproductive toxicity) compared to the previous IATA (OECD QSAR toolbox, endocrine disruptome). Regarding the in vitro data, full transcriptomics data (versus selected genes previously) were considered as well as in vitro DART assays (using fish embryos).
- Toxicokinetics: PBK simulation was done here based on the exposure scenario. In vitro biokinetics was considered using the VIVID model.

The current case study is based on the integration of in silico and in vitro data for deriving NAM-based points of departure. These points of departure are compared with the considered exposure prediction based on the use scenario.

The outcome of both the safety assessment of the systemic toxicity of propylparaben in the current and the previous IATA are similar: conclusion on safe use of propylparaben in the considered exposure scenarios.

Sources of parabens in personal care products and in consumer goods were retrieved and summarized. Relevant and main routes of exposure were considered in this assessment.

Based on the aggregate exposure from the relevant routes of exposure a tiered assessment was performed using ASPA. The lowest tier (deterministic) provided sufficient confidence for the assessment. For the purpose of testing ASPA, probabilistic tiers (high tier) exposure estimates were evaluated for illustration purpose.

ADME parameters were estimated and then measured for estimating internal concentrations in a forward dosimetry. In vitro parameters were used to feed into the PBK model.

For toxicodynamics, in silico alerts relevant to systemic effects were collected. In vitro data generated in RISK-HUNT3R as well as from literature and past initiatives (Cosmetics Europe Long Range Science Initiative) were used to run the assessment.

Points of departure were derived using bioactivity data and bioactivity exposure ratios were calculated.

1 Regulatory Purpose / Context of use

The objective is to evaluate the applicability of ASPA workflow for OECD IATA for the safety assessment of consumer products. For propylparaben as the test agent, the safety assessment was performed according to the cosmetic regulation in Europe.

There are opinions on the safe use of propylparaben in consumer goods and personal care products (SCCS https://health.ec.europa.eu/system/files/2022-08/sccs_o_243.pdf and CIR https://www.cir-safety.org/sites/default/files/Parabens_0.pdf).

In the context of this IATA case study, the hypothetical problem formulated is the following:

Is the combined exposure to propylparaben at maximum allowed concentration in personal care products as well as consumer goods (facial mask, clay mask and body paint) safe for consumers in Europe?

Working assumptions are that data are available and show no concern on local effects, sensitization and genotoxicity. For systemic toxicity, no (relevant) analogues exist to allow a read-across exit.

Given this is a safety assessment for consumers, the combined exposure was estimated and translated to an internal concentration considering the sources, the products and routes of exposure of relevance to this assessment. The presence of propylparaben in cosmetics and other uses by adults and the dermal route were the most relevant for the assessment. A deterministic estimate was derived using values from the SCCS' notes of guidance.

In ASPA, this corresponds to the problem formulation section.

2 Endpoint addressed

Only systemic effects are addressed in this IATA case study. The approach taken is not intended to predict the specific target organ toxicity, but instead to adequately cover the biology associated with systemic toxicity to provide an exposure-led, protective approach that would identify any safety concerns. The tools selected are not aiming at replicating adverse effects at organ or organism level like the animal studies. The approach is based on the NGRA principles: exposure-led and hypothesis driven.

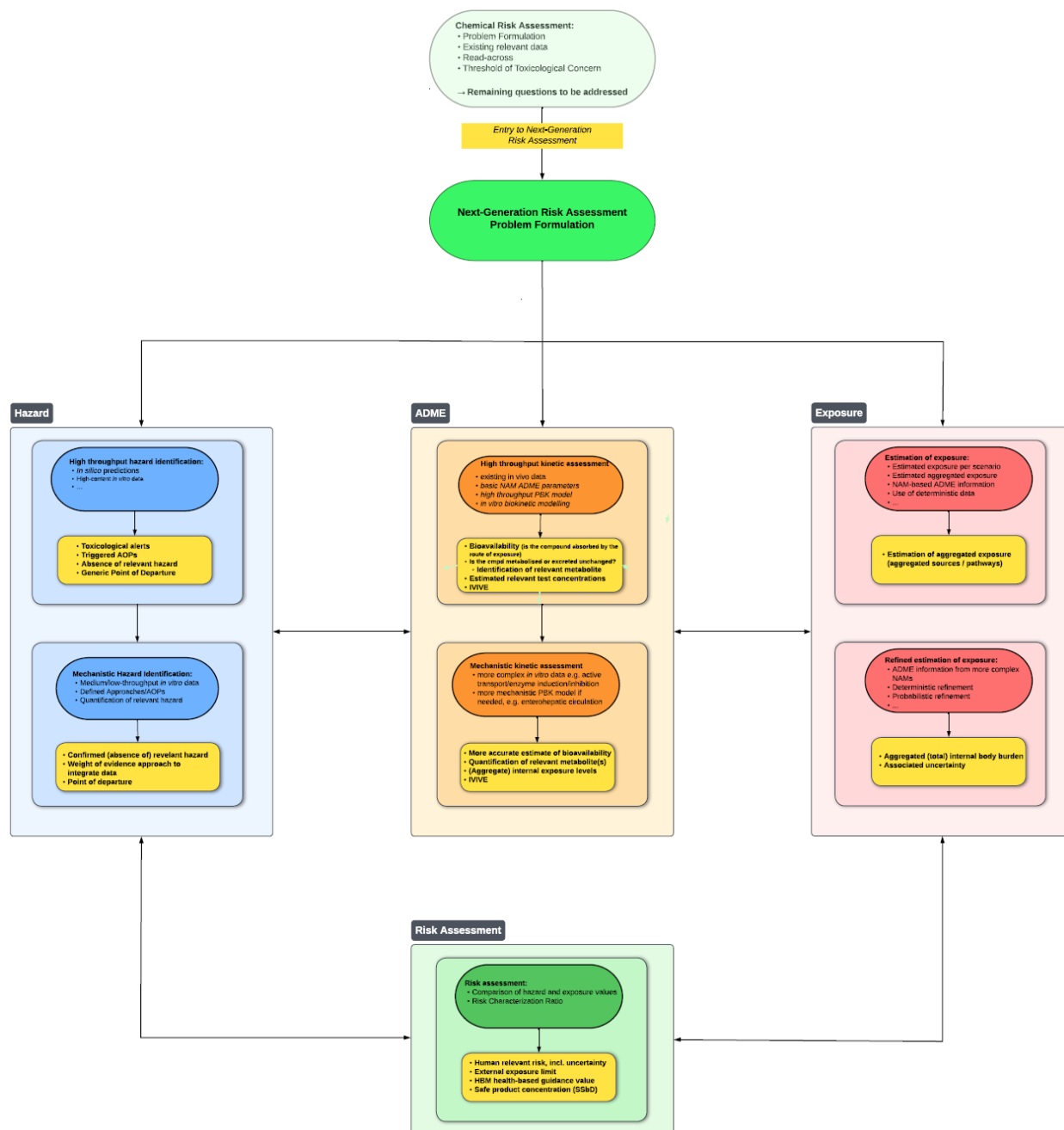
3 Description of workflow

The ASPA IATA general scheme is shown in Figure 1 below. ASPA consists of 3 modules that are eventually integrated into a fourth one for risk assessment. ASPA is a workflow that helps structure and integrate data in NGRA. Based on the context of use and the problem formulation, only relevant modules are considered in the assessment. In this case, the ASPA workflow has been adapted to the (similar) OECD IATA Framework.

For propylparaben, all modules were considered.

Figure 1. Generic ASPA IATA workflow

Consisting of 3 modules: hazard, kinetics (absorption, distribution, metabolism and excretion (ADME)) and exposure that are integrated at the bottom to perform a risk assessment based on NAM data.



Note: A high-resolution version is available at: <https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/assessment-of-chemicals/case-studies-iata-propylparaben-multiple-sources-using-aspis-safety-profiling-algorithm/generic-aspis-safety-profiling-algorithm-workflow-version-2-1.pdf>

Recently published in Leist et al. - ALTEX 2026;43(1):158-175 doi: 10.14573/altex.2509081- is provided below in section 6

4 Scientific rationale / Basis for IATA workflow

The following section provides a high-level description of ASPA. The detailed version can be found in the paper by Leist et al., 2025 (doi:10.14573/altex.2509081). This IATA workflow is based on traditional risk assessment that consists of hazard characterization and exposure assessment. To link external exposure to internal exposure, kinetics are included. Three modules; hazard, exposure and kinetics (ADME: absorption, distribution, metabolism and excretion) were considered in this case study and the output was integrated for the safety decision. The modules guide the evaluation and when appropriate the refinement of the hypotheses that are formulated. The decision is made when enough information is collected for making the decision.

As described above, depending on the problem formulation, modules can be included or excluded and the NAMs within each module can be selected based on their suitability for the chemical(s) and based on the problem formulation. In this case, we include all modules of ASPA. Within the hazard module, the working hypothesis is that propylparaben lacks in vivo data related to systemic effects and has no (relevant) analogues for considering a read-across assessment.

For each module, a tiered assessment is performed using when and where relevant non-testing methods (like in silico tools) and/or in vitro assays. For the latter, high-throughput assays are commonly used as starting point followed if/when relevant by bespoke assays as ways to refine the initial working hypothesis. The NAM-based bioactivity estimates were used as points of departure (PoDs).

For the exposure, given the use scenario considered, aggregate exposure from multiple products and routes of exposure were covered for propylparaben (Affourtit et al. manuscript in preparation).

In the ADME/tk module, the estimated doses applied were translated into internal concentrations by forward dosimetry. Measured ADME parameters were used in this module.

The assessment was performed by comparing the NAM-based PoDs with the internal exposure estimates.

Uncertainty characterization was performed in a semi-quantitative way for each assay used as well as for the propagation of the uncertainty to the overall decision.

The proposed RISK-HUNT3R uncertainty assessment framework was not used for the current propylparaben IATA case study.

5 Domain of applicability (technical and biological)

a. Technical domain of applicability

There are no technical limitations in testing propylparaben in vitro. For the in silico tools, weight was given to output that are in the applicability domain. In silico data that were out of the applicability domain were discarded.

Propylparaben is included in the training set of some of the models. In this case, actual data were used.

b. Biological domain of applicability

Given that the scope of this case study is to explore the applicability of this IATA for systemic toxicity (including DART), the tools considered were selected to cover as best as possible molecular and cellular events relevant for these endpoints. Regarding the biological domain of applicability, different tools were considered for propylparaben. The coverage was full genome at transcript level, endocrine bioactivity and stress response on biological processes (e.g. inflammation) and teratogenicity. The purpose is not to replicate apical effects from animal studies but to cover molecular and cellular events that might be involved in biological processes leading to systemic effects. The choice of the tools made here fulfils this hypothesis-driven approach. Also, this approach is still work in progress and no standards or guidance documents on how to apply it exist for the time being.

- In silico tools relevant for systemic effects:
 - o Nuclear receptor binding models, ER, AR, RAR, RXR, liver toxicity (HESS), NRF2, DART (developmental and reproductive toxicity).
 - o Chemical effect predictor (CEP): a knowledge graph linking chemical to proteins, biological functions and diseases. This tool is still in an early development phase.
- Hybrid models: computational models for estrogenicity and androgenicity from ToxCast
- In vitro models:
 - o Broad biological coverage: Transcriptomics: using HepaRG and TempSeq, full genome coverage, cell painting
 - o Focused assays:
 - Endocrine activity: from receptor binding, co-factors activation to gene transcription activity on different nuclear receptors: assays from ToxCast, CALUX assays from BDS
 - Other In vitro models: other ToxCast assays covering cell stress assays, stem cell-based assays, assays on zebrafish embryos

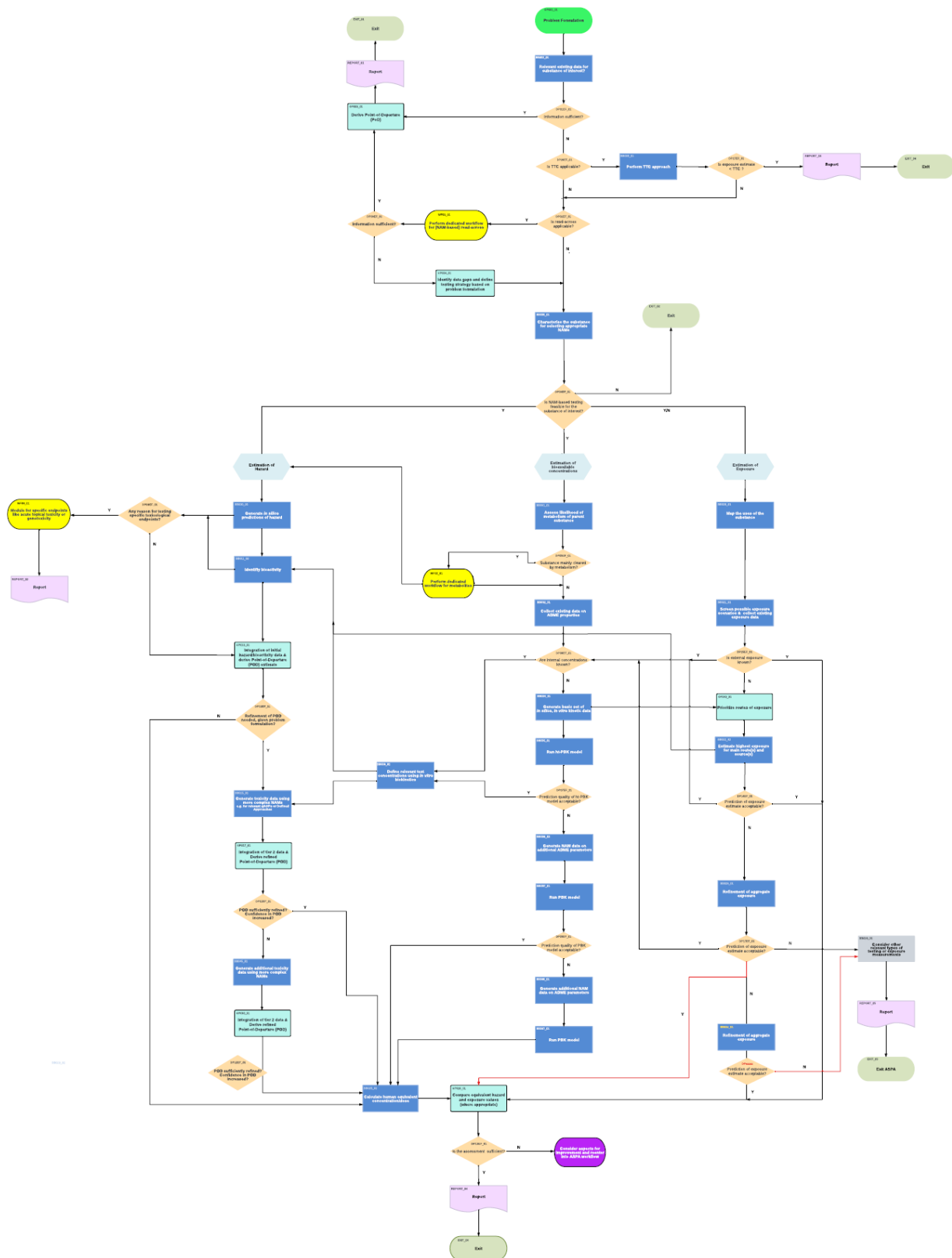
6 Modules

All four modules in ASPA were included in this OECD IATA case study.

The basis for the case study submitted is ASPA, “the ASPIS Safety Profiling Algorithm”. The ASPA framework is more elaborate, but similar to the 2025 OECD IATA Framework. The purpose of ASPA is to provide guidance to end-users for putting NGRA into practice, both for the generation and the assessment of data. In RISK-HUNT3R, ASPA will be used to demonstrate through case studies (as this one) the applicability of NGRA approaches and the utility of ASPA. Furthermore, the case studies are used to fill gaps, and to parametrize decision points and rule settings.

The current version of ASPA, i.e., v3.0, is depicted below. Given that the workflow should be applicable for regulatory decision-making, it comprises the standard elements for chemical safety assessment. Following problem formulation, ASPA defines a tiered approach on how to use NAMs for generating data, and how to assess the data. Each tier will also include assessment of associated uncertainties. As such, ASPA is intended to serve both industry and regulators. ASPA comprises a wide set of modules relevant for chemical safety assessment, aimed at toxicokinetic modelling and in vitro to in vivo extrapolation (IVIVE) approaches, hazard identification and characterization, exposure and risk assessment. Different modules may be activated or deactivated, depending on the problem formulation. Thus, ASPA is flexible; it allows for addressing multiple types of regulatory questions and frameworks, with a focus on European regulations, using different approaches.

Figure 2. Schematic representation of ASPA v3.0



Note: A high-resolution version is available at: <https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/assessment-of-chemicals/case-studies-iaa-propylparaben-multiple-sources-using-aspis-safety-profiling-algorithm/aspis-safety-profiling-algorithm-version-3-0-workflow.pdf>

Summary of building blocks of ASPA covered in this case study

Components of ASPA used for this case study		
Problem formulation		Is the combined use of propylparaben at maximum concentration from personal care products, consumer goods
Estimation of exposure		
	Map the uses of the substances	Preservative used in aqueous products
	Screen possible use scenarios	Exposures expected for consumer use, environmental exposure, exposure of sensitive populations and wide dispersive use
	Prioritize routes of exposure	Dermal
	Estimate highest exposure for relevant routes and sources	Low tier aggregate dermal exposure: 0,76 mg/d/Kg
	Prioritize sources of exposure	Dermal
Estimation of bioavailable concentration		
	Are ADME properties known	Yes -several existing NAMs data from Cosmetics' Europe Long Range Science Strategy
	Assess likelihood of metabolism of parent substance	Yes - done here based on existing data
	Run PBK model	Done using the in vitro input data
	Prediction quality of PBK model acceptable?	Yes -after calculating bioactivity:exposure ratios

Estimation of Hazard		
	Generate in silico predictions of hazard	Done using OECD QSAR toolbox, Case Ultra, Leadscope, VEGA, tools from Danish Technical University: alerts on ER activity and DART
	Identify bioactivity	In vitro NAMs from ToxCast (transcriptomics, cell painting, in vitro pharmacology profiling, DART from devTox and fish embryo assay), Calux assay on steroid receptors
	Integration of initial hazard/bioactivity data & derive Point-of-Departure	PoDs calculated from in vitro NAMs related to ED activity
Compare equivalent hazard and exposure values (where appropriate)		PoDs from NAMs are compared to the exposure estimates from the PBK predictions. All bioactivity occurred at concentrations exceeding the exposure estimates
Is the assessment sufficient?		Yes - level of confidence was qualified

Scientific rationale/basis for IATA workflow

In recent years, various efforts have been made worldwide to contribute to NGRA approaches, including design of new concepts building on integration of exposure and hazard assessment models and implementation of new approach methodologies (NAMs) [Ball et al, 2022; Berggren et al, 2017; Pastoor et al, 2014; National Research Council, 2007]. Generally, NGRA is considered feasible given the advances made in science and technology, exemplified by the development of models for rapid exposure predictions, high-throughput toxicokinetic modelling, and innovative (high-throughput) in vitro approaches for toxicity testing. A number of case studies demonstrating the applicability of NGRA approaches for regulatory risk assessment has been published over the years. However, (full) operationalization of NGRA concepts is limited thus far. Therefore, there is a strong need for a workflow, to serve as guidance on both data generation and data interpretation, aimed at implementation of NGRA approaches. RISK-HUNT3R aims at developing such a workflow called ASPA.

Since ASPA comprises a wide set of elements/ modules relevant for chemical risk assessment: toxicokinetic modelling and in vitro in vivo extrapolation (IVIVE) approaches, hazard identification and characterization modules, exposure and risk assessment.

ASPA does not prescribe the specific NAMs to be used, since these depend on the specific problem formulation. Instead, ASPA shows, how the different modules are interlinked, build upon each other and

lead through defined decision points to the ultimate goal, e.g., risk assessment. The entry into ASPA usually leads to first insights into the properties of a substance to be assessed (screening-like modules leading to information with potentially higher uncertainty). At this level, NAMs to be used can be various in silico models, panels of receptor activation assays, stress pathway assays or omics approaches. To reduce uncertainty and refine the assessment, further data can be generated in following tiers of testing. Consequently, for each case study, the specific NAMs to be used need to be defined, especially for higher tiers.

7 Information sources in each module

All details on the information sources can be found in Annex I.

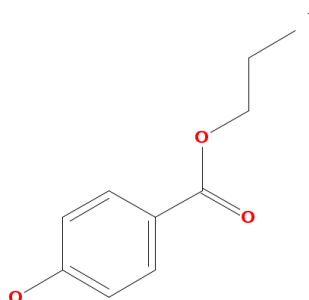
7.1 Problem formulation

Is combined exposure to propylparaben at maximum allowed concentrations in personal care products as well as consumer goods (facial mask, clay mask and body paint) safe for consumers in Europe? Safe here means that no bioactivity (based on the hypothesis-driven approach) is observed at relevant consumer exposure levels. Currently, no guidance document exists on the use of bioactivity/exposure ratios for risk assessment.

Working assumptions: data available and showing no concern on local effects, sensitization, genotoxicity. For systemic toxicity, no (relevant) analogue exists to allow a read across exit.

7.2 Exposure assessment

Propylparaben (PrP) is used as a preservative in aqueous products in cosmetics, drugs, consumer goods and toys in Europe. It is not allowed as a food additive in Europe but can be used as food contact material (FCM). The exposure in drugs was not considered in this IATA.



Exposure to propylparaben is expected for consumers (including sensitive populations) as well as the environment with wide dispersive use. The focus here is on adult consumers only.

The combined and aggregate exposure estimates were obtained based on the problem formulated and building on recent sources (Brand et al 2017 - literature review, Affourtit et al 2022 Modelling aggregate exposure to parabens).

Identified sources and exposure routes in Europe are the following:

- Personal care products (PCP) (dermal)
- Other (non-PCP) consumer products (feminine hygiene products, all sorts of paper products (dermal)

- Children's toys (dermal, oral)
- House dust (oral)
- Food contact material (oral)

In terms of physical-chemical properties relevant to systemic effects, the information was retrieved from the literature and from in silico predictions.

Table 1. Physical-chemical properties of propylparaben

Parameter	Propylparaben
Molecular weight	180.2
Melting Point (°C)	97 °C (b)
Volatility (mmHg at 25°C)	0.000307 (a)
Log P	3.04 (a), 2.34 - 3.04 (b)
pKa	7.91 - 8.87 at 25 °C (b)
Aqueous solubility (mg/L)	500 at 25 °C (a, b); 518 at 32 °C (c)

Note: From a: EPA EPI Suite; b: from OECD QSAR Toolbox, and from published literature (Dearden & Worth, 2007; Spicer et al., 2002; Wu et al., 2010); c: from Grégoire et al. (2017).

Considering the personal care products, the most relevant and majority of exposure comes from cosmetics, cf. table 2 below from the European Scientific Committee on Consumer Safety (SCCS) Notes of guidance. Oral exposure from toothpaste and lipstick remains minor.

Table 2. Deterministic exposure estimates to propylparaben

Table 5: Product exposures for the deterministic calculation of aggregate exposure for preservatives through cosmetic use. Note that these values can also be used for other ingredients when aggregate exposure calculations are needed for one or more classes of cosmetic products.

<i>Type of cosmetic product exposure</i>	<i>Product category</i>	<i>Daily Exposure E_{product} (g/d)</i>	<i>Relative daily exposure E_{product} / bw¹ (mg/kg bw/d)</i>
<i>Rinse-off Skin & hair cleansing products</i>	Shower gel	0.19	2.79
	Hand wash soap	0.20	3.33
	Shampoo	0.11	1.51
	Hair conditioner	0.04	0.67
<i>Leave on Skin & hair cleansing products</i>	Body lotion	7.82	123.20
	Face cream	1.54	24.14
	Hand cream	2.16	32.70
	Deodorant non-spray	1.50	22.08
<i>Make-up products</i>	Hair styling	0.40	5.74
	Liquid foundation	0.51	7.90
	Make-up remover	0.50	8.33
	Lipstick	0.06	0.90
	Eye make-up	0.02	0.33
	Mascara	0.025	0.42
<i>Oral care Products²</i>	Eyeliners	0.005	0.08
	Toothpaste	0.14	2.16
	Mouthwash	2.16	32.54
Aggregate exposure		17.4	269

1. The specific bw of the persons involved in the study is used and not the default value of 60kg

2. Oral care product categories are not corrected and are presumed here to only represent dermal exposure (mucosa)

Source: Table 5 from the SCCS notes of guidance

Starting with the low-tier aggregate dermal exposure (estimates from SCCS), the assumption is that all products are used together in high amounts and with high frequency and contain the substance at the maximum allowed concentration, for PrP 0.14% as acid, which equates to 0.18% as ester.

Exposure from products described in the SCCS notes of guidance: 0.53 mg/kg bw/d

(Details of the exposure from cosmetic products **SED** = $A * C * \text{DAP} / \text{bw}$ = $17.4\text{g} * 0.18\% * 100\% / 60 \text{ kg}$ = **0.53 mg/kg/d**. SED: Systemic Exposure Dosage A: amount of the product applied (g/d) C: concentration of the ingredient (%) DAP: Dermal Absorption (%) bw: Body weight (kg))

For other consumer products not in the SCCS notes of guidance, data from monitoring studies were gathered (Ficheux et al. 2015, 2016 & Bremmer et al., 2006. - 95th percentiles from Ficheux. Adults (bw of 60 kg)).

Table 3. Exposure to propylparaben from other consumer products

Products	mg/use	frequency per day	Concentration (%)	SED (mg/kg bw/d)
Body paint	59000	6/365	0.18	0.025
Facial mask	13383.8	0.14	0.18	0.049
Clay mask	27660	0.14	0.18	0.10
Eye contour cream	480	2	0.18	0.025
Aggregate				0.23

Total aggregated exposure to propylparaben from personal care products $0.53 + 0.23 = \mathbf{0.76 \text{ mg/kg bw/d}}$

Comprehensive survey data across the EU Cosmetics Industry in 2016 were available on real use levels and occurrence data of propylparaben in cosmetic products. These values were used in a higher-tier probabilistic external dose exposure assessment performed on the basis of real European consumers' habits & practices (H&P) data in the Creme Care and Exposure model (<https://www.cremeglobal.com/>) in a previous IATA (from Ouedraogo et al., 2022).

For information, refined estimates for exposure to propylparaben were calculated but not used in the current assessment (because no refinement was needed for the safety margins derived from the assessment):

- Tier 2 for dermal exposure: 0.56 mg/kg bw/d – only personal care + body paint products with measured concentrations - maximum concentration (37% + 19%)
- Tier 3 for combination of dermal and oral, assuming dermal uptake was estimated at 37%, and oral at 100%: 0.2 mg/kg bw/d – probabilistic estimate - 95th percentile

7.3 ADME/Tk

In order to translate the external dose to internal concentrations, PBK modelling was performed. For propylparaben, literature as well as data generated in past initiatives (Cosmetics' Europe long-range science strategy -LRSS) data were available (Ouedraogo et al. 2022). The PBK model was based on the model published by Campbell et al. (2015) with the incorporation of a mechanistic dermal absorption model. The simulated results after oral and dermal exposure of propylparabens were in reasonable agreement with the human data published by Shin et al (2019; 2023).

The following paragraphs are adapted from Ouedraogo et al. 2022:

Understanding absorption, distribution, metabolism, and excretion (ADME) properties and the relative rate and extent of biotransformation of propylparaben is key in this assessment. This will help guide data generation and interpretation of toxicodynamics data required to inform safety assessment.

The metabolism of parabens in humans is well-studied (Abbas et al., 2010; Janjua et al., 2008; Ozaki et al., 2013; Moos et al., 2016). It is understood that after oral and dermal exposure, propylparaben undergoes ester hydrolysis to form a primary metabolite, para-hydroxybenzoic acid (pHBA), and the corresponding linear aliphatic alcohol: propyl alcohol. It is useful to consider in what amount propylparaben penetrates across the skin following dermal application and absorbs into the systemic circulation and in what form.

Experiments (following OECD guideline 428 for skin penetration (OECD 2004)) in which propylparaben was applied to viable human skin explants and incubated for 24 h showed that it is extensively metabolized by cutaneous enzymes, such that, after 24 h, nearly all propylparaben applied to human skin was subsequently present in the medium as metabolites (Genies et al., 2019). Only 0.2% of the administered dermal dose was measured after 24 h in receptor fluid as parent propylparaben. These *ex vivo* and *in vitro* studies indicate that (i) very low amounts of parent paraben enter the systemic circulation after topical application of propylparaben due to first-pass metabolism in the skin, and (ii) the major metabolite entering the systemic circulation is pHBA (Genies et al., 2019). A pragmatic value of 1% can be used in risk assessment for the systemic delivery of parent propyl by the dermal route. The measured value for skin penetration through human skin was $0.2 \pm 0.2\%$, but to allow for uncertainty and variability, given small sample numbers in this study and the potential for a very small amount residual in the skin tissue that could go on to be absorbed, a value of 1% is considered appropriately conservative.

The metabolism of parabens in liver S9 and EpiSkin S9 was compared under the same incubation conditions. These incubations were undertaken as a screening assay to provide an indication of the metabolic stability of the chemical in liver and skin. In addition, this assay provided some comparative information on the xenobiotic metabolizing enzymes that were responsible for paraben metabolism in liver- and skin-based models (Eilstein et al., 2019 and table 4). Metabolism in skin was slower than in liver. This is probably due to the carboxylesterase isoform, carboxylesterases-2 (CES2), known to be mainly expressed in the skin (Fagerberg et al., 2014). CES2 prefers lipophilic substrates with a large alcohol group (Laizure et al., 2013; Taketani et al., 2007) rather than small alcohol groups as are present in propylparaben. pHBA and propanol are the major metabolites via the action of esterases, whereas cytochrome P450 enzymes are insignificant for propylparaben's metabolism.

Table 4. Metabolism of parabens

Parent Compound	Metabolized via	Primary	Metabolized via	Secondary	Metabolized via	Tertiary
Paraben	Chemical Enzymatic Hydrolysis	4-Hydroxybenzoic acid + Alcohol	Glycine	4-Hydroxyhippuric acid		
			UGT	Ester glucuronide		
			UGT	Ether glucuronide	Chemical Enzymatic Hydrolysis	4-Hydroxybenzoic acid
	UGT	Ether-glucuronide	Chemical Enzymatic Hydrolysis			
	Sulfuric acid	4-Carboxyphenyl sulfate		Stable		

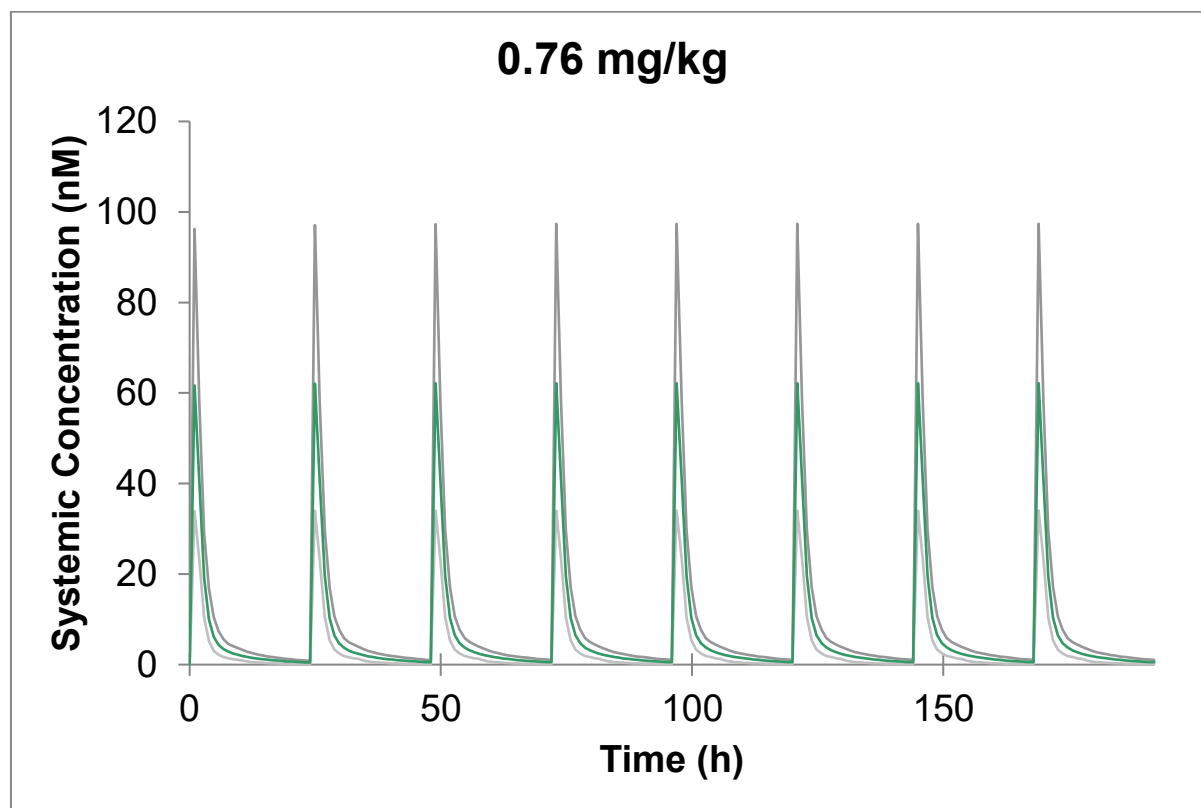
Source: the literature from Abbas, S., Greige-Gerges, H., Karam, N., Piet, M., Netter, P. and Magdalou, J. (2010). Metabolism of Parabens (4-Hydroxybenzoic Acid Esters) by Hepatic Esterases and UDP-Glucuronosyltransferases in Man. *Drug Metabolism and Pharmacokinetics*, 25(6), pp.568-577. Available at: https://www.jstage.jst.go.jp/article/dmpk/25/6/25_DMPK-10-RG-013/_pdf

Simulation of internal concentrations were performed by Certara using SimCyp considering both dermal (from cream) and oral exposure (from lipstick). Oral exposure was negligible compared to the dermal and was not used in the assessment (figure 6).

Starting with the low tier deterministic exposure scenario, here are the simulated concentrations:

- Combined consumer exposure of 0.76 mg/kg bw/d: simulation of a population of 100 adult subjects aged between 20-50; with 50% female and 50% males. The concentrations are mean, 5th and 95th percentiles.

Figure 3. Simulated concentrations for propylparaben for a combined exposure of 0.76 mg/kg bw/d via dermal route



Note: The green line represents the mean value and the grey lines the 5th and 95th percentile.

Table 5. Simulated concentrations for propylparaben for a combined exposure of 0.76 mg/kg bw/d via dermal route

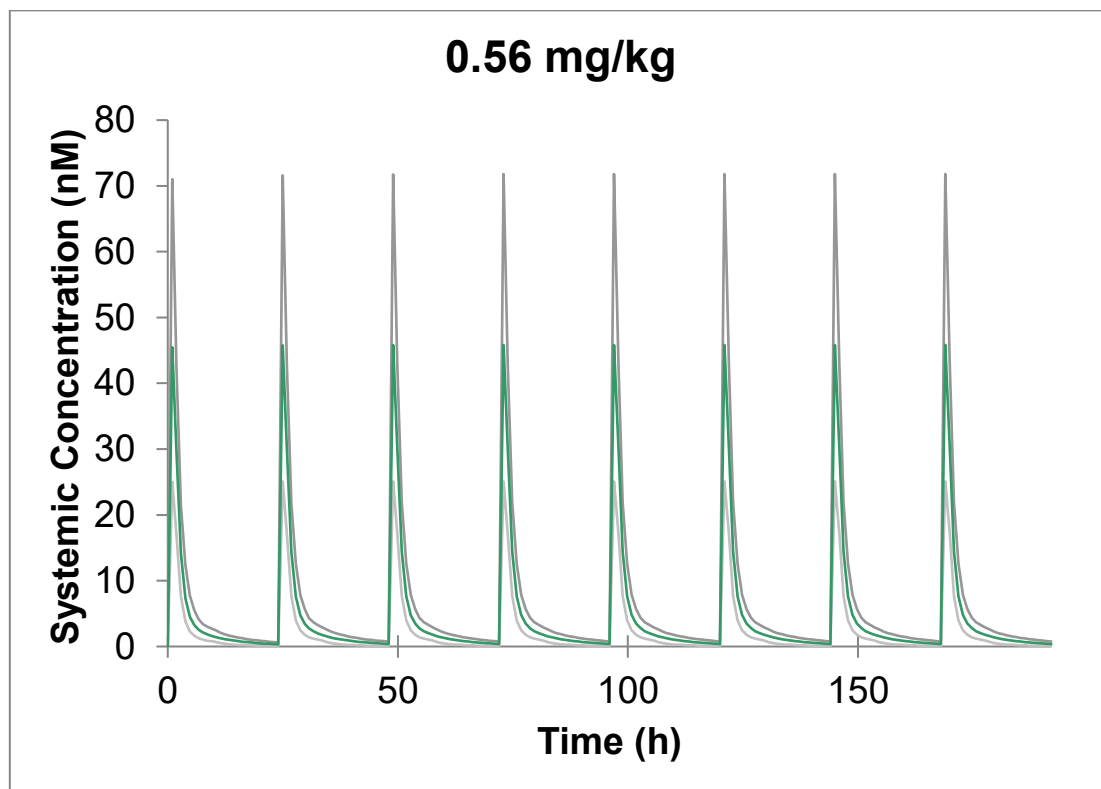
Metric	Geometric Mean	SD	5 th %ile	95 th %ile
C _{max} (nM)	61.7	18.9	36	97
AUC (nM*h)	158	48	88	237
C _{avg} (nM)	6.6	2.0	3.7	9.9

Considering a fraction unbound (F_u) of 0.083, the geometric mean free C_{max} concentration of propylparaben is 5 nM (61.7×0.083). This is the value used for the safety assessment in this IATA.

For information purpose the refined exposure estimates were lower and the simulated exposure at these exposure levels are the following:

- Dermal exposure at 0.56 mg/kg bw/d

Figure 4. Simulated concentrations for propylparaben for a combined exposure of 0.56 mg/kg bw/d via dermal route



Note: The green line represents the mean value and the grey lines the 5th and 95th percentile.

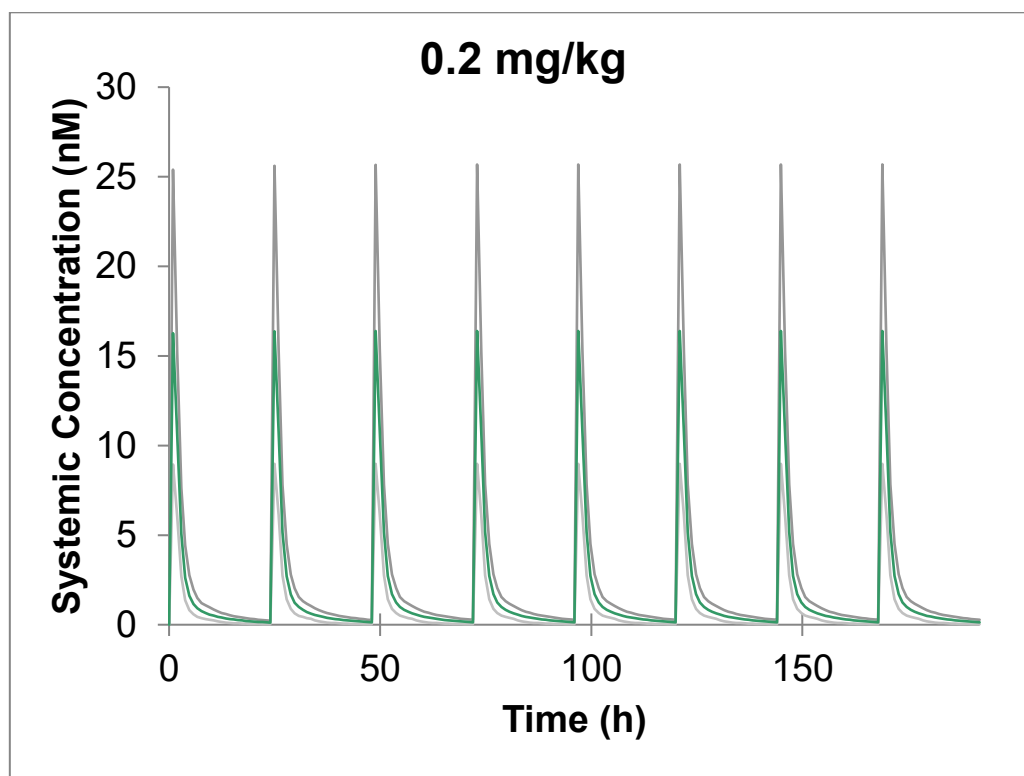
Table 6. Simulated concentrations for propylparaben for a combined exposure of 0.56 mg/kg bw/d via dermal route

Metric	Geometric Mean	SD	5 th %ile	95 th %ile
C _{max} (nM)	45.5	14.0	27	72
AUC (nM*h)	116	35	65	175
C _{avg} (nM)	4.9	1.5	2.7	7.3

The free C_{max} is 3.8 nM (45.5 x 0.083).

- Further refinement of exposure considering dermal (0.2 mg/kg) and oral (0.05 mg/kg):

Figure 5. Simulated concentrations for propylparaben for a dermal exposure of 0.2 mg/kg



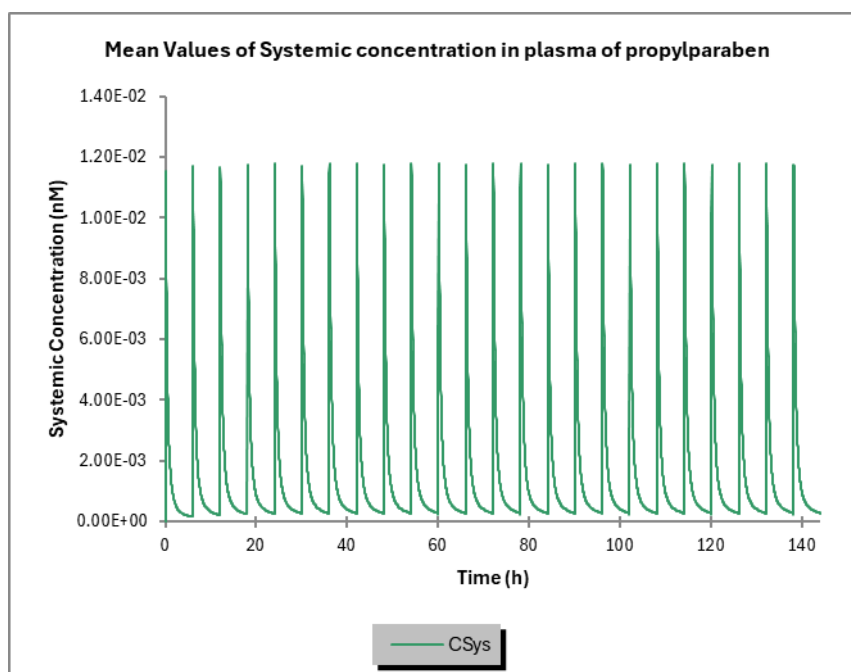
Note: The green line represents the mean value and the grey lines the 5th and 95th percentile.

Table 7. Simulated concentrations for propylparaben for a dermal exposure of (0.2 mg/kg)

Metric	Geometric Mean	SD	5 th %ile	95 th %ile
C _{max} (nM)	16.3	5	9.6	25.7
AUC (nM*h)	41.6	12.6	23	63
C _{avg} (nM)	1.7	0.5	0.97	2.61

The geometric free concentration of propylparaben is 1.35 nM.

Figure 6. Oral exposure after application of lipstick



Note: 0.01g per application containing 0.6 mg/g propylparabens) with a worse case assumption that all of the dose is swallowed

The mean C_{max} was 0.012 nM (free C_{max} = 0.00098 nM) after oral exposure.

A detailed description of the PBK modelling with all input parameters and the confidence level of the level's assessment is provided as an annex document.

7.4 Toxicodynamic data

Considering the information collected on the ADME/tk properties, in silico models relevant to systemic effects were used to evaluate propylparaben and its main metabolite para-hydroxybenzoic acid and the corresponding alcohol 1-propanol. The following in silico systems together with profilers (on ER) from the OECD QSAR Toolbox version 4.7 were used for the in silico evaluation:

- Precalculated predictions from the freely available Danish (Q)SAR Database and Models website (<https://qsar.food.dtu.dk>, version March 2025) developed, supported and hosted by the Technical University of Denmark (DTU).
- Predictions were generated in a few additional QSAR models developed by DTU in the RISK-HUNT3R project. Documentation (QMRFs) is not available yet, as manuscripts are in preparation for the models.

Another in silico tool that is still under development at RISK-HUNT3R, the Chemical Effect Predictor CEP – a decision tree built using a knowledge graph - was used to identify alerts on potential adverse effects.

The main in silico alerts identified are endocrine bioactivity (estrogenicity) and teratogenicity from the DART ontology in the OECD QSAR toolbox. Further information (ideally in vitro data) is needed to assess the relevance of these alerts.

The following tables (8-13) summarize the in silico data collected.

7.4.1 Endocrine activity existing data and predictions from models available in the Danish (Q)SAR Database / Models website and additional DTU models

Different targets related to endocrine activity were evaluated for propylparaben, para-hydroxybenzoic acid and the corresponding alcohol: estrogen, androgen, progesterone (via aromatase inhibition), thyroperoxidase, Sodium/iodide symporter (NIS), Thyroid Receptor α Binding, Thyroid Receptor β Binding, Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ), Arylhydrocarbon Receptor (AhR), Pregnane X Receptor (PXR), Constitutive Androstane Receptor (CAR). A number of the training sets have been modelled in three different QSAR development software systems which apply different complementary machine learning methods. Predictions are based on a battery call requiring at least two of the three predictions for a substance to be in domain and agree on the prediction.

Most of the (Q)SAR models (except some with manuscripts in preparation) are documented in the (Q)SAR Model Reporting Format (version from the software “QMRF editor”) and freely available on the website. A table with an overview of the statistical external performances as determined by robust cross-validation is furthermore available from the website, from the top menu point “Information”, sub-point “Model documentation”, (except for the VEGA reproductive toxicity model) see print-screen excerpts from this table below:

Pregnane X receptor binding (human in vitro)	631	Sens=72.4, Spec=83.9, Conc=78.5	CASE Ultra
		Sens=80.4, Spec=80.4, Conc=80.4	Leadscope
		Sens=79.9, Spec=82.7, Conc=81.4	SciQSAR
Pregnane X Receptor (PXR) Binding (Human in vitro) NEW	1504	Sens=86.6, Spec=98.5, Conc=92.6 (2*5-fold cross-validation)	Leadscope
Pregnane X Receptor (PXR) Activation (Human in vitro)	2176	Sens=89.1, Spec=98.6, BA=93.9 (2*5-fold cross-validation)	Leadscope
Pregnane X Receptor (PXR) Activation (Rat in vitro)	2330	Sens=86.5, Spec=97.4, BA=92.0 (2*5-fold cross-validation)	Leadscope
CYP3A4 Induction (Human in vitro)	2271	Sens=86.7, Spec=98.2, BA=92.5 (2*5-fold cross-validation)	Leadscope
Arylhydrocarbon (AhR) Activation – Rational final model	4625	Sens=75.4, Spec=87.0, BA=80.8 (2*5-fold cross-validation)	Leadscope
Arylhydrocarbon (AhR) Activation – Random final model	4625	Sens=86.4, Spec=91.5, BA=88.9 (2*5-fold cross-validation)	Leadscope
Constitutive Androstane Receptor (CAR) activation at max. 20 µM	924	Sens=72.2, Spec=93.5, BA=82.8 (2*5-fold cross-validation)	Leadscope
Constitutive Androstane Receptor (CAR) activation at max. 50 µM	1903	Sens=78.4, Spec=91.4, BA=84.9 (2*5-fold cross-validation)	Leadscope
Constitutive Androstane Receptor (CAR) inhibition at max. 20 µM	1408	Sens=58.4, Spec=97.1, BA=77.8 (2*5-fold cross-validation)	Leadscope
Constitutive Androstane Receptor (CAR) inhibition at max. 50 µM	1870	Sens=72.4, Spec=91.6, BA=82.0 (2*5-fold cross-validation)	Leadscope
Teratogenic potential in Humans	323	Sens=65.0, Spec=85.1, Conc=76.4	CASE Ultra
		Sens=72.0, Spec=85.5, Conc=80.1	Leadscope
		Sens=64.6, Spec=92.7, Conc=81.4	SciQSAR
Developmental/Reproductive Toxicity library (PG) v1.1.2	685	NA	VEGA

The performances of the models were considered when providing a call for the alerts.

Binding to the estrogen receptor as well as transactivation were predicted for the parent compound but not for the degradation products. Furthermore, the parent compound was part of the training sets for ER-related models as a positive and in AR-related models as a negative (see column marked 'Exp' in Table 8a).

Table 8a. Predictions for endocrine and Molecular Endpoints for propylparaben - excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human in vitro)	POS	POS_IN	POS_IN	POS_IN	NEG_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human in vitro)	POS	POS_IN	POS_IN	POS_IN	POS_IN
Estrogen Receptor α Activation (Human in vitro)	POS	POS_IN	POS_IN	POS_IN	POS_IN
Estrogen Receptor Activation, CERAPP data (in vitro)	POS	N/A	N/A	POS_IN	N/A
Estrogen Receptor α Activation (Human in vitro)		N/A	N/A		N/A
Estrogen Receptor β Activation at max. 20 μ M (Human in vitro)		N/A	N/A	NEG_IN	N/A
Estrogen Receptor β Activation at max. 50 μ M (Human in vitro)		N/A	N/A	POS_IN	N/A
Estrogen Receptor β Inhibition at max. 10 μ M (Human in vitro)		N/A	N/A	POS_IN	N/A
Estrogen Receptor β Inhibition at max. 50 μ M (Human in vitro)		N/A	N/A	POS_IN	N/A
Androgen Receptor Inhibition (Human in vitro)	NEG	NEG_IN	NEG_IN	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_OUT	N/A
Thyropoxidase (TPO) Inhibition QSAR1 (Rat in vitro)	NEG	N/A	N/A	INC_OUT	N/A
Thyropoxidase (TPO) Inhibition QSAR2 (Rat in vitro)	NEG	N/A	N/A	INC_OUT	N/A
Sodium/iodide Symporter (NIS) Inhibition, higher sensitivity (Human in vitro)	NEG	N/A	N/A	NEG_OUT	N/A
Sodium/iodide Symporter (NIS) Inhibition, higher specificity (Human in vitro)	NEG	N/A	N/A	NEG_OUT	N/A
Deiodinase type 1 (DIO1) Inhibition (Human in vitro)*	NEG			NEG_IN	
Deiodinase type 2 (DIO2) Inhibition (Human in vitro)*	NEG			NEG_IN	
Deiodinase type 3 (DIO3) Inhibition (Human in vitro)*	NEG			NEG_IN	

Thyroid Stimulating Hormone Receptor (TSHR) Inhibition (Human <i>in vitro</i>)*				NEG_IN	
Thyroid Receptor α Binding (Human <i>in vitro</i>)					
- mg/L			28825.64	472.7689	145.2041
- μ M			159955.8	2623.433	805.7493
- Positive for IC ₅₀ $\leq$ 10 μ M					
- Positive for IC ₅₀ $\leq$ 100 μ M					
- Domain		OUT	OUT	OUT	OUT
Thyroid Receptor β Binding (Human <i>in vitro</i>)					
- mg/L			5831.484	40.08934	301.4248
- μ M			32359.38	222.459	1672.631
- Positive for IC ₅₀ $\leq$ 10 μ M					
- Positive for IC ₅₀ $\leq$ 100 μ M					
- Domain		OUT	OUT	OUT	OUT
Aromatase Inhibition at max. 10 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Aromatase Inhibition at max. 50 μ M (Human <i>in vitro</i>)		N/A	N/A	INC_OUT	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Activation at max. 20 μ M (Human <i>in vitro</i>)		N/A	N/A	INC_OUT	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Activation at max. 50 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Inhibition at max. 10 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Inhibition at any concentration (Human <i>in vitro</i>)		N/A	N/A	POS_IN	N/A
Retinoic Acid Receptor (RAR) Inhibition at max. 10 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Retinoic Acid Receptor (RAR) Inhibition at any concentration (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A

Hedgehog (HH) pathway Inhibition at max. 10 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Hedgehog (HH) pathway Inhibition at max. 50 µM (Human <i>in vitro</i>)		N/A	N/A	POS_OUT	N/A
Arylhydrocarbon Receptor (AhR) Activation, Rational final model (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation, Random final model (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Binding (Human <i>in vitro</i>)	N/A	NEG_IN	NEG_IN	NEG_IN	NEG_IN
Pregnane X Receptor (PXR) Binding (Human <i>in vitro</i>) NEW	NEG	N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Rat <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
CYP3A4 Induction (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A

Note: DTU-developed models

Table 9b. OECD QSAR Toolbox v.4.2 profilers' predictions – Propyl paraben, excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

Estrogen Receptor Binding, alerts in:	
- parent only	Moderate binder, OH group
- metabolites from <i>in vivo</i> Rat metabolism simulator only	Weak binder, OH group
- metabolites from Rat liver S9 metabolism simulator only	Weak binder, OH group
rtER Expert System - USEPA, alerts in:	
- parent only	Parabens
- metabolites from <i>in vivo</i> Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found

Note: Profiler predictions are supporting information to be used together with the relevant QSAR predictions.

Table 10a. Endocrine and Molecular Endpoints – para-hydroxybenzoic acid, excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

	Exp	Battery	CASEUltra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human in vitro)	NEG	NEG_IN	NEG_IN	NEG_OUT	NEG_IN
Estrogen Receptor α Binding, Balanced Training Set (Human in vitro)		POS_IN	POS_IN	POS_IN	NEG_IN
Estrogen Receptor α Activation (Human in vitro)		INC_OUT	POS_IN	POS_OUT	NEG_IN
Estrogen Receptor Activation, CERAPP data (in vitro)	NEG	N/A	N/A	INC_OUT	N/A
Estrogen Receptor α Activation (Human in vitro)		N/A	N/A		N/A
Estrogen Receptor β Activation at max. 20 μ M (Human in vitro)		N/A	N/A	NEG_IN	N/A
Estrogen Receptor β Activation at max. 50 μ M (Human in vitro)		N/A	N/A	POS_OUT	N/A
Estrogen Receptor β Inhibition at max. 10 μ M (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Estrogen Receptor β Inhibition at max. 50 μ M (Human in vitro)	NEG	N/A	N/A	NEG_OUT	N/A
Androgen Receptor Inhibition (Human in vitro)	NEG	NEG_IN	NEG_IN	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Thyropoxidase (TPO) Inhibition QSAR1 (Rat in vitro)		N/A	N/A	POS_IN	N/A
Thyropoxidase (TPO) Inhibition QSAR2 (Rat in vitro)	POS	N/A	N/A	POS_IN	N/A
Sodium/iodide Symporter (NIS) Inhibition, higher sensitivity (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Sodium/iodide Symporter (NIS) Inhibition, higher specificity (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Deiodinase type 1 (DIO1) Inhibition (Human in vitro)*				NEG_IN	
Deiodinase type 2 (DIO2) Inhibition (Human in vitro)*	NEG			NEG_IN	
Deiodinase type 3 (DIO3) Inhibition (Human in vitro)*				NEG_IN	
Thyroid Stimulating Hormone Receptor (TSHR) Inhibition (Human in vitro)*				NEG_IN	

Thyroid Receptor α Binding (Human *in vitro*)

- mg/L		11116.04	22093.09	215.1773	138.9814
- μ M		80481.02	159955.8	1557.901	1006.237
- Positive for $IC_{50} \leq 10 \mu$ M					
- Positive for $IC_{50} \leq 100 \mu$ M					
- Domain		IN	IN	OUT	IN

Thyroid Receptor β Binding (Human *in vitro*)

- mg/L		2837.197	4469.478	63.25671	1204.916
- μ M		20541.54	32359.38	457.9837	8723.687
- Positive for $IC_{50} \leq 10 \mu$ M					
- Positive for $IC_{50} \leq 100 \mu$ M					
- Domain		IN	IN	OUT	IN

Aromatase Inhibition at max. 10 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Aromatase Inhibition at max. 50 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Activation at max. 20 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Activation at max. 50 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Inhibition at max. 10 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Inhibition at any concentration (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Retinoic Acid Receptor (RAR) Inhibition at max. 10 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Retinoic Acid Receptor (RAR) Inhibition at any concentration (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Hedgehog (HH) pathway Inhibition at max. 10 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Hedgehog (HH) pathway Inhibition at max. 50 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation, Rational final model (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation, Random final model (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Binding (Human <i>in vitro</i>)	N/A	NEG_IN	NEG_IN	NEG_IN	NEG_IN
Pregnane X Receptor (PXR) Binding (Human <i>in vitro</i>) NEW		N/A	N/A	NEG_IN	N/A

Pregnane X Receptor (PXR) Activation (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Rat <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
CYP3A4 Induction (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A

Note: DTU-developed models

Table 11b. OECD QSAR Toolbox v.4.2 profilers - para-hydroxybenzoic acid, excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

Estrogen Receptor Binding, alerts in:	
- parent only	Weak binder, OH group
- metabolites from <i>in vivo</i> Rat metabolism simulator only	
- metabolites from Rat liver S9 metabolism simulator only	Weak binder, OH group
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from <i>in vivo</i> Rat metabolism simulator only	
- metabolites from Rat liver S9 metabolism simulator only	No alert found

Note: Profiler predictions are supporting information to be used together with the relevant QSAR predictions.

Table 12a. Endocrine and Molecular Endpoints – propyl alcohol, excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human in vitro)		NEG_IN	NEG_IN	NEG_OUT	NEG_IN
Estrogen Receptor α Binding, Balanced Training Set (Human in vitro)		NEG_IN	NEG_IN	NEG_OUT	NEG_IN
Estrogen Receptor α Activation (Human in vitro)		NEG_OUT	NEG_IN	NEG_OUT	NEG_OUT
Estrogen Receptor Activation, CERAPP data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Estrogen Receptor α Activation (Human in vitro)		N/A	N/A		N/A
Estrogen Receptor β Activation at max. 20 μ M (Human in vitro)		N/A	N/A	NEG_IN	N/A
Estrogen Receptor β Activation at max. 50 μ M (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Estrogen Receptor β Inhibition at max. 10 μ M (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Estrogen Receptor β Inhibition at max. 50 μ M (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human in vitro)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) Inhibition QSAR1 (Rat in vitro)		N/A	N/A	NEG_OUT	N/A
Thyroperoxidase (TPO) Inhibition QSAR2 (Rat in vitro)	NEG	N/A	N/A	NEG_OUT	N/A
Sodium/iodide Symporter (NIS) Inhibition, higher sensitivity (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Sodium/iodide Symporter (NIS) Inhibition, higher specificity (Human in vitro)	NEG	N/A	N/A	NEG_IN	N/A
Deiodinase type 1 (DIO1) Inhibition (Human in vitro)*	NEG			NEG_IN	
Deiodinase type 2 (DIO2) Inhibition (Human in vitro)*	NEG			NEG_IN	
Deiodinase type 3 (DIO3) Inhibition (Human in vitro)*	NEG			NEG_IN	
Thyroid Stimulating Hormone Receptor (TSHR) Inhibition (Human in vitro)*				NEG_IN	

Thyroid Receptor α Binding (Human *in vitro*)

- mg/L		4845.832	9613.344	949.3891	78.32032
- μ M		80629.48	159955.8	15796.82	1303.167
- Positive for $IC_{50} \leq 10 \mu$ M					
- Positive for $IC_{50} \leq 100 \mu$ M					
- Domain		IN	IN	OUT	IN

Thyroid Receptor β Binding (Human *in vitro*)

- mg/L		1128.012	1944.799	25.40179	311.225
- μ M		18768.92	32359.38	422.6588	5178.453
- Positive for $IC_{50} \leq 10 \mu$ M					
- Positive for $IC_{50} \leq 100 \mu$ M					
- Domain		IN	IN	OUT	IN

Aromatase Inhibition at max. 10 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Aromatase Inhibition at max. 50 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Activation at max. 20 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Activation at max. 50 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Inhibition at max. 10 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Peroxisome Proliferator-Activated Receptor gamma (PPAR- γ) Inhibition at any concentration (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Retinoic Acid Receptor (RAR) Inhibition at max. 10 μ M (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Retinoic Acid Receptor (RAR) Inhibition at any concentration (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Hedgehog (HH) pathway Inhibition at max. 10 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Hedgehog (HH) pathway Inhibition at max. 50 μ M (Human <i>in vitro</i>)	NEG	N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation, Rational final model (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Arylhydrocarbon Receptor (AhR) Activation, Random final model (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Binding (Human <i>in vitro</i>)	N/A	NEG_IN	NEG_IN	NEG_IN	NEG_IN
Pregnane X Receptor (PXR) Binding (Human <i>in vitro</i>) NEW		N/A	N/A	NEG_IN	N/A

Pregnane X Receptor (PXR) Activation (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Rat <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
CYP3A4 Induction (Human <i>in vitro</i>)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (Human <i>in vitro</i>)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (Human <i>in vitro</i>)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (Human <i>in vitro</i>)		N/A	N/A	NEG_IN	N/A

Note: DTU-developed models

Table 13b. Endocrine and Molecular Endpoints – propyl alcohol, excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, non cyclic structure
- metabolites from <i>in vivo</i> Rat metabolism simulator only	Non binder, non cyclic structure
- metabolites from Rat liver S9 metabolism simulator only	Non binder, non cyclic structure
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from <i>in vivo</i> Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found

Note: Profiler predictions are supporting information to be used together with the relevant QSAR predictions.

Different models were used to evaluate the developmental and reproductive toxicity of propylparaben, para-hydroxybenzoic acid and propyl alcohol.

One positive alert was evidenced for propylparaben and para-hydroxybenzoic acid with the Case ultra model. For propyl alcohol, a positive outcome for an experimental study was found in VEGA (Table 13b).

Table 14a. Teratogenicity alerts – propylparaben

	Battery	CASE Ultra	Leadscope	SciQSAR
Teratogenic Potential in Humans	INC_OUT	POS_IN	NEG_IN	NEG_IN

DTU-developed models based on commercial training set.

Note: Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database

PG from VEGA software predicts developmental and reproductive toxicity of compounds.

Table 15b. Teratogenicity alerts – propylparaben

.	Exp	Prediction_Domain
Developmental/Reproductive Toxicity library (PG) v1.1.2		POS_mod_OUT

VEGA model

Note: Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database.

Table 16a. Teratogenicity alerts – para-hydroxybenzoic acid

	Battery	CASE Ultra	Leadscope	SciQSAR
Teratogenic Potential in Humans	INC_OUT	POS_IN	INC_OUT	NEG_IN

DTU-developed models based on commercial training set.

Note: Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database.

Table 17b. Developmental/reproductive toxicity alerts - para-hydroxybenzoic acid

.	Exp	Prediction_Domain
Developmental/Reproductive Toxicity library (PG) v1.1.2		NEG_low_OUT

VEGA model

Note: Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database.

From the DART scheme profiler in the OECD QSAR toolbox (V 4.7.1) this compound is predicted as " Not Known precedent for reproductive and developmental toxic potential.

Table 18a. Teratogenicity alerts – propyl alcohol

	Battery	CASE Ultra	Leadscope	SciQSAR
Teratogenic Potential in Humans	NEG_IN	NEG_IN	POS_OUT	NEG_IN

DTU-developed models based on commercial training set.

Note: Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database.

Table 19b. Developmental/reproductive toxicity alerts – propyl alcohol

Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database.

.	Exp	Prediction_Domain
Developmental/Reproductive Toxicity library (PG) v1.1.2	POS	POS_NA

VEGA model

Note: Excerpt from the downloadable prediction file from the freely available Danish (Q)SAR Database.

Profiler-type of predictions to be used as supporting information together with relevant QSAR predictions

Evaluation of estrogenicity, androgenicity, effects on thyroid, steroidogenesis and retinoid receptors-EATS-R- and reproductive toxicity predictions from the Danish (Q)SAR Database

Propylparaben:

E: Positive predictions in domain for ER alpha binding and activation, CERAPP activation and ER beta activation (based on alerts for lower potency actives). No negative predictions in domain. Alerts for parent found in both QSAR Toolbox ER-related profilers (predicted to be a moderate binder). Included as positive in training sets for ER alpha binding and activation models and CERAPP activation model. Overall positive Result for ER-related activation activity.

A: Negative predictions in domain for AR binding and inhibition models. No positive predictions. Included as negative in training sets for AR binding and inhibition models. Overall negative Result for AR-related inhibition activity in relation to the endpoints/models covered.

T: No predictions in domain for TPO or NIS inhibition. The substance is included in the training sets as a negative. Negative predictions in domain for DIO1, DIO and DIO3 inhibition. The substance is included in the training sets as a negative. Negative prediction in domain for TSHR inhibition. No predictions in domain for TR alpha and beta binding. Overall negative Result for T-related activity in relation to the endpoints/models covered.

S: Negative prediction in domain for aromatase 25% inhibition at max. 10 µM. The substance is included in the training set as a negative. Negative prediction for PPAR-γ 25% activation at max. 50 µM. The substance is included in the training set as a negative. Negative prediction for PPAR-γ inhibition based on positive class with 25% absolute inhibition at max. 10 µM, and positive prediction for PPAR-γ inhibition based on positive class with 25% absolute inhibition at max. 50 µM. Overall mixed Result for S-related activity in relation to the endpoints/models covered.

R: Negative prediction in domain for RAR inhibition. Overall negative Result for T-related activity in relation to the endpoints/models covered.

Reproductive toxicity: No prediction in domain could be obtained in the battery call for teratogenicity due to conflict in the underlying three models. No prediction in domain could be obtained in the Reproductive/developmental toxicity profiler-type VEGA model. No overall "Result" could be obtained.

Para-hydroxybenzoic acid:

E: Positive predictions in domain for ER alpha binding based on balanced training set and negative prediction for ER alpha binding based on full training set. The substance is included as a negative in the full training set. No battery prediction in domain for ER alpha could be obtained due to conflicting predictions in the underlying models. No prediction in domain could be obtained for CERAPP activation. The substance is included in the model as a negative. Negative prediction in domain for ER beta activation at max. 20 µM based on alerts for higher potency actives, and no prediction in domain for ER beta activation based on alerts also for lower potency actives. Alert for parent found in one out of two QSAR Toolbox ER-related profilers (predicted to be weak binder). Overall mostly negative / uncertain Result for ER-related activation activity in relation to the endpoints/models covered.

A: Negative predictions in domain for AR binding and inhibition models. No positive predictions. Included as negative in training sets for AR binding and inhibition models. Overall negative Result for AR-related inhibition activity the endpoints/models covered.

T: Positive predictions in domain for TPO inhibition. The substance is included as a positive in the full model (QSAR2). Negative predictions in domain for NIS inhibition. The substance is included in the training

sets as a negative. Negative predictions in domain for DIO1, DIO and DIO3 inhibition. The substance is included in the training sets as a negative for DIO2. Negative prediction in domain for TSHR inhibition. Positive predictions in domain for TR alpha and beta binding, however the potency is predicted to be very low, i.e. $IC_{50} > 100 \mu M$. Overall positive Result for T-related activity based on the predictions in the TPO inhibition models.

S: Negative prediction in domain in models for aromatase inhibition. The substance is included in the training sets as a negative. Negative predictions for PPAR- γ activation and inhibition. The substance is included in the training sets as a negative. Overall negative Result for S-related activity in relation to the endpoints/models covered.

R: Negative predictions in domain for RAR inhibition. Overall negative Result for T-related activity in relation to the endpoints/models covered.

Reproductive toxicity: No prediction in domain could be obtained in the battery call for teratogenicity due to conflict in the underlying models. No prediction in domain could be obtained in the Reproductive/developmental toxicity profiler-type VEGA model. No overall "Result" could be obtained.

Propyl alcohol:

E: Negative predictions in domain for ER alpha binding. No battery call in domain could be obtained for ER alpha activation due to two of the underlying three models being out of domain, however the third model gave a negative prediction in domain. Negative prediction in domain for CERAPP activation. The substance is included in the model as a negative. Negative prediction in domain for ER beta activation. The substance is included as a negative in the model for ER beta activation at max. $50 \mu M$. No alerts for activity in the two QSAR Toolbox ER-related profilers (predicted to be non-binder). Overall negative Result for ER-related activation activity.

A: Negative predictions in domain for AR binding and inhibition models. No positive predictions. Included as negative in CoMPARA training sets for AR binding and inhibition models. Overall negative Result for AR-related inhibition activity in relation to the endpoints/models covered.

T: No predictions in domain could be obtained for TPO inhibition. The substance is included as a negative in the full model (QSAR2). Negative predictions in domain for NIS inhibition. The substance is included in the training sets as a negative. Negative predictions in domain for DIO1, DIO and DIO3 inhibition. The substance is included in the training sets as a negative for the three DIO models. Negative prediction in domain for TSHR inhibition. Positive predictions in domain for TR alpha and beta binding, however the potency is predicted to be very low, i.e. $IC_{50} > 100 \mu M$. Overall negative or very weak Result for T-related activity in relation to the models / endpoints covered.

S: Negative predictions in domain in models for aromatase inhibition. The substance is included in the training sets as a negative. Negative predictions for PPAR- γ activation and inhibition. The substance is included in the training set as a negative for one of the PPAR- γ inhibition models. Overall negative Result for S-related activity in relation to the endpoints/models covered.

R: Negative predictions in domain for RAR inhibition. The substance is included in the training set as a negative for one of the RAR models. Overall negative Result for T-related activity in relation to the endpoints/models covered.

Reproductive toxicity: Negative prediction in domain in the battery model for teratogenicity in Human based on negative predictions in domain for two of the three underlying models, with the prediction from the third model being out of domain. Positive prediction (no domain call for this model) in the Reproductive/developmental toxicity profiler-type VEGA model (which has high sensitivity and low specificity, i.e. in general giving many false positives). The substance is included in the training set as a positive. Overall positive "result" based on the prediction and training set value in the model for reproductive/developmental toxicity.

7.4.2 Chemical Effect Predictor - CEP

The CEP is a machine learning tool that uses a knowledge graph (KG) to predict potential health effects of chemicals. The KG links drugs/chemicals, proteins, biological functions, and diseases.

For propylparaben, two approaches were used:

- **Data-driven approach:** This approach uses available data on propylparaben interactions with proteins retrieved in resources like ChEMBL (<https://www.ebi.ac.uk/chembl/>), Papyrus, CTD (<https://ctdbase.org/>), the comparative toxicogenomic database (CTD <https://ctdbase.org/>) and CALUX data. Four different combinations of proteins were considered here: ChEMBL original filter (**ALDH1A1 – Propylparaben**), ChEMBL loose filter (**ALDH1A1, AR, ESR1, PPARG Propylparaben_1**), ChEMBL + Papyrus + CTD (**AHR, ALDH1A1, AR, CYP19A1, ESR1, ESR2, NR1I2, PPARG, SHBG, THRA, THRB – Propylparaben_2**) and CALUX (**ESR1, AR, TTR - Propylparaben_4**)
- **Ab initio assessment:** where there is limited/no data available, this approach uses predicted protein interactions leveraging data from structurally related compounds. The **ToxProfiler** tool (<https://toxpro.bhsai.org/login>) provided three receptors interacting with propylparaben: **ESR1, ESR2, PPARG**. This target combination is referred to as **Propylparaben_3**.

The enrichment analysis in different disease classes (Disease Set Enrichment Analysis - DSEA) highlighted **Endocrine disorders and Reproductive and Breast disorders** are the top features.

A detailed description of CEP as well as the evaluation performed for propylparaben is provided as an annex document.

7.5 In vitro data

Based on the in silico alerts identified, follow-up is needed on endocrine bioactivity (especially estrogenicity) and teratogenicity. However, to cast a broad net on the biological applicability domain (and given the limited biological coverage of the in silico tools used), assays addressing biological processes at large scale like transcriptomics and cell painting were considered.

The in vitro assays were collected from the literature (ToxCast) and from the Cosmetics' Europe LRSS initiative.

Types of assays from ToxCast considered: in vitro pharmacology profiling, cell stress, transcriptomics, cell painting, assays on stem cells and zebrafish embryo assay. The transcriptomics data were obtained from 3 cell types (MCF7 cells which possess nuclear receptors for estrogens, HepaRG cells that have some biotransformation capacity and U2OS cells) at full genome level. U2OS cells were also used to monitor several morphological features in the cell painting assay. In the ToxCast set of assays, receptor binding, enzyme inhibition, co-culture assays monitoring inflammation, cell stress assays related to organelles' health as well as assays in zebrafish embryos were considered. The latter is informative of teratogenicity while the others do cover biological processes broadly at molecular and/or cellular level, all relevant for this case study.

Data from LRSS: CALUX assays from BDS.

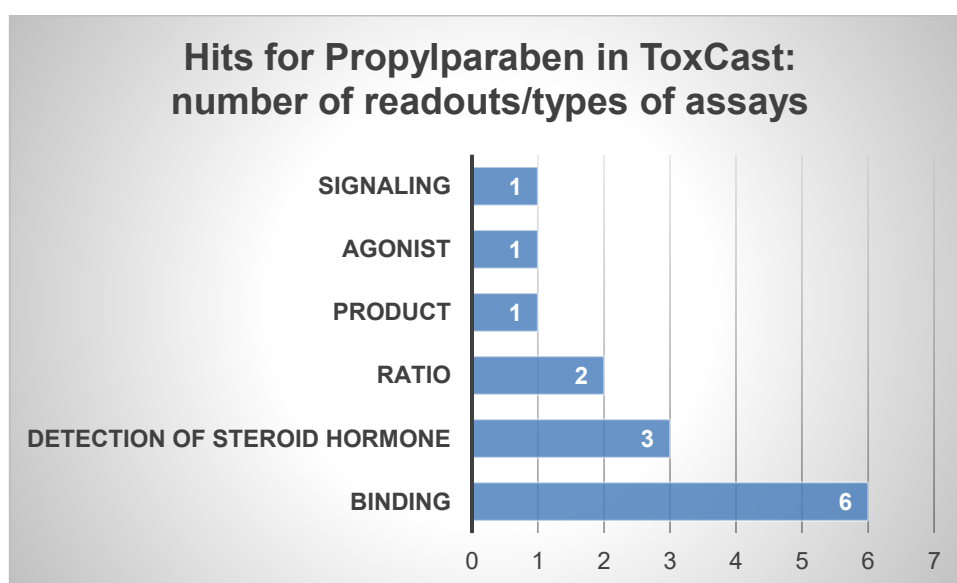
7.5.1 ToxCast data

Propylparaben was tested in 656 assays and 14 had an outcome showing there was a hit after discarding those where flags (on potential confounders) could be identified. Hits are those assays where biological

activity was confirmed after verifying there was no interference in the readouts and that the concentrations considered were not cytotoxic. Confounders like PoDs detected at cytotoxic concentrations were excluded. Based on these assays, propylparaben had a 2.13% hit rate.

Most of the active hits are related to endocrine bioactivity (mostly estrogenicity) from the signaling, gene reporter, binding, agonist assays and the detection of steroid hormones. A flag on teratogenicity in zebrafish embryos from the assays performed in Tanguy's and Padilla's laboratories was dropped due to flags for potential confounders (effect observed at cytotoxic concentrations) (assay described in Feshuk et al., 2024 EPA Publication Number 601B24001 on TOXICITY FORECASTER (TOXCAST) ASSAY DESCRIPTION DOCUMENTATION).

Figure 7. Summary of hits in ToxCast assays for propylparaben



These assays confirm the alerts identified in the in silico alerts on ER activity for the ester. All ToxCast assays are described according to the OECD guidance document 211. The description of the assays can be retrieved in the following locations:

<https://clowder.edap-cluster.com/files/66e2e177e4b0a7c65d2f7a8b>

<https://doi.org/10.23645/epacomptox.6328037.v11>

Para-hydroxybenzoic acid, and 1-propanol trigger a single hit respectively on the thyroid peroxidase activity (TPO) at the highest concentration and on the PXR receptor. The activity on TPO was not confirmed in the CALUX assay. Both alerts given their isolated nature are of low priority for follow-up.

These results show that the bioactivity is led mostly by the parent compound and not by the degradation products.

The benchmark dose for hits with propylparaben ranged from 2.69 μ M (ER signaling in T47D cells) to 30.03 μ M (antagonism of progesterone receptor in HEK293T cells) (Table 14).

Table 20. Summary of hits for propylparaben in ToxCast assays

NAME	BMD	ASSAY_FUNCTION_TYPE
ACEA_ER_80hr	2,6943506	signaling
CEETOX_H295R_OHPREG	2,99434184	detection of steroid hormone
CEETOX_H295R_PROG	3,25357704	detection of steroid hormone
CEETOX_H295R_OHPROG	3,35652986	detection of steroid hormone
OT_ER_ERaERa_0480	3,59988206	binding
OT_ER_ERaERa_1440	4,02849467	binding
OT_ER_ERaERb_0480	4,88688272	binding
OT_ER_ERaERb_1440	5,43008494	binding
OT_ER_ERbERb_0480	10,7963135	binding
OT_ER_ERbERb_1440	14,6593725	binding
TOX21_ERa_BLA_Agonist_ch2	18,2621947	product
TOX21_ERa_BLA_Agonist_ratio	21,2068811	ratio
TOX21_ERa_LUC_VM7_Agonist	27,9032937	agonist
TOX21_PR_BLA_Antagonist_ratio	30,033127	ratio

The cell painting data were generated by measuring several cellular features in U2OS cells. No activity was observed for propylparaben nor for p-hydroxybenzoic acid or 2-propanol.

Toxicogenomics data were also generated by the US EPA covering full genome by TempoSeq (BioSpider) on HepaRG, MCF7 and U2OS cells.

For the gene transcription in HepaRG cells, eight concentrations ranging from 0.03 to 100uM were evaluated. Only 5 genes were significantly regulated with benchmark doses ranging from 1.43 to 90.90 uM at gene level.

In tempoSeq full genome transcription assays performed on MCF7 (6h incubation) and U2OS cells (24h incubation), no differentially regulated gene was observed (data not shown).

Overall, the data retrieved in ToxCast shows low activity (ER especially) occurring in vitro.

7.5.2 CALUX data

The CALUX (Chemically Activated Luciferase gene eXpression) battery is a set of bioassays used for evaluating different nuclear receptors.

“In investigating potential MoAs for reproductive toxicity, an obvious consideration is [steroid hormones](#) and their receptors, particularly the androgen and oestrogen receptors. These receptors can be modulated in their activity by synthetic chemicals and other [xenobiotics](#), as well as by endogenous molecules. Based on this notion, the low binding alerts and binding activity of the parabens observed in the *in silico* profilers and the bioactivity (ToxCast) data already gathered, specific CALUX® transactivation assays ([OECD 2016](#)) were selected to examine the similarities and differences in the endocrine activity of parabens. As endocrine activities represent molecular initiating events rather than more downstream key events in some reproductive toxicity adverse outcome pathways (AOPs), evaluating endocrine activity is a way to survey many potential AOPs simultaneously. As such, interaction with receptors for oestrogen-, androgen-, thyroid signalling and steroidogenesis (EATS) are relevant to potential MoAs based on *in vitro* endocrine activity.

A range of CALUX assays, complemented with specific assays to measure thyroid- and steroidogenesis interferences, was selected to create a complete EATS panel in which the parabens were evaluated.” (adapted from Ouedraogo et al, 2022)

Transactivation triggered by propylparaben was observed in the estrogenic gene transactivation assay. The activity decreased in presence of S9 where only phase I cofactors were present. No activity was observed for para-hydroxybenzoic acid (cf table 15).

The endocrine bioactivity is driven by the ester. Here, only phase I enzymes were used in the CALUX assay. In realistic exposure conditions, phase II enzymes are also present. Given the cleavage of propylparaben by carboxylesterases, the likelihood of triggering this molecular initiating event in a realistic exposure condition is low.

Table 21. CALUX assay on propylparaben and para-hydroxybenzoic acid: Summary of EATS testing results

End point	PP		pHBA	
	-S9	+S9	-S9	+S9
Cytotoxicity	-3.5	>	>	>
(Anti-) estrogenic and (anti-) androgenic assays				
ER α CALUX	-6.0	-5.1	>	>
anti-ER α	>	>	>	>
CALUX	>	>	>	>
AR CALUX	>	>	>	>
anti-AR CALUX	-4.5	-4.2	>	>
Thyroidogenic assays				
TR β CALUX	>	>	>	>
anti-TR β	>	>	>	>
CALUX	>	>	>	>
TTR	-4.5	nd	>	nd
hTPO	>	nd	-3.0	nd
Steroidogenesis				
H295R-E2	-5.0	nd	-3.0	nd
H295R-T	-4.0	nd	>	nd

Note: PC10 (for agonistic tests)/PC20 (for antagonistic tests) values are shown in -Log M; the color indicates the potency (yellow < orange < red). PP = propylparaben, pHBA= para-hydroxybenzoic acid.

“Incubations with S9 decreased bioactivity in the CALUX EATS panel. This is consistent with the fact that the major metabolite, pHBA, is devoid of significant biological activity and only shows slight activity in the TPO- and H295R assay at millimolar concentrations. Propylparaben had estrogenic and anti-androgenic activity *in vitro*. Little direct effect on thyroid receptor signalling and hTPO inhibition was observed but TTR binding was found positive and some able to steroid production was observed according to the H295R assay. The parent paraben exhibited measurable activity as agonist in the oestrogen receptor assay when tested at high concentrations, while being antagonists in the AR assay at high concentrations. This linked activity has been noted before in other endocrine active substances. While it can be argued that anti-androgenic activity in some cases may contribute to the oestrogenicity of a substance *in vivo*, in the case of the short linear chain parabens the anti-androgenic activity observed in the EATS panel is of comparatively low potency relative to the observed oestrogenic activity. Both the oestrogenic and anti-androgenic effect of the propylparaben decreased significantly in the presence of rat liver S9, suggesting that it is readily metabolised to inactive metabolites. The results from the EATS assays, with and without metabolic activity, supported the earlier findings of ER activity from *in silico* alerts and ToxCast data, where for the

latter differences are greater in the absence of metabolism. However, it has to be emphasised that in all EATS assays, parabens are many orders of magnitude less potent compared to the natural oestrogen 17 β -estradiol ([Golden et al., 2005](#)).” (adapted from From Ouédraogo et al., 2022)

Overall, the toxicodynamic data collected on propylparaben and its degradation products point to low bioactivity (estrogenic activity).

7.6 Data integration and risk characterization

The previous sections on exposure and ADME/tk provide information on how the data were integrated. The in silico and the in vitro tools used were chosen to cover molecular and cellular processes relevant for systemic toxicity and DART. The assessment is meant to derive points of departure in order to identify the margin of safety.

For the toxicodynamic part, in silico alerts were evaluated together with in vitro data in a weight of evidence approach. In this IATA, existing in vitro data were leveraged for the assessment.

Starting with the in silico tools, alerts on endocrine activity and DART were identified.

Given the limited biological coverage of the in silico tools (nuclear receptors, DART), toxicogenomics data as well as ToxCast data were analyzed in order to follow up the in silico alerts and to expand the biological coverage. Alerts related to estrogenicity were identified in the ToxCast assays. However, given the limited biotransformation capacity of the cell types used in the assays related to endocrine activity in ToxCast, assays using metabolic transformation on nuclear receptors related to ER, AR, steroidogenesis and thyroid hormone transport were performed. These assays highlighted the role of the ester in driving the estrogenicity (CALUX assays).

Given that propylparaben is readily cleaved by esterases, the likelihood of a remaining amount of ester triggering a cascade of events leading to potential adverse events is low.

For alerts related to DART, data on zebrafish embryos were retrieved in ToxCast for propylparaben. The measured data were flagged, showing they were not reliable. Zebrafish embryo assay data were not available for para-hydroxybenzoic acid and 1-propanol. There is some uncertainty related to the ability of zebrafish embryos to fully metabolize propylparaben.

Overall, the toxicodynamic data point to potential residual estrogenicity for propylparaben.

The estimated internal concentrations were compared with the points of departure derived from the in vitro bioactivity.

The bioactivity exposure ratios were calculated using the reliable bioactivity data (related to ER activity mainly, cf table 15). $BER = BMD \text{ assay} / C_{max}$. Different values of C_{max} (from Table 5) were used for calculating the BERs: the free C_{max} value, C_{max} geometric mean, C_{max} 5th percentile and C_{max} 95th percentile.

The bioactivity – exposure ratios -BERs- observed using the ToxCast data for propylparaben ranged from 27.77 to 6006.62 considering different metrics for the internal concentrations (free C_{max} , geometric mean of C_{max} , 5th or 95th percentile for C_{max}).

The following figures recapitulate the calculated BERs:

Figure 8. Bioactivity exposure ratios for propylparaben using free Cmax

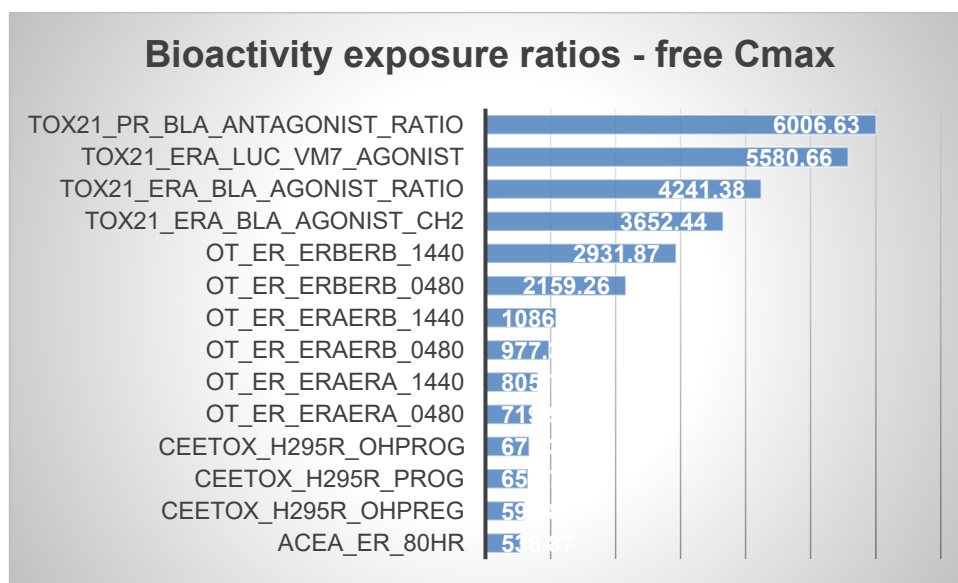


Figure 9. Bioactivity exposure ratios for propylparaben using the geometric mean of the Cmax

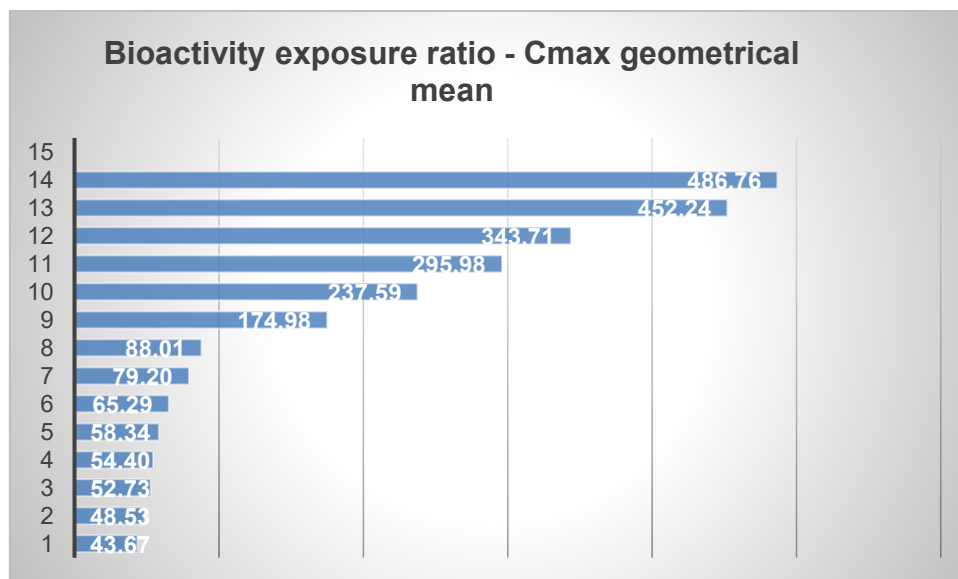


Figure 10. Bioactivity exposure ratios for propylparaben using the 5th percentile of the Cmax

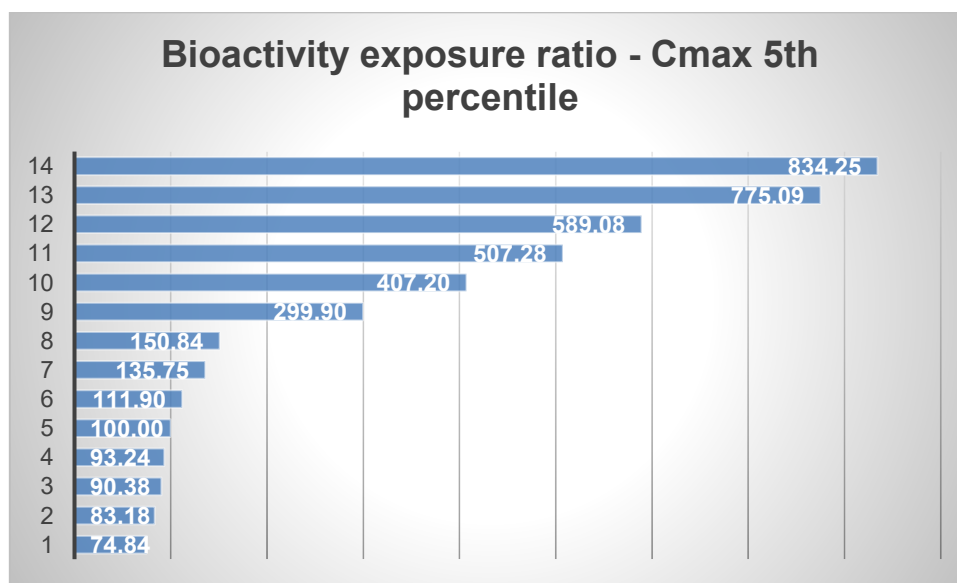
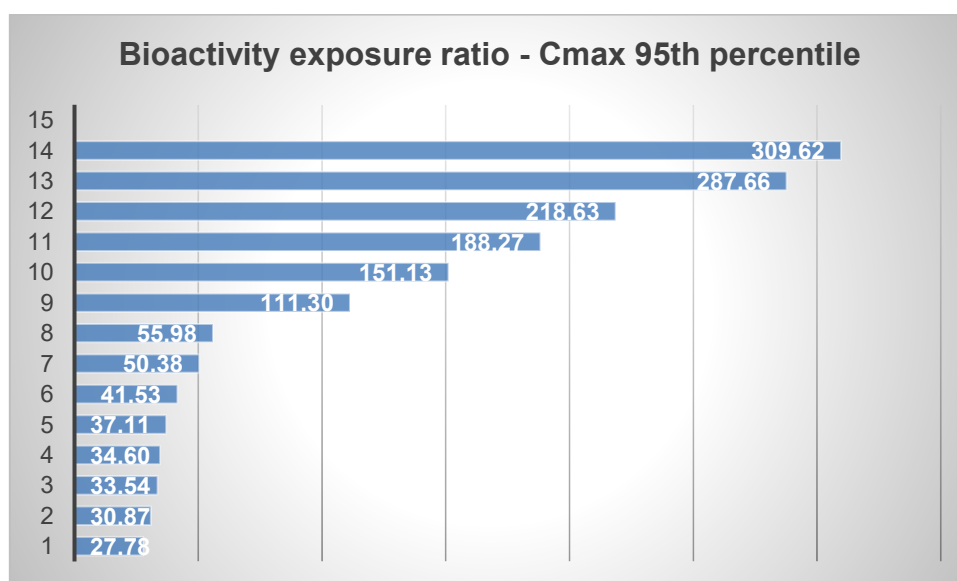


Figure 11. Bioactivity exposure ratios for propylparaben using the 95th percentile of the Cmax



All bioactivity exposure ratios are above 1, meaning that in vitro activity occurs at concentrations far beyond the estimated exposure to consumers. Given that these are processes occurring at molecular or cellular level and we do not know if they could eventually trigger irreversible unwanted effects, we are on the conservative side. Orthogonal data especially covering teratogenicity could strengthen the outcome of this assessment. The actual point of departure for propylparaben from legacy in vivo studies in repeated dose toxicity is 1000 mg/Kg/day and the SCCS allows its use as preservative in cosmetics up to 0.18% as ester.

Work published in the literature using bioactivity exposure ratios (Cable et al. 2025) show that based on the toolbox selected, it is possible to assess the level of protectiveness for human health.

8 Data selection / gathering

The data in this IATA case study were selected to address the problem formulated: is the use of combined use of propylparaben as preservative at maximum concentration in consumer products and toys safe?

Measured and estimated physical-chemical parameters relevant to systemic toxicity were collected from the literature and in silico tools.

Given this is a safety assessment for consumers, the combined exposure was estimated and translated to an internal concentration considering the sources, the products and routes of exposure of relevance to this assessment. The presence of propylparaben in cosmetics and the dermal route were the most relevant for the assessment. A deterministic estimate was derived using values from the SCCS' notes of guidance. Using the ASPA workflow, this is the very first tier for assessing exposure. Exposure from the oral route was evaluated and it was negligible compared to the exposure levels from the dermal route. It was decided to work on this IATA using the exposure values derived from the dermal route.

The external exposure was translated into internal dose metrics (geometric Cmax, AUC and Caverage) using in vitro measured data as input parameters. The first tier which is based on use of in silico parameters was not used, given that measured data were available. The second tier in the ADME/tk pillar was used here.

For the toxicodynamic data, both in silico tools relevant to systemic toxicity were used. In vitro data were available for propylparaben and its main metabolite para-hydroxybenzoic acid in ToxCast, including transcriptomic data in three cell lines (MCF7, U2OS, HepaRG) and in the CALUX assays. The data collected were relevant to the assessment of propylparaben.

9 Data Evaluation

A first hypothesis was formulated based the initial in silico alerts and the existing data collected for propylparaben.

For in silico alerts, only outputs that were in domain were considered. They were used in a weight of evidence manner.

In vitro data were evaluated for their robustness in terms of reliability and fitness-for-purpose for this assessment.

A sensitivity analysis was performed on the PBK input parameter to identify which ones were driving the partitioning and distribution of propylparaben.

10 Data integration and interpretation

Data for this IATA were integrated at different level:

- Exposure: the combined exposure to propylparaben from different products via the dermal route.
- Translating the exposure to internal concentrations was performed by using in vitro input parameters in the SimCyp model and performing a sensitivity analysis to identify the most impactful parameters. The distribution of propylparaben and its metabolites were useful for generating and interpreting the in vitro toxicodynamic data.
- For the toxicodynamics, in silico and invitro data were assessed in a weight of evidence manner. The in vitro assays were selected to cover the alerts identified with the in silico tools and broader in terms of biological processes. Higher tier assays were also available and considered for this IATA. Most of the bioactivity observed was due to the propylparaben and not by the degradation products that showed little/no activity in the assay battery used.
- Bioactivity exposure ratios were calculated using the benchmark doses derived from the in vitro assays and the Cmax values. All the bioactivity exposure/ratios were above 1 and the majority > 100.

11 Measures of reliability

For the in silico data, reliability were assessed according to the OECD QAF recommendations.

For in vitro assays, validity criteria like reproducibility, reliability, fitness for purpose were considered. Details for ToxCast assays are provided in the assays' description referenced in annex I.

A team from RISK-HUNT3R (Ullrika Sahlin and Zheng Zhou, Lund University) worked on a statistical approach to assess the variability between different biological replicates of some of the assays used like the CALUX assay. Over different bioassays and modes, the reliability varies from 0.17 to 0.93 (with 95CIs).

The sensitivity analysis performed for the PBK modelling is also a way of addressing the robustness of the evaluation.

12 Expert judgement & alternative interpretations

Generally, less weight was given to tools and assays that do not fully comply with OECD standards like the ones described in the test guidelines or the guidance document 211 or GIVIMP.

The chemical effect predictor was given less weight than models from the OECD QSAR toolbox.

Also, assays that address upstream events like molecular initiating events were given less weight than those addressing events that are downstream events closer to adversity when their output was not consistent across the biological scale. Therefore, not all molecular initiating events were further evaluated with higher tier assays.

One caveat (or advantage?) in this IATA is that all the in vitro data were available and no toxicodynamic data was generated de novo. An assay on teratogenicity using mammalian cells like the most up to date version of Stemina's devTox assay or the reprotracker assays from Toxys could be a valuable addition in the weight of evidence assessment. Ideally the evaluation would be performed on propylparaben, para-hydroxybenzoic acid and 1-propanol.

Known PoDs from legacy in vivo data for propylparaben from available (sub)chronic studies have a NOAEL of at least 1000 mg/Kg/day, except one study (OECD 422) with NOAEL = 981 mg/Kg/day (Source: ECHA).

13

Description of uncertainties

A certain degree of uncertainty exists in terms of biological coverage of the toolbox used in this IATA. This is not specific to this case study but to the application of the bioactivity/exposure ratio concept for the safety assessment of consumer product. So far, this concept was successfully applied in prioritization cases by organization like the US EPA and Health Canada (Paul Friedman et al., 2020, 2025, [Health Canada 2021](#)) but may not be sufficient for regulated substances with a broad range of legal information requirements.

There is no consensus in the scientific community on how to define an acceptable bioactivity/exposure ratio. Work found in the literature illustrates examples on how to derive BER to address the evaluation of systemic effects (Baltazar et al. 2020, Cable et al., 2025, Hewitt et al., 2022).

Another source of uncertainty which is well documented is the lack of proper metabolic capability in most of the ToxCast assays. The retrofitting of some of the ToxCast data with some metabolic enzymes was not used here. Therefore, the output of the assays relates to the parent compound propylparaben, which is known to be degraded by carboxylesterases in skin as well as in liver. The CALUX assays in presence of phase I metabolic enzymes as well as the transcriptomic data in HepaRG were valuable in covering these metabolic processes. However, no in vitro biokinetics was performed to verify what the free concentrations of propyl paraben and its degradation products were in the assays performed.

Based on the estimates of free propylparaben in the toxicokinetic studies, the BER values derived varied from 27.77 to 6006.62 depending on the in vitro assay considered.

The following table summarizes the uncertainty assessment of this case study:

Table 22. Qualitative confidence assessment: medium/high in this use scenario

Data type	Assumptions	Level of confidence (low, medium, high)	Comments
Exposure data	The exposure estimate exceeds consumer exposure in reality	High	Predicted exposure using a deterministic estimate that is highly conservative and much more than consumers are exposed to in reality
ADME	Propylparaben readily cleaved by esterases to pHBA, the main metabolite. Bioactivity carried by parent compound, ester.	High	In silico predictions as well as actual data (EATS fro Calux, ToxCast) differentiate pHBA from the ester). On the other hand, pHBA is not covered in the PBPK modelling and there is no estimate of internal exposure to pHBA which leaves some uncertainty.
PBK	PBK model will provide the data on internal exposure of the target chemical based on an external exposure scenario.	High	Adapted from Ouédraogo et al., 2022: "PBK model developed and used to estimate the internal plasma concentrations. Model previously published and validated (Campbell et al. 2015). Human data available to benchmark estimates from PBK model

In silico data (mostly EATS and DART)	Alerts relevant to systemic toxicity, incl. DART can be identified with the tools selected	High	This is a peculiar situation where the compounds were part of the majority of the training sets of the models used.
Chemical effect predictor	Alerts on biological plausibility of adversity can be identified	Low	Tool still in development, performances not yet assessed
ToxCast data	ToxCast can be used as follow up to alerts identified with in silico tools and a way to cover molecular and cellular events relevant to systemic effects, incl. DART	Medium	Existing data: low bioactivity observed, mainly low potency ER activity for ester. For DART, no evidence of activity based on zebrafish data retrieved. A NAM based assay (e. DevTox, Reprotracker) might strengthen the confidence in the absence of activity.
CALUX	Assays provide good quality data on EATS activity as follow up to in silico alerts	High	Adapted from Ouédraogo et al., 2022: "assay performed according to OECD TG by an experienced lab with track record of high reproducibility, low variability. CALUX assays are based on using U2-OS cells, which have no endogenous receptors. This makes the assay highly specific and reduces the uncertainty. U2-OS cells have limited metabolic capacity, which might lead to false negative results if active metabolite would be produced in vivo, or false positive results if an active parent molecule would be readily metabolised in vivo. This uncertainty was reduced by performing the assays +/- liver S9 extract. Good quality data, with low potential to cause overestimation or underestimation"
NAM based PoDs	NAMs can be used to derive PoD	High	As the only robust bioactivity was observed on estrogenicity, BMCs were derived from molecular and cellular assays related to this pathway.
Bioactivity exposure ratios	BER calculated are protective	High	All bioactivities observed are well above the exposure estimates. Acceptable levels of BERs depend on the toolbox used to make the assessment, the problem formulated and a given context of use. Given this is based on biological activity, there is no mean to know if the estrogenicity observed could translate into adversity. Evidence gathered here shows no likelihood of adversity. Orthogonal assays could confirm this. So far, BER was used for prioritization in regulatory dossiers. For safety assessment, a dossier is currently under review by SCCS. Safety assessments based on legacy in vivo studies confirm that the outcome is protective of human health.

14 Conclusion

To the question if the combined exposure of propylparaben as preservative at maximum levels in consumer products and toys is safe for consumers?

The in silico and in vitro data gathered in the IATA using different tiers in the ASPA workflow led to decision that the use of propylparaben in these exposure scenarios is safe with high probability using bioactivity/exposure ratios. The low bioactivity observed in the confirmed hit calls together with the low potency when compared to reference compounds (e.g., binding to estrogen receptor, estrogenicity using the US EPA computational model) supports this conclusion. Further evidence is brought by the lack of bioactivity in the teratogenicity assay performed using the zebrafish embryo assay. This is in line with the opinion on the safe use of propylparaben (up to a maximum of 018%) available from SCCS.

Some uncertainty remains on what an acceptable BER is for risk assessment. An effort is ongoing at WPHA to address this topic.

As stated in the introduction, the purpose of this case study was to evaluate the applicability of ASPA workflow for OECD IATA for the safety assessment of consumer products. This goal was successfully achieved using propylparaben for demonstration purpose. At the same time, this case study highlighted further development needs that remain to be addressed such as qualifying biological coverage, predictivity of in silico methods, availability of reliable in vitro and non-animal data, as well as agreement on how to qualify a protective bioactivity exposure ratio -BER-.

15 Learnings and Lessons

The OECD IATA Case Studies illustrate the application of New Approach Methods (NAMs), including Integrated Approaches for Testing and Assessment (IATAs), in specific regulatory contexts. To support knowledge-sharing and reuse of experience across jurisdictions, the OECD IATA Case Study Project includes structured expert review and discussion of each submission.

Following discussion at the OECD IATA Case Study Project meeting in November 2025, it was agreed that key learnings and lessons arising from the review of each case study would be documented directly within the individual case study reports. This approach aims to enhance transparency, usability, and practical relevance, and to facilitate understanding of strengths, uncertainties, and considerations relevant to regulatory application.

The learnings from this case study, particularly regarding differences, similarities, and the relative advantages and limitations of the ASPA-based approach compared with an earlier OECD case study using a read-across approach to assess dermal exposure to propylparaben in cosmetic products, are described in Section 7.5.2.

Annex I. Reporting individual information sources*

The [in-silico models from DTU are in an annex document](#) in the QMRF format.

Other documents as appendices of annex I

- [PBK modelling and results obtained](#)
- [Description of the Chemical Effect Predictor and prediction for propylparaben](#)

Information on ToxCast data and links for accessing them are below:

ToxCast assays: described according to the OECD GD211

To summarize, “*In vitro* bioactivity data from the US EPA ToxCast program (Dix et al., 2007; [Judson et al., 2010](#)) were obtained from the database *invitrodb* (version 3.4, <https://doi.org/10.23645/epacomptox.6062623.v7>) and summarized as detailed in [Suppl. Fig S4](#). The database contains potency estimates and activity calls ('hitcall') for chemical x assay endpoint pairs. For chemicals tested more than once in a given assay, results were averaged using a weighted approach that gives less weight to concentration-response curves with more caution flags, as caution flags tend to be indicative of less reliable data/curve fits. Subsequently, an *in vitro* point-of-departure (POD) was calculated from the lowest three potency estimates (i.e., benchmark concentration, BMC) out of all active assays for a given chemical, again by using a weighted average using the number of caution flags as weights.” (Nyffeler et al., 2023)

The detail description of the assays can be retrieved following these links:

<https://doi.org/10.23645/epacomptox.6328037.v11>

Feshuk M., Ko A., Vahanan M. M., Carstens K., Harrill A. , Paul Friedman K. EPA Publication Number 601B24001 on TOXICITY FORECASTER (TOXCAST) ASSAY DESCRIPTION DOCUMENTATION: <https://clowder.edap-cluster.com/files/66e2e177e4b0a7c65d2f7a8b?dataset=66df2b8ce4b0a7c65d2ebb36&space=>

Cell painting assay (information retrieved from the US EPA Chemistry dashboard on November 6th, 2025: CompTox Chemicals Dashboard v2.6.0)

Assay Description

HTPP Bioactivity High-throughput phenotypic profiling (HTPP) with the Cell Painting assay Nyffeler et al. 2020, ([PMID: 31899216](#)) uses high content imaging of cells labeled with organelle-targeting fluoroprobes to profile chemical effects on cell morphology. The information displayed in this tab are results from global, category-level, and feature-level modeling of HTPP data as described in Nyffeler et al. 2021 ([PMID: 32862757](#)). Endpoints were subject to concentration-response modeling using the *tcplfit2* package described in Sheffield et al. 2021([PMID: 34791027](#)).

Category Level Summary Points are plotted according to the potency (i.e. BMD, x-axis) and efficacy (i.e. Top/Cutoff, y-axis) determined from concentration-response modeling of global or category-level endpoints based on Mahalanobis distance modeling approaches described in Nyffeler et al. 2021. For each Sample ID × cell type × seeding density × exposure duration combination tested, up to 49 category-level endpoints and 1 global endpoint may be displayed on this plot if active (i.e. a hit). An example of a category-level endpoint is Mito_Texture_Cytoplasm, a latent variable measuring the texture of mitochondrial labeling in the cytoplasm compartment of the cells. If the global endpoint was active for a Sample ID × cell type × seeding density × exposure duration combination, the BMD is displayed as a large purple point. In addition, if category-level endpoint(s) for a Sample ID × cell type × seeding density × exposure duration combination were affected, the BMD of the most-sensitive category is also displayed as a large point color-coded to match the fluorescent channel / organelle from which it was derived. Points are shape coded based the compartment from which they were derived (i.e. cytoplasm, nuclei, membrane, ring or cell). Mouse over each point to display metadata and modeling results associated with that point. Click the cell type, exposure duration and/or seeding density checkboxes to display data associated with those conditions.

Feature Level Summary Points are plotted according to the potency (i.e. BMD, x-axis) and efficacy (i.e. Top/Cutoff, y-axis) determined from concentration-response modeling of normalized and z-scaled feature-level data. Up to 1300 feature-level endpoints may be displayed on this plot if active for each Sample ID × cell type × seeding density × exposure duration combination tested. If present on the graph, large purple points represent the global BMD and large not purple points represent the most sensitive category-level BMD for a given Sample ID × cell type × seeding density × exposure duration combination. Points are shape coded based the compartment from which they were derived (i.e. cytoplasm, nuclei, membrane, ring or cell). Mouse over each point to display metadata and modeling results associated with that point. Click the cell type, exposure duration and/or seeding density checkboxes to display data associated with those conditions.

The US EPA teams “developed a widely used R-language application called *tcpl* (ToxCast Pipeline; [Filer et al., 2017](#)) which manages large collections of high-throughput screening (HTS) data from the raw data management through concentration–response modeling and graphical results presentation. The *tcplfit2* package addresses two shortcomings in the original implementation of *tcpl*. The first is that the collection of curve-fitting methods in *tcpl* was limited to a constant model, a Hill model (sigmoidal) and a gain-loss model (a rising Hill model followed by falling Hill model). We have now added several exponential and power law models which are commonly used in concentration–response modeling, including all models used in the BMDExpress program used for modeling concentration–response transcriptomics data ([Phillips et al., 2019](#); [Yang et al., 2007](#)). Additionally, benchmark dose (BMD) modeling capabilities have been added. The BMD is the dose or concentration where the model curve crosses the benchmark response (BMR) level, which is defined as some multiple of the standard deviation of the background noise level (often 1.349). The second goal of developing *tcplfit2* is to make a stand-alone package with this comprehensive functionality which is independent of the complex data management capabilities and database integration tasks carried out by *tcpl*. One major new use of *tcplfit2* is to model concentration–response in transcriptomics at the gene and pathway levels (Harrill et al., 2021)” (from [Sheffield et al, 2022](#) Bioinformatics, Volume 38, Issue 4, February 2022, Pages 1157–1158, <https://doi.org/10.1093/bioinformatics/btab779>).

Transcriptomics data (from Rogers et al., 2025 Environmental Health Perspectives, Volume 133, Issue 6, <https://doi.org/10.1289/EHP1602>)

“HTTr screens of 1,201 chemicals in U2OS and HepaRG cell lines were used as source data for all transcriptomic analyses. Chemicals were selected to reflect those in the US EPA ToxCast chemical inventory including agricultural and industrial use compounds as well as a selection of pharmaceuticals. The U-2 OS cell line was chosen for its previous characterization and use in HTTr, and the HepaRG cell line was chosen for its increased metabolic competency and higher similarity in expression profiles to primary human hepatocytes vs. previous cell lines. Both screens used the same experimental design and

bioinformatics pipeline for initial derivation of transcriptional potency values. In brief, dimethylsulfoxide (DMSO)-solubilized chemical stock solutions were provided frozen from the US EPA ToxCast chemical inventory management contractor (EvoTec; Princeton, New Jersey) and stored at -80°C prior to dose plate preparation. U-2 OS osteosarcoma and HepaRG hepatocyte cell lines were used for chemical exposure (see Excel Table S2 for cell culture details). Chemicals were tested at 8 nominal concentrations ranging from 0.03–100 μM using 0.5-log spacing with a final DMSO concentration of 0.05%. All exposures were conducted in triplicate using independent cell cultures. At 24 h after chemical exposure, cell lysates were prepared for TempO-Seq analysis as previously described. The TempO-Seq human whole transcriptome v2 assay (hWTV2) with cell line-specific custom attenuation was performed by BioSpyder, Inc.

Raw FASTQ files were processed using a custom bioinformatics pipeline as previously described, including read alignment, sample quality control, differential expression analysis, and concentration–response modeling. Moderated $\log_2$ (fold change) values were generated from the *DESeq2* R package (version 1.24) for all concentrations of each chemical–gene pair. Concentration–response modeling was then conducted using the *tcplfit2* R package (version 0.1.5) by fitting $\log_2$ (fold change) values to nine models (constant, hill, poly1-2, power, exp2-5) and selecting the model with the minimum Akaike Information Criterion. Benchmark concentrations (BMCs) were computed for each chemical–gene pair based on benchmark response values representing departure from a null response distribution. The null distribution cutoff was defined as 1.349 times the standard deviation (SD) of responses from the lowest two concentrations of all test samples, which assumes that these concentrations will be inactive for a large majority of the chemical test set.²³

Key metrics from *tcplfit2* include continuous hitcalls, which represent confidence in winning model selection and that model responses exceed cutoffs, and normalized efficacy values (*top_over_cutoff*), which represent the signal-to-noise ratio. A hitcall ≥ 0.9 was chosen to match previous HTr and ToxCast screening studies. A *top_over_cutoff* ≥ 1.5 was chosen empirically to limit the potential for noise when fitting individual genes. Concentration-responsive (“active”) chemical–gene pairs were defined for downstream analyses using both criteria above. BMCs for active genes were bounded to be within 0.1 times the lowest concentration tested and 10 times the highest concentration tested after modeling to match previous signature-based analyses. »

CALUX assays

Details on the CALUX assays are provided in the JRC [DB-ALM Protocol n° 197](#)

Reference on ASPA

Leist, M., Tangianu, S., Affourtit, F., Braakhuis, H., Colbourne, J., Cöllen, E., Dreser, N., Escher, S., Gardner, I., Hahn, S., Hardy, B., Herzler, M., Islam, B., Kamp, H., Magel, V., Marx-Stoelting, P., Moné, M.J., Lundquist, P., Ottenbros, I., Ouédraogo, G., Pallocca, G., van de Water, B., Vinken, M., White, A., Pastor, M. and Luijten, M. (2025), An Alternative Safety Profiling Algorithm (ASPA) to Transform Next-Generation Risk Assessment into a Structured and Transparent Process, ALTEX, in press. <https://doi.org/10.14573/altex.2509081>

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Annex II. Reporting IATA Outcomes

Name of Chemical	<i>Propylparaben</i> CAS: 94-13-3 SMILES: <chem>C(=O)(c1ccc(O)cc1)OCCC</chem>
Purpose	<i>The regulatory context is the European cosmetic regulation. The problem formulated is the following: is the combined use of propylparaben as preservative at maximum concentration in consumer products and toys safe?</i> . <i>Given this is a safety assessment for consumers, the combined exposure was estimated and translated to an internal concentration considering the sources, the products and routes of exposure of relevance to this assessment. The presence of propylparaben in cosmetics and the dermal route were the most relevant for the assessment. A deterministic estimate was derived using values from the SCCS' notes of guidance.</i>
Description of IATA Framework, including all information sources used, and modules included in the specific approach	ASPA comprises a wide set of modules relevant for chemical safety assessment, aimed at toxicokinetic modelling and in vitro in vivo extrapolation (IVIVE) approaches, hazard identification and characterization, exposure and risk assessment. Different modules may be activated or deactivated, dependent on the problem formulation. Thus, ASPA is flexible; it allows for addressing multiple types of regulatory questions and frameworks, with a focus on European regulations, using different approaches. <u>Exposure</u>: use of data from the SCCS' notes of guidance, literature. Aggregate exposure modelled by RIVM <u>ADME-TK</u>: data from Cosmetics' Europe Long range science strategy and RISK-HUNT3R, PBK modeling adapted from literature by Certara <u>Toxicodynamics</u>: - in silico data from Danish EPA resources, OECD QSAR toolbox, VEGA - in vitro data from ToxCast and Calux assays

Data review	Measured and estimated physical-chemical parameters relevant to systemic toxicity were collected from the literature and in silico tools. The exposure data were collected from the literature and the aggregate exposure was modelled by RIVM. In silico and existing data on toxicodynamics were gathered by the Danish EPA. For ADME/tk, data were from the literature, the from the Cosmetics' Europe long range Science Strategy (ADME data) and the PBK modelling was performed by Certara. The toxicodynamic data were from ToxCast (US EPA chemical dashboard) and from the Cosmetics' Europe long range Science Strategy, LRSS (Calux assays). A first hypothesis was formulated based the initial in silico alerts and the existing data collected for propylparaben. For in silico alerts, only outputs that were in domain were considered. They were used in a weight of evidence manner. In vitro data were evaluated for their robustness in terms of reliability and fitness-for-purpose for this assessment. A sensitivity analysis was performed on the PBK input parameter to identify which ones were driving the partitioning and distribution of propylparaben.
Data Integration and Interpretation	Data for this IATA were integrated at different level: - Exposure: the combined exposure to propylparaben from different products via the dermal route. - Translating the exposure to internal concentrations was performed by using in vitro input parameters in the SimCyp model and performing a sensitivity analysis to identify the most impactful parameters. The distribution of propylparaben and its metabolites were useful for generating and interpreting the in vitro toxicodynamic data. - For the toxicodynamics, in silico and invitro data were assessed in a weight of evidence manner. The in vitro assays were selected to cover the alerts identified with the in silico tools and broader in terms of biological processes. Higher tier assays were also available and considered for this IATA. Most of the bioactivity observed was due to the propylparaben and not by the degradation products that showed little/no activity in the assay battery used. - Bioactivity exposure ratios were calculated using the benchmark doses derived from the in vitro assays and the Cmax values. All the bioactivity exposure/ratios were above 1 and the majority > 100.
Expert Judgement	<i>Expert judgement was used to select the appropriate tiers in the different pillars and in the weight of evidence assessment. The working hypotheses are also driven by expert judgement.</i>

Uncertainties in the specific workflow followed	<i>ASPA is new and still needs to be proof-checked with different contexts of use.</i>
Uncertainties specific to the chemical assessed	A certain degree of uncertainty exists in terms of biological coverage of the toolbox used in this IATA. This is not specific to this case study but to the application of the bioactivity/exposure ratio concept for the safety assessment of consumer product. So far, this concept was successfully applied in prioritization cases by organization like the US EPA and Health Canada (Paul Friedman et al., 2020, 2025, Health Canada 2021). There is no consensus in the scientific community on how to define an acceptable bioactivity/exposure ratios. Work found in the literature illustrates examples on how to derive BER to address the evaluation of systemic effects (, Baltazar et al. 2020, Cable et al., 2025, Hewitt et al., 2022). Another source of uncertainty which is well documented is the lack of proper metabolic capability in most of the ToxCast assays. The retrofitting of some of the ToxCast data with some metabolic enzymes was not used here. Therefore, the output of the assays relates to the parent compound propylparaben, which is known to be degraded by carboxylesterases in skin as well as in liver. The CALUX assays in presence of phase I metabolic enzymes as well as the transcriptomic data in HepaRG were valuable in covering these metabolic processes. However, no in vitro biokinetics was performed to verify what the free concentrations of propyl paraben and its degradation products were in the assays performed. Based on the estimates of free propylparaben in the toxicokinetic studies, the BER values derived varied from 112 to 53068 depending on the in vitro assay considered. Considering only the assays in zebrafish embryos, the BER ratios ranged from 11 to 13000 considering different readouts and different Cmax values (free concentration, mean, 5th or 95th percentile).
Outcome of the Assessment	To the question if the combined exposure of propylparaben as preservative at maximum levels in consumer products and toys is safe for consumers? The in silico and in vitro data gathered in the IATA using different tiers in the ASPA workflow led to decision that the use of propylparaben in these exposure scenarios is safe with high probability using bioactivity/exposure ratios. Some uncertainty remains on what an acceptable BER is for risk assessment. An effort is ongoing at WPHA to address this topic.
Discussion of other interpretations (if applicable)	<i>Describe possible alternative interpretations (likely based on expert judgement).</i>