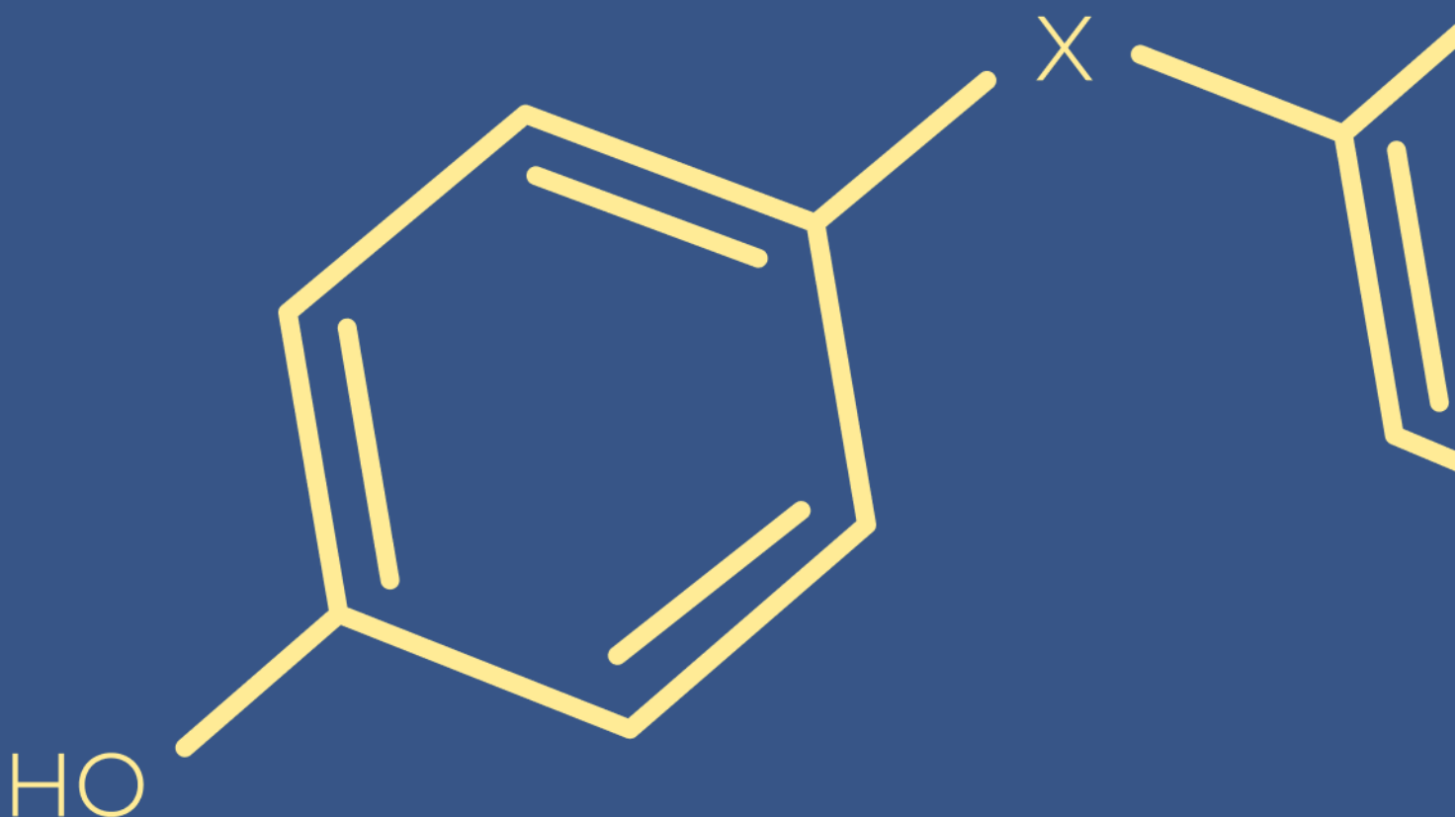




Nordic Council
of Ministers

Development of recommendations for grouping of endocrine disruptors



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Abbreviations

AD	Applicability Domain
ADME	Absorption, distribution, metabolism and excretion
AE	Assessment Element
AOP	Adverse Outcome Pathway
AR	Androgen receptor
ARN	Assessment of Regulatory Needs
BFRs	Brominated Flame Retardants
BP	Butylparaben
BPR	Biocidal Products Regulation
CAG	Cumulative Assessment Group
CAS RN	Chemical Abstracts Service Registration Number
CLP	Classification, Labelling and Packaging of substances and mixtures Regulation
CLH	Harmonized Classification and Labelling
CMR	Carcinogenic, Mutagenic or toxic for Reproduction
CRA	Cumulative risk assessment
DIO	Iodine-induced thyroid dysfunction
DTU	Technical University of Denmark
EATS	Estrogen, Androgen, Thyroid, Steroidogenesis
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EFSA	European Food Safety Authority
ER	Estrogen receptor

GD	Guidance Document
IC	Index compound
IPB	Isobutylparaben (isobutyl 4-hydroxybenzoate)
MoA	Mode of Action
NIS	Sodium/Iodide symporter
OECD	Organisation for Economic Co-operation and Development
PBT/vPvB	Persistent, Bioaccumulative and Toxic/very Persistent, very Bioaccumulative
PFAS	Per- and polyfluoroalkyl substances
PMT/vPvM	Persistent, Mobile and Toxic/very Persistent, very Mobile
PPPR	Plant Protection Products Regulation
PXR	Pregnane X Receptor
(Q)SARs	(Quantitative) Structure-Activity Relationships
RAAF	Read-across Assessment Framework
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation
SMILES	Simplified Molecular-Input Line-Entry System
SVHC	Substance of Very High Concern
Tox21/ToxCast	Toxicology in the 21st century/Toxicity Forecaster (chemical screening)
TPO	Thyroid Peroxidase
UVCB	Substance of Unknown or Variable composition, Complex reaction products or Biological materials

Definitions

Definitions used in this project are built on the definitions and terminology provided in the ECHA RAAF and the OECD Guidance Document (GD) 194. We refer to the ECHA RAAF (ECHA 2017) and the OECD GD 194 (OECD 2014) for additional definitions and explanations of relevant terms.

Analogue approach

Grouping approach which aims to fill a data gap for one substance in the group based on information from one or a few source substances.

The ECHA RAAF defines that the term analogue approach is used when read-across is employed between a few, very structurally similar substances for which it is not possible to establish a trend or a regular pattern. As a result of the structural similarity, a given (toxicological or other) property of one substance (the source) is used to predict the same property for another substance (the target), for which this property is not available but is needed to fulfil a REACH information requirement. The outcome of a study conducted with the source substance is read-across for all investigated parameters to the target substance. A worst-case approach may also be used (ECHA 2017).

The OECD GD 194 defines that the term analogue approach is used when the focus is to fill data gaps for one specific chemical, based on empirical data from one or more similar chemicals. The analogue approach is specified to be useful when the target and source chemicals share a known common mode (or mechanism) of action and the adverse effects resulting from this is evaluated (OECD 2014).

Applicability Domain

The set of inclusion/exclusion rules that identify the ranges of values within which a reliable prediction can be made for category members (ECHA 2017).

Assessment Element (AE)

A critical scientific aspect of a read-across to be assessed through a number of questions to be answered (ECHA 2017).

Categorization

In this document used when defining a group of similar substances. Can be used interchangeably with the term grouping, which is the preferred one in this document.

Category approach

Grouping approach which aims to fill gaps for a given property for multiple substances in the category based on available information from the source category members.

The ECHA RAAF defines that the term category approach is used when read-across is employed between several substances with structural similarities. These substances are grouped together based on defined structural similarities and differences between the substances. As a result of the structural similarity, one or more (toxicological or other) properties are proposed to be similar or to follow a regular pattern. The predictions are made within the group for the target substance(s) based on the observed regular pattern. Alternatively, the prediction is based on a read-across from a category member in a conservative manner (worst case) (ECHA 2017).

The OECD GD 194 defines that the term category approach is used when chemicals whose physico-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern due to structural similarity. In this approach, the properties of the individual chemicals within a category are assessed based on evaluating the category as a whole (OECD 2014).

Category hypothesis

Explanation as to why property(ies) of category members may be predicted from reference substances within the category. This explanation must be based on a relationship between structural similarity and the predicted property(ies) (ECHA 2017).

Category justification

Reasoning and associated supporting evidence that are provided to verify the scientific validity and robustness of the category hypothesis (ECHA 2017).

Group

Under REACH, substances that are structurally similar with physicochemical, toxicological, ecotoxicological and/or environmental fate properties that are likely to be similar or to follow a regular pattern may be considered as a group of substances. Within a group of substances, a data gap might be filled by read-across, as described below (ECHA 2017).

Grouping

In this document used when defining a group of similar substances. Can be used interchangeably with categorization, but grouping is preferred in this document.

QSAR

Mathematical model (often a statistical correlation) relating one or more qualitative and/or quantitative parameters (molecular descriptors) derived from the chemical structure to a qualitative or quantitative measure of a property or activity. QSARs are quantitative models yielding a continuous or categorical result. For regulatory use, QSARs should be validated according to the OECD (Q)SAR Validation Principles, hereunder also associated with a defined applicability domain (AD).

SAR

A qualitative relationships that relates a (sub)structure and/or other molecular descriptors to the presence or absence of a property or activity of interest, by expert judgement but most often also by use of statistical considerations. For regulatory use, SARs should be validated according to the OECD (Q)SAR Validation Principles. Often, the SAR expert systems incorporates mechanistic interpretation with literature references into the system.

(Q)SAR

A combination of SAR and QSAR, as they are very overlapping concepts, both based on the similarity principle, i.e. the hypothesis that substances with similar molecular structure will have similar properties, hereunder biological activities.

Read-across

Method used to fill data gaps for substances in an analogue or a category approach.

Under REACH, read-across is a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s)). Consequently, the read-across is considered as property-specific (ECHA 2017).

Read-across hypothesis

Hypothesis on the basis of which property(ies) of target substance(s) may be predicted from source substance(s). This hypothesis must be based on a relationship between structural similarity and the predicted property(ies) and needs to be supported by a read-across justification (ECHA 2017).

Read-across justification

The reasoning and associated supporting evidence that are provided to verify the scientific validity and robustness of the read-across hypothesis (ECHA 2017).

Sub-grouping

In this document used when defining a smaller group of similar substances out of a larger group.

Summary

Minimizing exposure to endocrine disruptors (EDs) is high on the political agenda, both in the EU and in the Nordic region. One tool to speed up these efforts is by regulating groups of substances. This project analysed case examples of groupings of EDs. For each case example, advantages, challenges, uncertainties and regulatory perspectives were discussed and learnings were extracted. On this basis, recommendations for grouping of EDs were developed and incorporated in a simplified workflow, which can guide grouping of EDs. Main findings were that EDs can be grouped like other chemicals, their complex hazard profiles require special attention, and groupings should start with the broadest group possible. To facilitate competence building for grouping of EDs in EU regulatory process, the report was presented to chemical authorities across the EU at a webinar in November 2024.

Recommended workflow

Step 1 Define purpose and hypothesis

- Define the regulatory purpose
- Define the grouping hypothesis

Step 2 Include relevant substances

- Start with the broadest group possible. Use (Q)SARs to check if additional substances should be included.
- Include substances under regulatory scrutiny (**For EDs: Regulatorily identified EDs**) or registered in high tonnages under REACH for use as source substances if possible
- Use well-argued inclusion and exclusion criteria, refrain from excluding substances based on current regulatory status or use.
- Make sure that all substances are characterized in detail, including impurity profiles.

Step 3 Produce a data matrix

- Include in silico, in vitro, in vivo data
- Include relevant metabolites and ADME data
- **For EDs: Consider complex hazard profiles, including different effects depending on timing of exposure and investigation, low dose and non-monotonic effects etc.**

Step 4 Redefine the group and define sub-groups, as appropriate

- **For EDs: Consider that different molecular moieties may be relevant for different mechanisms and thereby modes of action and adverse effects. Some overlap may occur, but this should ideally be analyzed and described.**
- **For EDs: Consider that many endocrine disruptors act through more than one mechanism/mode of action and that several different mechanisms/mode of actions can lead to the same adverse effects.**
- Consider that if in vitro data are used to define sub-groups, ADME must be considered and that if ADME/in vivo information is considered, the sub-groups may have different boundaries.
- Use QSARs to check if more substances could be added to the (sub-)group.

Step 5 Read-across to fill data gaps

Step 6 Conclude on results, revisit the hypothesis, as appropriate

Figure 1 Recommended workflow for (sub-)grouping of endocrine disruptors with the ultimate aim of filling data gaps by read-across. General recommendations are provided at relevant steps in the workflow. Recommendations specific for endocrine disruptors are highlighted in bold.

Introduction

In the EU Commission's "Chemicals Strategy for Sustainability - Towards a toxic-free environment" from 2020, one of the goals is to introduce a generic approach to risk management, where the most harmful substances, including endocrine disruptors, are not allowed in consumer products. Until this generic approach is implemented, the aim is to prioritize broad restrictions encompassing groups of these substances.

Following up on this, several activities are ongoing to identify groups of substances as endocrine disruptors. One example is the European Commission's "Restrictions Roadmap under the Chemicals Strategy for Sustainability" from 2022, which identifies several priority substances and groups of substances that are in the process towards regulation or under discussion in the EU, including groups of endocrine disruptors. Another example is the continuous development of "Assessment of Regulatory Needs" (ARNs) prepared by ECHA on groups of substances with the purpose of helping authorities conclude on the most appropriate way to address the identified concerns and any intermediate steps, such as data generation, needed to initiate and introduce regulatory measures^[1]. Several of the ARNs contain (sub)groups of substances suspected of being endocrine disruptors.

The ED hazard classes are new in the harmonized classification and labelling system (EU 1272/2008; EU 2023/707), enabling harmonized hazard classification of EDs, which will trigger risk assessment and/or management in other relevant legislation and labelling requirements along the supply chain.

There are examples of harmonized classification for groups of substances from the past. Nickel and its compounds, comprising a total of ~ 140 substances, have an array of harmonized classifications, including for carcinogenicity. Nickel (and nickel powder) are classified as suspected human carcinogens (Carc. 2), as is nickel tetracarbonyl; all other nickel compounds have harmonized classifications as known human carcinogens (Carc. 1A) (EC 2018). Further, smaller groups of Boron compounds have a harmonized classification due to reproductive toxicity (Repr. 1B) (EC 2018; EC 2021).

Assigning harmonized classifications for a whole group of substances can be an efficient regulation approach if the whole group is found to possess one or more hazardous properties and a possible means to avoid regrettable substitution (i.e. one substance with a harmonized classification is substituted with another

1. A description of the ECHA's work with assessments of regulatory needs can be found here: <https://echa.europa.eu/understanding-assessment-regulatory-needs>

structurally very similar substance, which is later found to have similar hazardous effects).

It can be foreseen that, in the coming years, groups of endocrine disruptors will be identified and regulated, including under the CLP Regulation. Work has just started, and the methods and their regulatory acceptability, which will set a precedent for many years to come, are under development.

In this project, we explored approaches and developed recommendations for formation of (sub-) groups of substances with the ultimate aim of filling data gaps by read-across. Some of the case examples did not in their objectives aim at data gap filling, but their category formation approaches were still found to provide valuable learnings generally applicable for forming (sub-)groups with this purpose.

Additional broad grouping approaches in regulatory contexts, without an aim of data gap filling by read across, can be exemplified by the EFSA Cumulative Assessment Group (CAG) approach (EFSA 2012; EFSA 2014; EFSA 2019) and the proposal for a restriction of PFAS substances under REACH (ECHA 2023b). None of these are analysed in detail in this report, but their potential use and relevance for EDs is put into perspective in the discussion.

The OECD Guidance Document (GD) 194 on the Grouping of Chemicals is under revision. It provides guidance, stepwise procedures and reporting formats for different types of grouping approaches. The guidance aims to support the development of grouping and read-across for use in hazard or risk assessment of substances. It draws on approaches known to have been accepted by regulators or have been developed by regulators or registrants under voluntary programs (OECD 2014)

ECHAs Read-Across Assessment Framework (RAAF) is a tool for examining predictions, based on read-across, of the human health, environmental fate and environmental hazard properties of chemical substances in the context of the REACH Regulation. It can be used as guidance for consistent evaluation of the scientific aspects of a proposed read-across case (ECHA 2017).

Under REACH, registrants are responsible for ensuring that the chemicals they place on the EU market do not pose a risk to human health and the environment. Registrants are also obliged to consider and, where they can, use appropriate alternative approaches to fulfil the REACH information requirements on vertebrate animal studies. Read-across approaches are often used by Registrants to fulfil legal information requirements for toxicological endpoints and to avoid testing on animals. According to the 5th report on the use of alternatives to testing on animals for the REACH Regulation, 22,8% of the substances registered under REACH rely on read-across for at least one endpoint to comply with the standard information requirements, making read-across the most commonly used adaption if experimental data are not available (ECHA 2023). Although REACH registrants

are responsible for the safety of the chemicals, they place on the EU market, the EU authorities are obligated to check whether a certain number of the registration dossiers are in compliance with the legal requirements. REACH was amended in 2020 to raise the minimum percentage of registration dossiers selected for compliance checks defined in Article 41(5) from 5% to 20% (EC 2020). Other regulatory actions such as substance evaluation, restriction proposals and testing proposals can also lead to authorities evaluating read-across cases. The RAAF provides guidance for evaluating whether the applied read-across is valid, or if testing of the specific registered substance is needed to fulfil the standard information requirements.

By including both the OECD GD 194 and the ECHA RAAF as frameworks for our analyses, we sought to examine and discuss as many aspects of the case examples as possible.

This report aims to analyse existing examples of groupings of endocrine disruptors (or other relevant substances using a relevant methodology), identify advantages and challenges in the case examples and develop recommendations for (sub-)grouping of endocrine disruptors for regulatory purposes with the ultimate aim of filling data gaps by read-across and/or regulate groups of substances, hereunder assign harmonized classifications.

Methodology

Five case examples were included in the project. The examples were chosen to represent both analogue and category approaches and also include cases developed for both scientific and regulatory purposes.

It was not possible to find many different examples of groupings of endocrine disruptors for regulatory purposes, and this affected the case selection for the project. Please note that the case examples of Isobutyl and Brominated Flame Retardants were developed by our own team at DTU Food. Therefore, in these instances, we assess our own work, while in other cases, we evaluate projects developed by external parties.

Several regulatory proposals for grouping of endocrine disruptors are under preparation but not yet available for analyses and inclusion in this project as case examples. We therefore had to focus on available examples, and this project includes two case examples for bisphenols and one for a paraben, which all had a clear regulatory scope. ECHA has published many Assessments of Regulatory Needs (ARNs) for different groups of substances, which potentially include endocrine disruptors. The ARN for bisphenols was included in this project to complement the two other case examples on bisphenols. The Isobutylparaben case was included as it is an example of application data gap filling by read-across to identify an endocrine disruptor. In addition, a comparative study of endocrine activity of bisphenols was included as a case example as it was referenced in the other case examples for bisphenols. Finally, we included an example of preliminary structural grouping of brominated flame retardants, supported by (Q)SAR predictions, which ultimately focused on (genotoxic) carcinogenicity as endpoint.

Even though the main focus of this work was not endocrine disruption, we included it here as a learning example using an approach that could also be applied in groupings of endocrine disruptors.

In this report, for each case example, the purpose of the assessment and the methodology applied was described in brief. Each case example was analysed using the frameworks of the OECD GD 194 on Grouping of Chemicals (OECD 2014) and the ECHA RAAF (ECHA 2017). The OECD GD on Grouping of Chemicals lays out stepwise procedures for analogue and category approaches. The RAAF provides a framework for examining read-across cases in the context of the REACH Regulation with specified assessment elements (AEs) depending on the relevant assessment scenario (1–6), as outlined in Figure A in Annex 1.

Both documents discriminate between the use of analogue and category approaches. For each case example it was therefore identified which of these approaches was appropriate and applied. In an analogue approach, the aim of the

assessment is to fill a data gap for one target substance. In a category approach, the aim is to assess a given property of all the individual substances in the category based on available information for the category source substances and fill data gaps for the target substances. A 'category' in these documents is a group of structurally similar substances. Within an established group of structurally similar substances, a data gap might be filled by read-across. In practical terms, the use of the phrases 'category' and 'group' are often used interchangeably (see also section on definitions).

Both documents describe several critical aspects of the read-across with specific aspects and questions to be considered. For the RAAF, all the assessment steps can be concluded with an assessment of the overall reliability (score 1–5) as shown in Figure B in Annex 1.

The five different case examples included in the report had different objectives, and therefore different approaches were used. Two of the bisphenol cases (the ECHA ARN case and the restriction proposal case) did not aim at data gap filling, but rather focused on category formation. The last bisphenol case was a comparative study, which did not even aim at category formation, but rather on identifying structural alerts for specific ED mechanisms of action of bisphenols. However, the bisphenols in the comparative study were part of the in the category formation in the two other bisphenol case examples. In contrast, the aim of the isobutylparaben case and the brominated flame retardants case was data gap filling and therefore both category formation steps and additional steps recommended in the OECD GD 194 and the ECHA RAAF were included in these cases. However, in all case examples, the included steps, including the category formation steps, were analysed according to the OECD GD 194 and the ECHA RAAF, respectively. This meant that even though the objective of some of the case examples was not data gap filling by read-across, the category formation conducted was evaluated in the perspective of potential subsequent data gap filling by read-across.

On this basis, for all case examples, advantages, challenges, uncertainties and regulatory perspectives were discussed. Learnings, also those specific to endocrine disruptors, were extracted.

Based on the analyses and discussion of the case examples, recommendations for (sub-)grouping of endocrine disruptors were developed with the ultimate aim of filling data gaps by read-across.

Analyses of case examples: Grouping of bisphenols

Three cases of grouping of bisphenols were analysed, as described in the following.

Case 1: Bisphenols, Assessment of Regulatory Needs

Purpose of assessment

The purpose of the ECHA Assessments of Regulatory Needs (ARN) on bisphenols was to help authorities to conclude on the most appropriate way to address identified concerns for this group of substances within the EU regulatory system. It considered combinations of regulatory risk management instruments needed to introduce regulatory measures, including intermediate steps such as data generation. For different sub-groups of bisphenols, it was concluded based on available data that different regulatory actions were required at EU level, including identification as substances of very high concern (SVHCs), harmonized classification for Repr. 1B, specific restriction proposals and data generation (ECHA 2021).

Description of methodology applied

148 substances were included in the report based on structural similarity, with the presence of the para-bisphenol moiety being the unifying factor for bisphenols (see Figure 1).

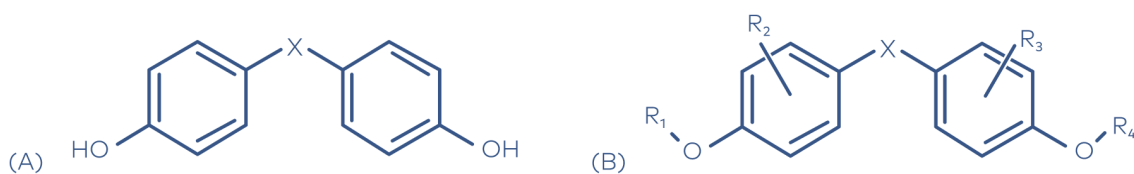


Figure 2 Generic structure of bisphenols adapted from the ECHA ARN (ECHA 2021)

(A) Generic "bisphenol" structure, with "X" representing the bridge between phenyl rings. There may be additional groups attached to the bridging atom(s). (B) General "bisphenol derivatives" structure. R_n can be the same/ different groups (R_n does not cover halogen substituents).

These 148 substances were divided into 6 sub-groups. 4 out of the 6 sub-groups were based on a common bisphenol bridge (denoted X in Figure 1), while the last two were characterized as either "Miscellaneous bisphenols" or "Other aliphatic or aryl bridged bisphenol derivatives" based on practical reasons:

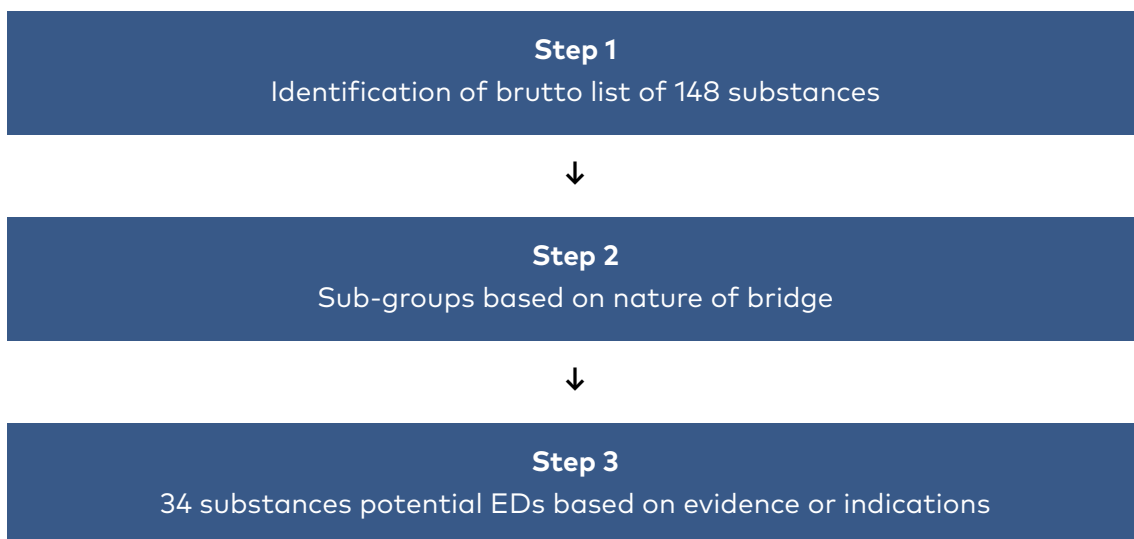
- BPA and BPA derivatives – 84 substances, of which 35 were not registered under REACH.
- BPS and BPS derivatives – 11 substances
- BPF and BPF derivatives – 17 substances
- BPAF and its derivatives – 9 substances
- Miscellaneous bisphenols – 17 substances
- Other aliphatic or aryl bridged bisphenol derivatives – 10 substances.

Bisphenols and bisphenol derivatives were further defined by the substituents of the phenyl rings and/or the substitution of the phenyl hydroxy groups (represented by R_n in Figure 1). For example, BPA and all BPA derivatives share a common bisphenol bridge, but while the phenyl rings of BPA contain hydroxy groups only (in addition to the bisphenol bridge) the BPA derivatives have different substituents at the phenyl rings or may have the phenolic hydroxyls derivatized (hydrogens are substituted, e.g. with ether bonded groups (R-O-R')) (ECHA 2021).

Further limits were set on the bisphenols included in the report. A molecular weight limit was arbitrarily set to 600 or below. Some specific bisphenols (TBBPA and TBBPA-derivatives) were excluded due to their use as flame retardants and information suggesting a possible distinct hazard profile (ECHA 2021).

In a third step, 34 of the 148 substances were identified as a sub-group with a need for further risk management measures, ultimately in the form of a restriction based on the substances being known or potential EDs or substances that could be identified as toxic for reproduction. The identification was related to experimental evidence or indications of ED effects, including:

- substances already identified as SVHCs for ED for human health or reproductive toxicity (3 substances)
- substances with information evaluated to be possibly sufficient for identification as SVHCs for ED for human health or reproductive toxicity (including in some cases read-across considerations) (12 substances)
- substances with data suggesting ED properties, but where further data generation may be required (9 substances)
- substances containing BPA, BPF and BPS (10 substances)



Workflow 1 Bisphenols, Assessment of Regulatory Needs (ECHA 2021)

Comparison with procedures described in the OECD GD 194

The purpose of the ARN was to help authorities conclude on the most appropriate way to address the identified concerns for a group of substances, or a single substance, based on available information about their known or potential hazards. No attempt was made to fill data gaps in the bisphenol assessment of regulatory needs. The case example could in the framework of the OECD GD 194 be interpreted as an identification of category members in a larger group (step 1) and smaller sub-groups (step 2). In contrast, step 3 was a selection of substances with available data sufficient for further regulation or data generation. The identification of category members is described in step 1 of the stepwise approach in the OECD GD 194, see table 1.

Table 1 Analysis of case 1 (Bisphenols, Assessment of Regulatory Needs) according to the stepwise approach recommended in the OECD GD 194.

Step	Stepwise approach recommended in the OECD GD 194.	Analysis of case 1 (Bisphenols, Assessment of Regulatory Needs)
0	Check whether the substance is a member of an existing category	Not performed.
1	Develop category hypothesis and definition and identify category members	In the first step, the 148 members of the group of bisphenols were identified based on structural similarity, with the common moiety being the "bisphenol" structure composed of two phenyl rings with a bridge between them and the hydroxyl groups being in the para-position. One physico-chemical property was used in the definition of the group; A molecular weight of 600 was arbitrarily set as cut off. These elements were in line with the recommendations in the OECD GD 194. However, the group of bisphenols was non-exhaustive as inclusion in the group required registration under REACH or a CLP notification. Furthermore, some specific substances were excluded (TBBPA and derivatives) due to their specific uses as flame retardants and some specific hazard information. The arguments for excluding substances with bromine are not reported. It can be valid to exclude substances based on specific toxicological properties but the arguments and justification for exclusions should be included in a transparent manner. If substances were excluded only based on their use, this seems like a pragmatic approach which is not in line with recommendations in the OECD GD 194. As in step 0, this discrepancy can be explained by the purpose of the ARN, which was not to build groups for subsequent data gap filling by read-across. In the second step, the substances were sub-grouped based on a structural feature, i.e. the nature of the bisphenol bridge. While the grouping of the 148 bisphenols was based on a concern for the toxicological properties of this group of substances, the sub-grouping (based on the nature of the bisphenol bridge) was not argued to be linked to any specific properties (e.g. specific toxicological effects or mechanisms) of the substances. The nature of the bisphenol bridge is recognized to be linked to modulation of e.g. estrogenic activity, but the main driver for endocrine activity seems to be at least one OH-group in a para position (Kitamura et al., 2005).

Step	Stepwise approach recommended in the OECD GD 194.	Analysis of case 1 (Bisphenols, Assessment of Regulatory Needs)
		Using the bisphenol bridge to sub-group is thus not in line with the recommendations in the OECD GD 194. Other molecular features would be more relevant to use as structural basis for the sub-grouping. In the third step, 34 substances from different sub-groups were identified as having a need for further restrictions based on available data. This was not based on structural similarities but specific evidence or indications on endocrine disruption or reproductive toxicity available for each substance. This approach therefore did not follow the recommendations in the OECD GD 194, but a structural grouping was also not the intention. The data mapping conducted in this step, including knowledge of regulatorily identified EDs, could be valuable in future grouping approaches where data rich substances could be used as source substances. The subsequent steps recommended in the OECD GD 194 for category formation, including data gathering, evaluation of the category(ies) and documentation of the category approach were not conducted
2	Gather data for each category member	Not performed.
3	Evaluate available data for adequacy	Not performed.
4	Construct a matrix of data availability	Not performed.
5	Perform a preliminary evaluation of the category and fill data gaps	Not performed.
6	Perform and/or propose testing	Not performed.
7	Perform a further evaluation of the category	Not performed.
8	Document the finalized category and refine the category rationale	Not performed.

Examination according to the ECHA RAAF

As for the comparison with the OECD GD 194, focus on the case example according to the ECHA RAAF was on the rationale for forming the category, which was evaluated by use of the first two assessment elements (AE) for category approaches: AE C.1 "Substance characterization" and AE C.2 "Structural similarity and differences within the category", see table 2.

Table 2 Analysis of case 1 (Bisphenols, Assessment of Regulatory Needs) according to approach recommended in the ECHA Read-Across Assessment Framework (RAAF).

Code	Approach recommended in the ECHA RAAF	Analysis of case 1 (Bisphenols, Assessment of Regulatory Needs)
AE C.1	Substance characterization Chemical identity and impurity profile of each category member are sufficiently detailed for assessment of the category approach.	This assessment element is judged to be acceptable with just sufficient confidence (notable reservations). Chemical identity was reported by EC numbers. CAS numbers were reported when available. Names, synonyms and structures were also reported, where available. Thus, the identity of the substances was generally well defined, but no impurity profiles were reported. Some of the substances were UVCBs with no further information on composition, and for some substances the structure was not publicly available.
AE C.2	Structural similarity and differences within category Structural similarities among all members are identified and structural differences allowed within the category are described.	This assessment element is judged to be acceptable with medium confidence (minor reservations) in the first two steps (see workflow 1 above). The third step was not based on structural similarities and is therefore not possible to assess for this element. In the first step, the 148 members of the group of bisphenols were identified based on structural similarity, with the common moiety being the "bisphenol" structure composed of two phenyl rings with a bridge between them and the hydroxyl groups being in the para-position. It was further defined that additional groups may be attached to the bridging atoms and that such additional groups may not be halogens. Thus, structural similarities among all members were identified and structural differences allowed within the category were described. In the second step, the substances were sub-grouped based on the nature of the bisphenol bridge. As in the first step, structural similarities among all members were identified and structural differences allowed within the category were described.

Code	Approach recommended in the ECHA RAAF	Analysis of case 1 (Bisphenols, Assessment of Regulatory Needs)
		In the third step, 34 substances from different sub-groups were identified as having a need for further restriction. This was not based on structural similarities but specific evidence or indications on endocrine disruption or reproductive toxicity for each substance. In this step, the assessment element in the RAAF cannot be used since the grouping was not based on structural similarity. Instead, substances were clustered in groups depending on regulatory readiness evaluated on the basis of available information in the registration dossiers. It is noted that no UVCBs were included in the sub-category of substances proposed for further regulatory actions.
AE C.3	Link of structural similarities and structural differences with the proposed regular pattern A category hypothesis has been provided and whether it applies to all category members.	Not performed.
AE C.4	Consistency of effects in data matrix Construct a data matrix for all category members vs. existing experimental data, arranged in suitable order to reflect trends or progression across the category.	Not performed.
AE C.5	Reliability and adequacy of the source study(ies) Study design of source substance(s) fulfills the information requirement and the test material(s) correctly represent source substance(s) in terms of purity and impurities.	Not performed.
AE C.6	Bias that influences the prediction Is inclusion of other structurally similar substances in the category possible or would they change the prediction of properties for the target substance(s)? The source substance(s) used for the predictions corresponds to the reliable study(ies) giving rise to the highest concern for the properties under consideration.	Not performed.

Discussion of the case example: Assessment of Regulatory Needs for bisphenols

The Assessment of Regulatory Needs (ARN) entailed a grouping of structurally similar substances based on the bisphenol moiety and a subsequent structural sub-grouping of different bisphenol types based on the nature of the bisphenols bridge. Finally, bisphenols were clustered into groups based on the regulatory readiness due to available hazard information in the registration dossiers. As illustrated in table 1 and 2, the grouping approach in the ARN focused on identification of category members and relied on available experimental data to conclude on the most appropriate way to address the identified concerns. Later steps recommended in the OECD GD 194 (step 2-8) and RAAF (AE C3-C6 and scenario specific AEs) were not performed. Data gap filling by read-across was not performed and not part of the scope of the ARN. Even though the objective of the case example was not data gap filling by read-across, the category formation conducted was evaluated in the perspective of potential subsequent data gap filling by read-across in order to extract general learnings.

Advantages

The ECHA ARN provided a good overview of the bisphenols registered under REACH and/or classified under CLP. The 34 substances identified as ready for (further) regulatory action may be valuable source substances in future category formations aiming at data gap filling by read-across (see also "Regulatory perspective" below).

Challenges

The initial list of 148 bisphenols was not exhaustive, since some bisphenols may be left out if they were not registered under REACH or classified under CLP. Furthermore, some substances were excluded due to an arbitrary molecular weight cut off and arguments about use (flame retardants) combined with possible distinct hazard profile (not substantiated). If considering category formation aiming at subsequent data gap filling by read-across, this exclusion of substances early in the grouping exercise is not recommended since it leaves substances out which may prove valuable source substances, even though they are not e.g. registered under REACH, classified according to CLP or have a slightly higher molecular weight than the cut-off.

The case example does not describe information about impurity profiles, and structures of some of the substances in the group are lacking. This information should be taken into consideration if subsequent data gap filling by read-across is considered. Further, the relatively large group of bisphenols that are UVCBs may prove challenging to include in subsequent data gap filling by read-across as knowledge about their specific composition is required to assess their effects.

ECHA has developed a read-across assessment framework targeted multi-constituent substances and UVCBs, which has not been part of the scope of this report (ECHA 2017b).

The considerations leading to the division of the 148 bisphenols into 6 sub-groups defined by the bisphenol bridge were not provided in the published ARN. It is therefore not possible to evaluate the reasoning or hypothesis behind this sub-grouping in detail. It is noted that, according to Kitamura et al., 2005 (which is further discussed in section 1.3 of this report), the nature of the bisphenol bridge is recognized to be linked to modulation of e.g. estrogenic activity, but the main driver for endocrine activity seems to be at least one OH-group in a para position (and further refined, depending on the specific mechanism of action in focus). Other molecular features than the bisphenol bridge could therefore be more relevant to use as structural basis for the sub-grouping when focus is on endocrine disruptive properties.

The general scope of ECHAs ARNs include selected hazards, namely CMR, sensitization, ED, PBT/vPvB or equivalent (such as PMT/vPvM) and aquatic toxicity. The main potential hazards for the group of 148 bisphenols were specified to be ED for human health and the environment, reproductive toxicity and skin sensitization, and for some also PBT/vPvB. Bisphenols may have other known or potential hazards that are not covered by the ARN. As grouping and read across is endpoint specific, different sub-groups of bisphenols may be relevant to form depending on the endpoint under evaluation. For each sub-group, the structural moiety(ies) used to define the sub-group should be relevant to the endpoint under scrutiny.

Uncertainties

The scope of the ARN was to assess the regulatory need for bisphenols. The identification of the 34 substances with a need for further restriction was not based on structural similarities but specific evidence or indications on endocrine disruption or reproductive toxicity for each substance. The potential uncertainty for the 34 substances included in this part of the assessment is therefore considered low/not significant. For the remaining bisphenols, the uncertainty about potential hazards is high as the ARN did not attempt to read across from data rich to data poor bisphenols.

Regulatory perspectives

The grouping in the ARN reports can be considered as a preparatory step to further regulatory actions, as the scope of the ARN groupings is not to fill data gaps by use of analogue or category approaches. The data mining leading to identification of 34 substances ready for (further) regulatory action provides valuable information. Regulating the bisphenols with available data adequate for harmonized classification as proposed by ECHA could be an important first step towards using

these data-rich substances (e.g. those identified as endocrine disruptors) as source substances to fill data gaps for less data rich bisphenols and facilitate regulation, as appropriate. The bisphenols with ongoing data generation, through e.g. substance evaluation, compliance check or testing proposals by registrants, could be included in such approaches as either source or target substances, depending on data available. The identification of substances with need for further restriction in the ARN can thus be seen as a preliminary sub-grouping where regulatory risk management for the data rich bisphenols could pave the way for subsequent scientifically solid sub-grouping hypotheses supported by read across for specific endpoints to fill data gaps for less data rich bisphenols.

Learnings

This case focused on category formation, but with potential subsequent data gap filling by read-across in mind, the following learnings were extracted:

- Lack of information about impurity profiles and chemical structures may challenge subsequent data gap filling by read-across.
- UVCBs may be challenging to include in subsequent data gap filling by read-across. ECHA has developed a specific guidance to do so (further consideration was out of the scope of this report).
- When aiming at data gap filling by read-across, it is important to start with broad groups of substances. Exclusion of substances early in the process based on arguments like use profiles or lack of CLP classification or REACH registration is not recommended since such substances may be valuable source substances for data gap filling.
- Sub-grouping should optimally be based on structural similarity considerations relevant to the endpoint under scrutiny. Different sub-groups may be relevant to form depending on the endpoint under evaluation.
- Data-rich substances (e.g. those identified as endocrine disruptors) may be valuable as source substances in future regulatory grouping approaches.

Learnings specific to endocrine disruptors

- Endocrine disruption was approached in the ARN in the same way as the other included hazards of concern.

Case 2: Proposal for restriction of bisphenol A and qbisphenols of similar concern for the environment

Purpose of assessment

The document presented a proposal for a restriction of bisphenol A and bisphenols of similar concern under the REACH Regulation based on the substances being endocrine disruptors in the environment.

Description of methodology applied

The group of substances was defined as those that had been identified as SVHCs for endocrine disruption in the environment (BPA+BPB) and substances that fulfilled the WHO/IPCS criteria for endocrine disruptors in the environment (BPS, BPF, BPAF and its salts). It is noted that BPS after the submission of the restriction proposal has been identified as an SVHC due to its endocrine disrupting properties for human health and the environment (ECHA 2022a).

Based on the group substances, structural boundaries were defined for possible inclusion of additional substances in the group in the future. The structural boundaries would be used as a requirement for inclusion together with a requirement that any additional substances had been identified as endocrine disruptors for the environment under REACH (SVHCs), CLP (category 1), Plant Protection Products Regulation (PPPR) or Biocidal Products Regulation (BPR).

The structural boundaries were based on information from the scientific literature. A primary source of information was a publication by Kitamura et al. 2005, which is further discussed in case 3. The structural alerts highlighted by Kitamura et al. were illustrated in the restriction proposal with reference to the scientific publication. See figure 3.

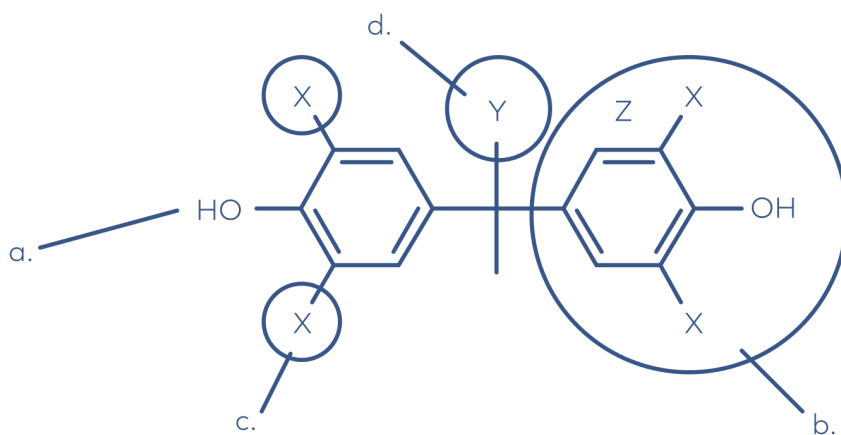


Figure 3 Illustration of structural requirement of bisphenol A and related substances for endocrine activity (adapted from the Restriction proposal (ECHA 2022) with reference to Kitamura et al., 2005).

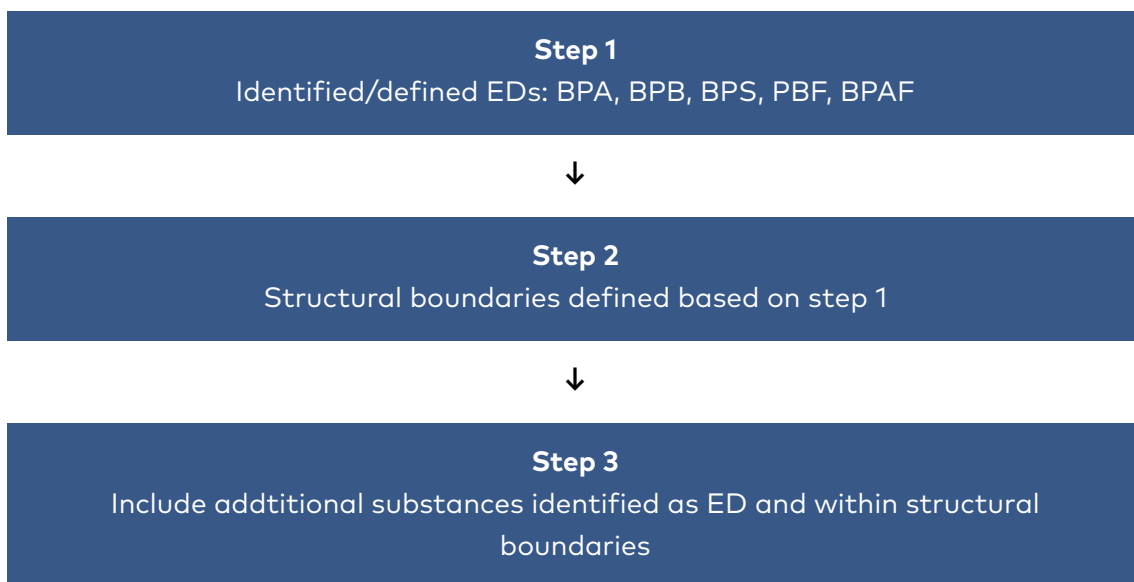
a.: Essential for estrogenic, antiandrogenic and thyroid hormonal activities. b.: Enhancing for estrogenic and thyroid hormonal activities. c.: Regulating for estrogenic and antiandrogenic activities. Essential for thyroid hormonal activity. d.: Regulating for estrogenic and antiandrogenic activity.

It was further elaborated that:

- Estrogenic activity required an unhindered hydroxyl group in the para-position and could be modulated by the distance between the para hydroxyl groups and the nature of the bisphenol bridge. Also, increasing polarity could reduce estrogenic activity.
- Anti-androgenic activity required an unhindered hydroxyl group in the para-position. Thyroid receptor activation required a hydroxyl group in the para-position and double substitution by a halogen or methyl group at the 3,5-positions of the one phenyl group.

Based on this, the group boundaries were defined as: "Bisphenols, HO-(R1)-R2-(R3)-OH with R1 and R3 being phenylene groups bearing any substituents at any ring position and R2 being a methylene group being unsubstituted or bearing any substituents or another bridging unit bearing unspecified substituents, which are listed in Appendix X and their salt". Appendix X lists the substances included in the group, i.e. BPA, BPB, BPS, BPF and BPAF, as described above.

Individual hazard assessments of the substances in the group were used to argue for their inclusion.



Workflow 2 Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment (ECHA 2022)

Comparison with procedures described in the OECD GD 194

Even though no attempt was made to fill data gaps in the proposal for restriction of bisphenols, the approach applied in step 3 of the workflow above could be interpreted as an identification of category members (described in step 1 of the stepwise approach in the OECD GD 194) and was analysed as such in the following, see table 3.

Table 3 Analysis of case 2 (Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment) according to the approach recommended in the OECD GD 194

Step	Approach recommended in the OECD GD 194	Analysis of case 2 (Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment)
0	Check whether the substance is a member of an existing category	Not performed.
1	Develop category hypothesis and definition and identify category members	The category formation was based on structural similarity with specified structural molecular alerts for endocrine disruption as shown by Kitamura et al. 2005 and with the structural boundaries of the group being HO-(R1)-R2-(R3)-OH, with R1 and R3 being phenylene groups bearing any substituents at any ring position, and R2 being a methylene group being unsubstituted or bearing any substituents or another bridging unit bearing unspecified substituents, which are listed in Appendix X (i.e. the included bisphenols and salts). The justification of the category included consideration of chemical structure, composition and functional groups with specific structural moieties being linked to specific common mechanisms. Further justification was based on similarity to substances already being identified as endocrine disruptors. This is as such in line with the recommendations in the OECD GD 194. However, adversity of bisphenols comprises a broad variety effects, which could potentially be induced by several different mechanisms and modes of action. Some degree of estrogenicity, anti-androgenicity and thyroid system interference could all be at play. Each of these modalities encompass many different mechanisms of action interlinked in a complex adverse outcome pathway (AOP) network. No attempt was made to link specific molecular moieties to specific mechanisms, modes of action or endpoints of concern. Further, the molecular moieties relevant to each mechanism, mode of action and adverse outcomes could potentially overlap, but such an analysis was also not included in the report and therefore not reflected in the identification of members of the bisphenol group. This is not in line with the OECD GD 194.
2	Gather data for each category member	As available from literature or used in regulatory hazard assessment such as SVHC identification or CLH proposals. This is in line with the recommendations in the OECD GD 194.
3	Evaluate available data for adequacy	Not performed.
4	Construct a matrix of data availability	Not performed.

Step	Approach recommended in the OECD GD 194	Analysis of case 2 (Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment)
5	Perform a preliminary evaluation of the category and fill data gaps	Not performed.
6	Perform and/or propose testing	Not performed.
7	Perform a further evaluation of the category	Not performed.
8	Document the finalized category and refine the category rationale	Not performed.

Examination according to the ECHA RAAF

As for the comparison with the draft OECD GD 194, focus of the examination according to the ECHA RAAF was on the rationale for forming the category in step 3 of the workflow, which was evaluated by use of the first two common assessment elements for category approaches, C.1 and C.2, see table 4.

Table 4 Analysis of case 2 (Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment) according to the approach recommended in the ECHA Read-across Assessment Framework (RAAF)

Code	Approach recommended in RAAF	Analysis of case 2 (Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment)
AE C.1	Substance characterization Chemical identity and impurity profile of each category member are sufficiently detailed for assessment of the category approach.	This assessment element is judged to be acceptable with high confidence (no reservations). Chemical identity was reported by EC numbers and CAS numbers. Names, synonyms and structures were also reported for all substances. For BPF, which is a multi-constituent substance, all constituents were reported with EC numbers, CAS numbers and structures. Thus, the identity of the substances was well defined, but no impurity profiles were reported, which could be a challenge in subsequent analogue or category approaches.

Code	Approach recommended in RAAF	Analysis of case 2 (Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment)
AE C.2	Structural similarity and differences within category Structural similarities among all members are identified and structural differences allowed within the category are described.	This assessment element is judged to be acceptable with high confidence. Bisphenols included in the group in step 3 in the workflow was defined as having to be identified as EDs and structurally within the defined boundaries: HO-(R1)-R2-(R3)-OH with R1 and R3 being phenylene groups bearing any substituents at any ring position and R2 being a methylene group which is unsubstituted or bearing any substituents or another bridging unit bearing unspecified substituents, which are listed in Appendix X (i.e. the included bisphenols and salts). Thus, structural similarities among all members were identified and structural differences allowed within the category were described.
AE C.3	Link of structural similarities and structural differences with the proposed regular pattern A category hypothesis has been provided and whether it applies to all category members.	Not performed.
AE C.4	Consistency of effects in data matrix Construct a data matrix for all category members vs. existing experimental data, arranged in suitable order to reflect trends or progression across the category.	Not performed.
AE C.5	Reliability and adequacy of the source study(ies) Study design of source substance(s) fulfills the information requirement and the test material(s) correctly represent source substance(s) in terms of purity and impurities.	Not performed.
AE C.6	Bias that influences the prediction Is inclusion of other structurally similar substances in the category possible or would they change the prediction of properties for the target substance(s)? The source substance(s) used for the predictions corresponds to the reliable study(ies) giving rise to the highest concern for the properties under consideration.	Not performed.

Discussion of the case example: Proposal for restriction of bisphenol A and bisphenols of similar concern for the environment

The proposed restriction covered a group of bisphenols defined by structural boundaries and with the single substances being identified as ED ENV under REACH (SVHC), CLP (category 1), PPPR or BPR in the future. The case example thus focused on identification of category members. Later steps recommended in the OECD GD 194 (step 2-8) and the ECHA RAAF (AS C3-C6), including data gap filling by read-across, were not performed. Even though the objective of the case example was not data gap filling by read-across, the category formation conducted was evaluated in the perspective of potential subsequent data gap filling by read-across in order to extract general learnings.

Advantages

The category formation conducted in this case example was in general compliant with the recommended steps in the OECD GD 194 and the ECHA RAAF.

Challenges

Inclusion in the group of restricted substances required both structural similarity to substances already included as well as regulatory identification of the substance as an endocrine disruptor. The last requirement usually requires substantial substance specific information, and thus limits the number of substances included in the group of bisphenols to data rich substances, already identified as EDs. Further, no attempt was made to analyse the link between the activity of the functional groups of the structural moieties to the effects on specific endpoints of concern and form broader groups of bisphenols based on the endpoints affected, possibly supported by use of (Q)SARs.

Adversity of bisphenols comprise a broad variety of effects, which could potentially be induced by several different mechanisms and modes of action. Some degree of estrogenicity, anti-androgenicity and thyroid system interference could all be at play. Each of these modalities encompasses many different mechanisms of action interlinked in a complex adverse outcome pathway (AOP) network. No attempt was made to link specific molecular moieties to specific mechanisms, modes of action or endpoints of concern. Further, the molecular moieties relevant to each mechanism, mode of action and adverse outcomes could potentially overlap, but such an analysis was also not included in the report.

Uncertainties

The restriction proposal has been withdrawn due to comments received in the public consultation. It is not known whether the scope of the restriction proposal will be revised before it is resubmitted.

Regulatory perspectives

From a regulatory perspective, the proposed restriction sought to open a direct path from ED identification to restriction under REACH. Included in the restriction would be substances regulatorily identified as EDs and within the structural boundaries defined in the proposal. Identification of EDs usually requires substantial substance specific information. However, a way to broaden the approach in future could be to use identified EDs as source substances for data gap filling by read-across to less data rich substances, which thereby could be identified as EDs and be included in the restriction.

Learnings

This case focused on category formation, but with potential subsequent data gap filling by read-across in mind, the following learnings were extracted:

- If possible, specific molecular moieties should be linked to specific mechanisms, modes of action or endpoints of concern. It should further be considered whether the molecular moieties relevant to the different relevant mechanisms, modes of action and adverse outcomes overlap.
- In the bisphenol restriction proposal case it was proposed to restrict the use of a group of structurally similar substances already identified as endocrine disruptors (i.e. information sufficient to conclude on this hazard property is already available). A way to broaden the approach in the future could be to use identified EDs as source substances for data gap filling by read-across to less data rich substances, which thereby could be identified as EDs and be included in such a restriction.

Learnings specific to endocrine disruptors

- Adversity of EDs may comprise effects through several adverse outcome pathways such as estrogenicity, anti-androgenicity and thyroid system interference. When developing sub-groups with a focus on ED properties, it is recommended to consider this aspect. Ideally, the importance of specific structural moieties for different mechanisms and thereby modes of action and adverse effects should be analysed.

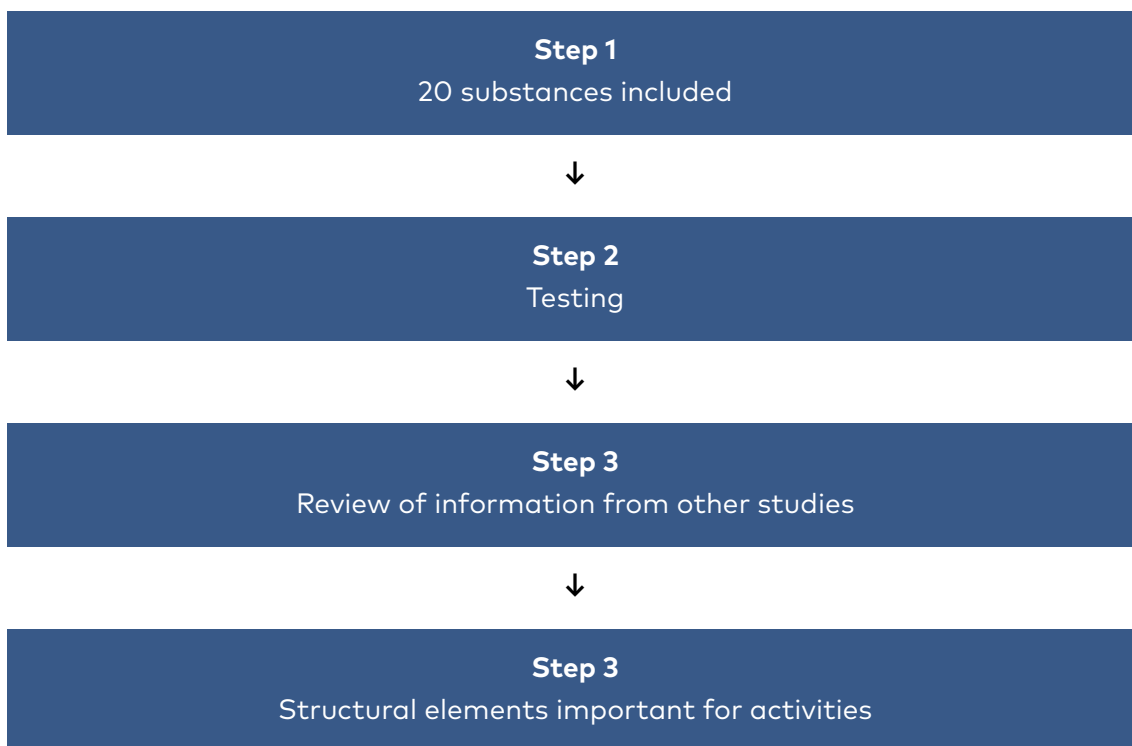
Case 3: Comparative study of 20 bisphenols

Purpose of assessment

The document is a peer reviewed publication. It is not presented as a grouping but as a comparative study where different endocrine activities were investigated *in vitro* for 20 different bisphenols: Estrogenic and anti-estrogenic activity in a reporter gene assay in MCF-7 cells (and for selected substances *in vivo* in the uterotrophic assay); Androgenic and anti-androgenic activity in a reporter gene assay in NIH3T3 cells and Thyroid disruptive activity in GH3 cells.

Description of methodology applied

Bisphenol A and 19 related substances were tested for estrogenicity, anti-estrogenicity *in vitro* and *in vivo*, androgenicity and anti-androgenicity *in vitro* and thyroid receptor activity *in vitro*. Based on the results and the structures of the tested substances and review of information from other peer reviewed studies, sub-structures of importance for the different activities were discussed.



Workflow 3 Comparative study of 20 bisphenols (Kitamura et al., 2005)

16 of the bisphenols induced estrogenic activity in the MCF-7 reporter gene assay, at varying concentrations.

Based on structural analyses of the substances positive versus negative for estrogenic activity and review of information from other peer reviewed studies, it was concluded that the following substructures influence the estrogenic activity of bisphenols: At least one hydroxyl group in para-position as well as the second phenyl group was found to be essential for estrogenic activity. Further, the bridge between the two phenylene rings was found to modify the estrogenic activity: When the bridge was more hydrophilic in nature than the propane bridge of BPA, the estrogenic activity was higher than for BPA, whereas it was lower when the bridge was more hydrophobic in nature.

Estrogenic activity was also investigated *in vivo* (in the uterotrophic assay) for some selected substances (TCBPA, TBBPA and BPA). All three substances induced uterotrophic effects *in vivo* with BPA being most effective, followed by TBBPA and TCBPA.

Two of the bisphenols induced anti-estrogenic activity in the MCF-7 reporter gene assay.

None of the bisphenols induced androgenic activity.

Fourteen of the bisphenols induced anti-androgenic activity in NIH3T3 assay.

Based on structural analyses of the substances positive versus negative for anti-androgen activity and review of information from other peer reviewed studies, it was concluded that the following substructures influence anti-androgenic activity of bisphenols: At least one 4-hydroxyl group was found to be essential for anti-androgenic activity. However, the second phenyl group was not. Further, 3,5-substituents were found to modify the activity, but not in the same direction; As TMBPA with methyl groups at the 3,5-positions had higher anti-androgenic activity than BPA, TCBPA and TBBPA with chloro- and bromine- groups, respectively, had no significant activity in this assay.

Three of the bisphenols (TMBPA, TCBPA, TBBPA) induced thyroid hormone dependent production of growth hormone in GH3 cells. All three have 3,5-substituents. It was concluded that a 4-hydroxyl group was essential for this activity, but that the bulky 3,5-group also play an important role in this activity.

Comparison with procedures described in the OECD GD 194

Even though no attempt was made to fill data gaps in this publication on bisphenols, the applied approach could be interpreted as a study to investigate structural features of importance for sub-categorization for different ED-related endpoints. The study therefore does not fit well into the OECD GD 194 for grouping, but relevant information is included for comparison where it fits best because the information is used in the other bisphenol grouping cases, see table 5.

Table 5 Analysis of case 3 (Comparative study of 20 bisphenols) according to the approach recommended in the OECD GD 194.

Step	Stepwise approach recommended in the OECD GD 194.	Analysis of case 3 (Comparative study of 20 bisphenols)
0	Check whether the substance is a member of an existing category	Not performed.
1	Develop category hypothesis and definition and identify category members	Structural boundaries for subcategories of bisphenols were defined based on testing of 20 bisphenols in vitro for (anti-)estrogenicity (also a few substances in vivo), (anti-)androgenicity and (anti-)thyroid receptor activity as well as review of information from other peer reviewed studies. In the first step, no reason was given for selecting the 20 specific bisphenols tested in the study (or exclusion of other bisphenols), which is not in line with the recommendations in the OECD GD 194. In the second step, chemical structure and functional groups were linked to specific common endocrine activities. This is in line with the recommendations in the OECD GD 194.
2	Check whether the substance is a member of an existing category	Not performed.
3	Evaluate available data for adequacy	Not performed.
4	Construct a matrix of data availability	Not performed.
5	Perform a preliminary evaluation of the category and fill data gaps	Not performed.
6	Perform and/or propose testing	Not performed.
7	Perform a further evaluation of the category	Not performed.
8	Document the finalized category and refine the category rationale	Not performed.

Examination according to the ECHA RAAF

As for the comparison with the OECD GD 194, the study does not fit well into the ECHA RAAF, but relevant information is included for comparison where it fits best because the information is used in the other bisphenol grouping cases, see table 6.

Table 6 Analysis of case 3 (Comparative study of 20 bisphenols) according to the approach recommended in the ECHA Read-across Assessment Framework (RAAF)

Code	Approach recommended in the ECHA RAAF	Analysis of case 3: Comparative study of 20 bisphenols
AE C.1	Substance characterization Chemical identity and impurity profile of each category member are sufficiently detailed for assessment of the category approach.	This assessment element is judged to be acceptable with minor reservations. Chemical identity was reported by name, abbreviation and structure. No impurity profile or CAS RN was reported for any of the included substances.
AE C.2	Structural similarity and differences within category Structural similarities among all members are identified and structural differences allowed within the category are described.	If this had been a grouping exercise and not a comparative study, this assessment element would have been judged to be not acceptable in its current form for the first step, where 20 bisphenol A derivatives were included in the study without justification (see workflow 3 above). Apart from the substances being referred to as "bisphenol A and related compounds", no reason for the initial selection of these 20 bisphenols was reported, and no detailed analysis in terms of structural similarity or differences within this group of substances was reported. This is not in line with the RAAF and leads to an uncertainty about whether other bisphenols could have been included. For the second step in the workflow (see workflow 3 above): If this had been a grouping exercise and not a comparative study, this assessment element would have been judged to be not acceptable in its current form based on test results and review of existing literature, substructures important for the (anti-)estrogenic, (anti-)androgenic and thyroid activities were discussed. For each subcategory, the structural boundaries were discussed, but neither the exact structural similarities required, nor the structural differences allowed within the subcategories were clearly identified.

Code	Approach recommended in the ECHA RAAF	Analysis of case 3: Comparative study of 20 bisphenols
AE C.3	Link of structural similarities and structural differences with the proposed regular pattern A category hypothesis has been provided and whether it applies to all category members.	Not performed.
AE C.4	Consistency of effects in data matrix Construct a data matrix for all category members vs. existing experimental data, arranged in suitable order to reflect trends or progression across the category.	Not performed.
AE C.5	Reliability and adequacy of the source study(ies) Study design of source substance(s) fulfills the information requirement and the test material(s) correctly represent source substance(s) in terms of purity and impurities.	Not performed.
AE C.6	Bias that influence the prediction - Is inclusion of other structurally similar substances in the category possible or would they change the prediction of properties for the target substance(s)? - The source substance(s) used for the predictions corresponds to the reliable study(ies) giving rise to the highest concern for the properties under consideration.	Not performed.

Discussion of the case example: Comparative study of 20 bisphenols

The comparative study of BPA and 19 related substances sought to define substructures of bisphenols relevant to endocrine ((anti-)estrogenic, (anti-)androgenic and (anti-)thyroid) activity based on *in vitro* (and for (anti-)estrogenicity also *in vivo*) findings. This can be interpreted as a category formation exercise and was analysed as such. Data gap filling by read-across was not performed and not part of the scope of this study. Even though the objective of the case example was not data gap filling by read-across, the category formation conducted was evaluated in the perspective of potential subsequent data gap filling by read-across in order to extract general learnings.

Advantages

Comparative studies like this are valuable for mechanistic understanding connected to molecular moieties of the chemical structures. They can be combined with e.g. docking simulations and (Q)SAR predictions for deeper understanding of the results.

Establishing links between specific molecular moieties and interaction with the estrogen, androgen and thyroid receptor could pave the way for formation of sub-categories of bisphenols with these mechanisms of action. In the future, this approach could be broadened to other sub-categories of bisphenols with other mechanisms of action relevant to endocrine disruption.

Challenges

It is acknowledged that this was a comparative study and not a grouping exercise. However, if the conducted category formation is considered to potentially be used for subsequent data gap filling by read-across, it would have been valuable to adhere to some of the recommended steps in the ECHA RAAF and/or the OECD GD 194.

The initial selection of substances for inclusion seemed arbitrary and was not well-argued, as no reason was given for selecting the 20 specific bisphenols tested in the study and excluding other bisphenols. This led to a non-exhaustive group of bisphenols.

The identity of the substances was generally well defined, but no impurity profiles were reported, which could challenge potential conductance of subsequent data gap filling by read-across.

Structural boundaries were discussed, but neither the exact structural similarities required, nor the structural differences allowed within the subcategories were clearly identified in the study.

Further, the formation of sub-categories was based on endocrine activity, mainly *in vitro*. A few substances were tested both *in vitro* and *in vivo* for estrogenicity and considerations about ADME (Administration, Distribution, Metabolism and Excretion) of the substances for extrapolation between *in vitro* and *in vivo* was discussed and highlighted as important. The sub-categorization suggested in the publication is therefore mainly relevant when considering endocrine activity *in vitro*, whereas extrapolation to the *in vivo* situation requires inclusion of ADME properties of the individual substances.

Uncertainties

It is noted that the structural elements used as a basis for this grouping approach were connected to receptor activation (estrogen receptor, androgen receptor and thyroid receptor) only. This narrow focus on receptor interaction may lead to the exclusion of relevant substances with ED properties. Since EDs may act through many other mechanisms and modes of action, it may be valuable in the future to consider also other mechanisms of action possibly connected to other molecular

moieties of importance for grouping. For example, for EAS-modalities, molecular moieties related to effects on steroid synthesis may be of relevance, and for thyroid activity, molecular moieties related to e.g. TPO (thyroid peroxidase) inhibition, interaction with NIS (Sodium/Iodide symporter), DIOs (Iodothyronine deiodinases) and transporters could be considered in addition to thyroid receptor activation.

The robustness of the conclusions on the role of the molecular moieties are not evident since the total number of substances included in the analysis of what is decisive for the observed estrogenic, anti-androgenic and thyroid activity is not reported clearly. References are also given to other studies with similar findings. It also not clearly reported whether docking studies are considered, or the conclusions are based on statistics or merely several studies pointing in the same direction. As an example, at least one hydroxyl group in para-position as well as the second phenyl group was found to be essential for estrogenic activity. However, all the bisphenols have a second phenyl group and only 1 bisphenol in the comparative study has only one ring (IPP), so within the publication itself there are not a lot of substances to base this conclusion on. It is possible that QSARs could be applied to confirm, reject or revise the conclusions on the role of molecular moieties.

It is mentioned that increasing polarity could reduce estrogenic activity. Further, both TCBPA, TBBPA and BPA, induced uterotrophic effects in vivo with BPA being most potent, followed by TBBPA and TCBPA. This could indicate that Br and Cl substituents may reduce estrogenic activity. This, however, would have to be assessed further and is not discussed in the publication.

Regulatory perspectives

The findings of the comparative study have been used to define the scope of the restriction proposal for bisphenols. Using comparative studies as the basis for understanding how specific molecular moieties contribute to the specific observed mechanistic effects of bisphenols could potentially be expanded to other chemical groups.

Comparative studies like this are valuable and could provide the foundation for the development of regulatory relevant (sub)groups of EDs, including bisphenols. (Q)SAR predictions and docking studies could be used to substantiate the hypothesis that specific molecular moieties are linked to specific mechanisms or modes of action. Adding ADME and other relevant toxicological information in a data matrix (including data rich source substances and less data rich target substances) could aid subsequent data gap filling by read-across, as recommended in the OECD GD 194 and the ECHA RAAF.

Learnings

This case focused on category formation, but with potential subsequent data gap filling by read-across in mind, the following learnings were extracted:

- Lack of impurity profiles and identifiers as CAS numbers may be a challenge for potential subsequent data gap filling by read-across.
- Care should be taken in the initial selection of substances for inclusion, so that this is done as broad as possible and with well-argued inclusion and exclusion criteria.
- Considering ADME (Administration, Distribution, Metabolism and Excretion) of the individual substances is important to consider when extrapolating from *in vitro* to *in vivo*.

Learnings specific to endocrine disruptors

- Comparative studies are valuable and could provide the foundation for development of (sub)groups of EDs, including bisphenols. (Q)SAR predictions and docking studies could be used to substantiate the hypothesis that specific molecular moieties are linked to specific mechanisms or modes of action.
- When developing sub-groups with focus on ED properties, it is recommended to consider that different molecular moieties may be relevant to different mechanisms and thereby modes of action and adverse effects. Some overlap may occur, but this should ideally be analysed and described. The complexity increases when considering that some substances act through more than one mechanism and mode of action (i.e. some degree of e.g. estrogenicity, anti-androgenicity and thyroid system interference could all be at play at the same time). Special care is therefore recommended in (sub-) grouping of endocrine disruptors, taking this complexity into consideration.

Analyses of case examples:

Other cases

Case 4: Identification of Isobutylparaben as a Substance of Very High Concern (SVHC)

Purpose of assessment

The aim of this assessment was to identify isobutylparaben as a substance of very high concern (SVHC) due to its endocrine disruptive properties relevant to human health. However, a low number of studies on endocrine effects of isobutylparaben prevented the assessment of isobutylparaben as a SVHC based solely on the evidence from this substance. Therefore, data gap filling by use of read-across from butylparaben as the source substance was applied. Butylparaben was already characterized as a SVHC due to endocrine disruption relevant to human health and has a very similar structure to isobutylparaben.

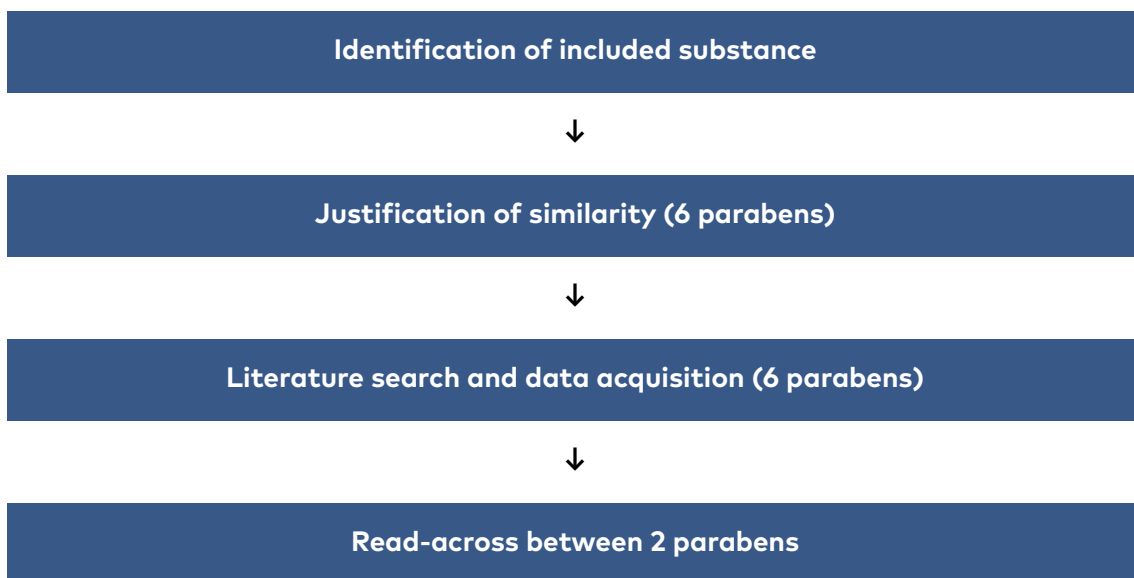
Description of methodology applied

This evaluation of isobutylparaben was based on read-across from butylparaben.

The first step of the read-across process was the justification of similarity between the two substances. The evaluation based the structural similarity on similarities in chemical structure, physico-chemical properties, toxicological effects and estrogenic properties. The two substances also have similar major metabolites. In addition to isobutylparaben and butylparaben, 4 other parabens (methyl, ethyl, propyl and isopropyl paraben) were included in the analysis, and trends across the category were analysed.

Following the identification and justification of the source substance, a thorough literature search was conducted, including studies from the open literature and data from ToxCast and ComTox chemicals dashboard as well as results from the Danish (Q)SAR database.

Mechanistic in vitro and in vivo data showed similar endocrine activity between the two substances (estrogenicity). Read-across from butylparaben to isobutylparaben was conducted to show adversity and a biologically plausible link between the two.



Workflow 4 Isobutylparaben. SVHC identification (ECHA 2022b)

Comparison with procedures described in the OECD GD 194

The assessment was an analogue approach, read-across from a source substance (butylparaben to a target substance (isobutylparaben), aiming to fill data gaps for isobutylparaben. For a comparison with the stepwise procedure recommended for analogue approaches in the OECD GD 194, each step in the procedure was outlined and assessed in table 9.

Table 9 Analysis of case 4 (Identification of Isobutylparaben as a Substance of Very High Concern (SVHC)) according to the approach recommended in the OECD GD 194

Step	Approach recommended in the OECD GD 194	Analysis of case 4 (Identification of Isobutylparaben as a Substance of Very High Concern (SVHC))
0	Check whether the substance is a member of an existing category	Not performed.
1	Identification of potential analogues	It was argued that IBP shared close structural similarity with butylparaben (BP), the only difference being that IBP had an isopropyl group at the end of the alkyl chain while BP had a butyl group. Four other parabens with shorter alkyl chain lengths were also identified, methyl-, ethyl-, propyl- and isopropylparaben. This is in line with the recommendations in the OECD GD 194.
2	Data gathering for the analogues	Data was gathered for the source substance BP. Data was also gathered for the four other structurally similar parabens identified, which in addition to BP could provide trend information relevant to the read-across. This is in line with the recommendations in the OECD GD 194.
3	Evaluation of available data for adequacy	Available data used to substantiate the read-across was evaluated to be reliable and adequate, as originating from either OECD TG studies or adequately reliable studies from the open literature. This is in line with the recommendations in the OECD GD 194.
4	Construct a matrix of data availability	Qualitative and to the extent possible, quantitative data matrixes were constructed for in vitro and in vivo endocrine activity and adverse effects for the group of six parabens, showing trends across the group with a particular emphasis on multiple lines of evidence for IBP and BP. This is in line with the recommendations in the OECD GD 194.

Step	Approach recommended in the OECD GD 194	Analysis of case 4 (Identification of Isobutylparaben as a Substance of Very High Concern (SVHC))
5	Assess the adequacy of the analogue approach and fill the data gap	The adequacy of the analogue approach was assessed as acceptable due to the demonstrated similarity in mechanistic information from a robust trend analysis including both the source substance and supported by 4 additional structural analogue substances in a congeneric series, data availability and results. It was argued that the lines of evidence showed correspondence between the results of in vitro and in vivo endocrine activity and available adversity endpoints in BP and IBP, justifying the read-across from the source substance BP to the target IBP for the data gap on adversity relating to effects on sperm quality in perinatally exposed rats. This is in line with the recommendations in the OECD GD 194. The data gap was filled in accordance with the recommendations in the OECD GD 194.
6	Document the analogue approach	The identification of IBP as an endocrine disruptor, including the information from the read-across in the analogue approach, was justified in accordance with the template for proposals for identifying substances of very high concern under the REACH Regulation (Annex VX Dossier template). This is in line with the recommendations of the OECD GD 194.

Examination according to the ECHA RAAF

In the RAAF, the analogue approach common AEs are also part of the category approach common AEs. For description of the common AEs, see table 10. The methodology for the case example of isobutyl paraben fits into both scenario 1 and 2; An analogue approach supported by biotransformation to common substances (scenario 1) and different substances having the same type of effects (scenario 2). The scenario-specific assessment elements are included in this case example, as a data matrix is constructed, and the relevant data gap(s) is filled by use of read-across.

The SVHC proposal for isobutylparaben describes that parabens are commonly metabolised (hydrolysed) by esterases with formation of a common metabolite parahydroxybenzoic acid (PHBA). Studies investigating the endocrine activity of the major metabolite PHBA show inconsistent results. With regards to metabolism of parabens in developing animals, limited capacity to metabolise BP has been found in offspring relative to dams during lactation. This suggests higher internal exposure levels of the parent molecule during the developmental phase (ECHA 2022b). In the examination according to the ECHA RAAF we therefore include scenario 2 (an analogue approach with different compounds having the same type of effect(s) as the most appropriate scenario as the read-across applied is for the data gap on adversity relating to effects on sperm quality in perinatally exposed rats.

The assessment elements (AE) specific for these scenarios are listed and assessed in table 10.

Table 10 Analysis of case 4 (Identification of Isobutylparaben as a Substance of Very High Concern (SVHC)) according to the approach recommended in the ECHA Read-across Assessment Framework (RAAF)

Code	Approach recommended in the RAAF	Analysis of case 4 (Identification of Isobutylparaben as a Substance of Very High Concern (SVHC))
AE A.1	Identity and characterization of source substance Chemical identity and impurity profile of each category member are sufficiently detailed for assessment of the category approach.	This assessment element is judged to be acceptable with high confidence. Chemical identity was reported by EC numbers and CAS numbers. Names, synonyms and structures were also reported for all substances. Both source and target substances are monoconstituent substances.
AE A.2	Link of structural similarities and differences with the proposed prediction A category hypothesis has been provided and whether it applies to all category members.	This assessment element is judged to be acceptable with high confidence due to the clear substance identification available for both source and target substances and the data availability <i>in vitro</i> and <i>in silico</i> predictions.
AE A.3	Reliability and adequacy of the source study(ies) Study design of source substance(s) fulfills the information requirement and the test material(s) correctly represent source substance(s) in terms of purity and impurities.	This assessment element is judged to be acceptable with high confidence as the source substance is well described in the literature and already identified as an SVHC based on its endocrine disrupting properties for human health.
AE A.4	Bias that influences the prediction Is inclusion of other structurally similar substances in the category possible or would they change the prediction of properties for the target substance(s)? The source substance(s) used for the predictions corresponds to the reliable study(ies) giving rise to the highest concern for the properties under consideration.	This assessment element is judged to be acceptable with high confidence as other parabens were assessed as potential source substances, and the final approach selected BP as the best suited source substance with supporting evidence from four other bisphenols providing information to a trend analysis.
Scenario 2-specific AEs		
AE 2.1	Substances the test organism is exposed to Have the substances the test organism is exposed to after administration of the source and target substances been identified?	This assessment element is judged to be acceptable with medium confidence (minor reservations). The level of mother substances and relevant metabolites, respectively, are not measured in all available <i>in vivo</i> studies. However, the major metabolite is identified to be similar between the target and source substances.
AE 2.2	Common underlying mechanism, qualitative aspects Has the common underlying mechanism for the observed effects been established and does it allow a prediction of qualitative similar effects?	This assessment element is judged to be acceptable with high confidence as a common underlying mechanisms has been identified. Qualitative aspects of the mechanisms of the source and target substances were shown in the data matrix.

Code	Approach recommended in the RAAF	Analysis of case 4 (Identification of Isobutylparaben as a Substance of Very High Concern (SVHC))
	Scenario 2-specific AEs	
AE 2.3	Common underlying mechanism, quantitative aspects Has it been established that the common underlying mechanism leads to the same quantitative outcome for the source and target substances?	This assessment element is judged to be acceptable with high confidence as potency of the mechanism is judged to be similar between the two substances. The quantitative aspects of the underlying mechanisms of the source and target substances were included in the data matrix, when available.
AE 2.4	Exposure to other substances than those linked to the prediction Has the possibility of other compounds than those linked to the prediction being present (impurities) or formed (intermediates, metabolites) been considered and if so, what their influence on the prediction is?	This assessment element is judged to be acceptable with high confidence as source and target substance were shown to have similar major metabolites, and their potential contribution to the effects observed was taken into consideration.
AE 2.5	Occurrence of other effects than covered by the hypothesis and justification Can other acting mechanisms of the source and/or target substance than the hypothesized one be present and contribute to the observed toxicity and can they impact the prediction?	This assessment element is judged to be acceptable with high confidence as mechanistic and toxicological information of source and target substances was described in detail.

Discussion

In this case example, a category was formed, a data matrix was developed and data gap filling by read-across is conducted. The outcome of the case was identification of isobutylparaben as a Substance of Very High Concern (SVHC) due to its endocrine disruptive properties relevant to human health under the REACH Regulation.

Advantages

The case example illustrated the value of developing a solid data matrix with all relevant data included. Although it was an analogue approach read-across between two substances, the case was supported by a trend analysis for four other structurally similar parabens with similar effects. The case is a solid example of a read-across that involves available *in silico*, *in vitro*, *in vivo* and ADME data to support the case. The approach was also supported by the fact that a lot of data was available for the included substances.

Challenges

When performing data gap filling by read-across, interpolation is preferred as it leads to a more robust read-across prediction, but extrapolation is often used in analogue approaches due to the limited number of substances involved, unless the hypothesis is that the source and target substances are transformed to common metabolites. In this case the read-across was an extrapolation performed between the two members of the paraben group with the longest carbon-backbone.

However, the read-across hypothesis was supported by the trend analysis and although this was not a category approach the available data allowed interpolation across the carbon-backbone lengths of all 6 parabens, ranging from IBP (3 carbons) to BP (4 carbons) as the source substance and the four other members (1–3 carbons).

Uncertainties

Not many uncertainties were identified in this case.

Regulatory perspective

This case is an example of a successfully applied read-across analogue approach in a regulatory context.

Learnings

- A solid data matrix (with ADME, *in silico*, *in vitro*, *in vivo* information) is a key tool for data gap filling.

Learnings specific for endocrine disruptors

- Similar structures combined with ADME, in silico, in vitro (and if available in vivo) data on endocrine activity can be used as a basis for read-across of adversity info for successful identification of endocrine disruptors based on data gap filling by read-across.

Case 5: Brominated flame retardants

Purpose of assessment

The aim of the project was to make preliminary structural groupings of brominated flame retardants (BFRs) for possible future work to address these by grouping approaches rather than as individual substances, as the regulation of a single substance is very time demanding and the process needs to start over if the molecular structure changes even slightly to have a new substance, possibly leading to regrettable substitution. One group was chosen for further work to explore identification of a critical health effect based on literature search and apply information from the experimental literature data and (Q)SAR predictions generated for all group members to make a preliminary justification for read-across for the critical effect in a category approach (Danish EPA, 2016).

Description of methodology applied

The grouping was conducted in several steps as illustrated in workflow 5.

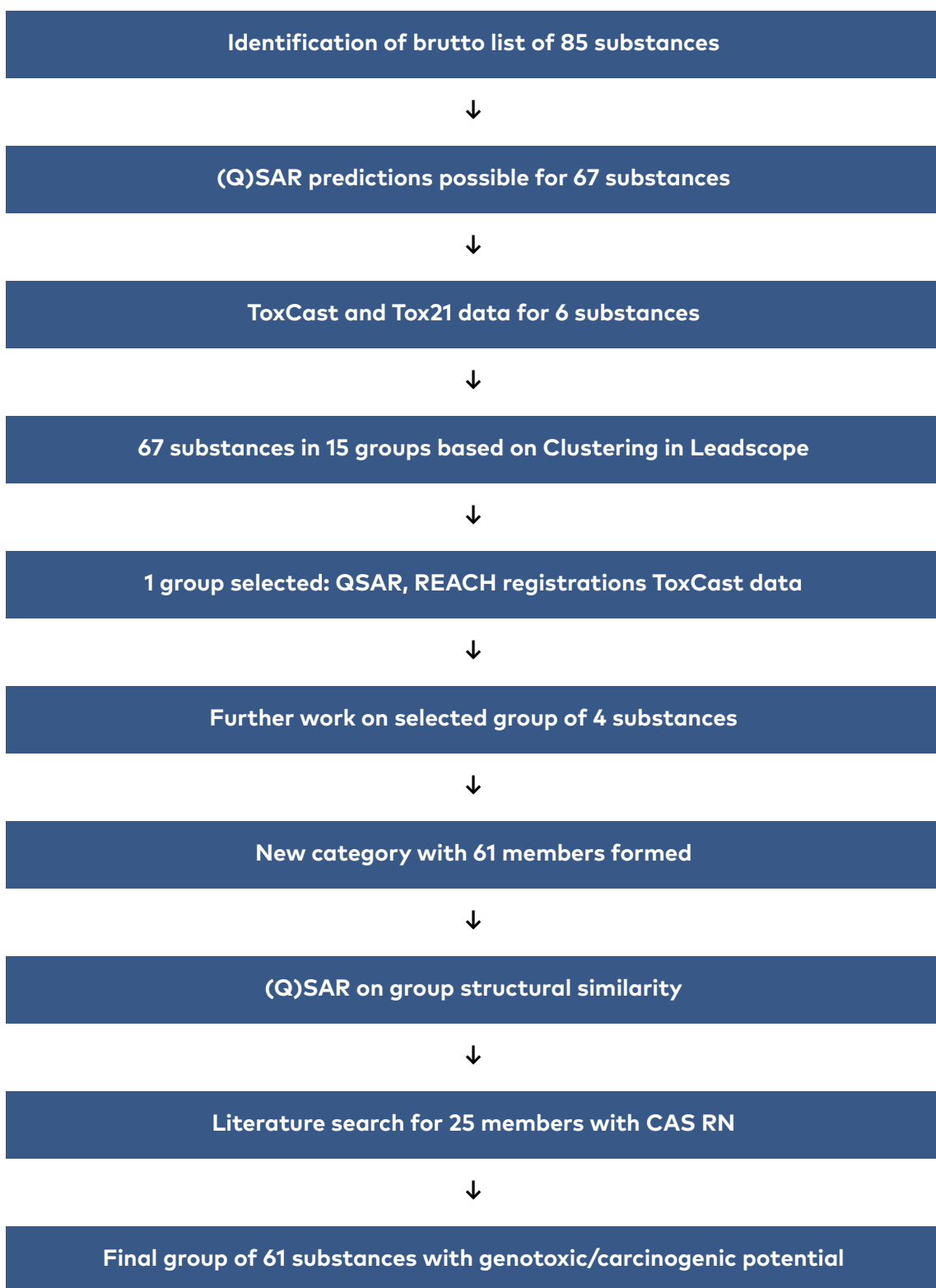
Initially, 85 BFRs were identified. Following the identification of the BFRs, structural information was gathered in the format of SMILES (Simplified Molecular-Input Line-Entry System) strings where possible, for use in (Q)SAR model predictions.

Substances incompatible with (Q)SAR analyses were excluded leaving 67 substances for further analysis. The 67 BFRs were predicted in >150 (Q)SAR models. In a next step, in vitro experimental data from the US programmes ToxCast and Tox21 were retrieved. The generated (Q)SAR predictions along with the ToxCast/Tox21 information was submitted to the Leadscape Predictive Data Miner software to group the substances. The grouping (termed clustering in Leadscape) was based on structural similarity alone or structural similarity plus (Q)SAR predictions. Clustering based on Tox21/ToxCast data did not yield meaningful groups as only 6 substances had such data.

The Leadscape clustering led to 15 groups and 7 singletons that could not be attributed to any of the groups:

- Aromatic substances – 10 substances
- Dibromo-(2,3-dibromopropoxy)benzene derivatives – 5 substances
- Cycloalkanes – 5 substances
- Phthalate acid and its anhydride – 2 substances
- Phenols and bisphenols – 5 substances
- Benzyl ethyl oxygen bridge derivatives – 2 substances
- Methoxy dibromobenzene derivatives – 5 substances
- Phthalates/Benzoate – 4 substances
- TBBPA ethers – 5 substances
- Small linear and branched brominated alkyl alcohols – 4 substances
- TBBPA esters/acrylate – 3 substances
- Phosphates – 2 substances
- Triazines – 3 substances
- Biphenyles – 2 substances
- Diphenyl ethers – 4 substances

The group “small linear and branched brominated alkyl alcohols” was chosen for further analysis due to positive (Q)SAR predictions of genotoxicity and cancer for all 4 members, REACH registrations for 2 members and Tox21/ToxCast data availability for 3 out of the 4 members. Based on the 4 members of this group, structural boundaries for a “category” were defined and all theoretical BFRs with structures living up to the category definition were determined, resulting in a category with 61 members. The 61 members of this new category were subjected to further (Q)SAR analyses using human health related (Q)SAR models and a literature / database search for the 25 members with a CAS RN was performed to attempt determining the critical human health effects. The literature / database search retrieved relevant results for 3 of the category members. The critical effect of these three members was identified to be multiple-organ carcinogenic effect, most probably exerted by a genotoxic mode of action either by the parent or metabolite(s). Read-across for the critical effect from the three category members with experimental data and the one member with a classification for the identified critical effect to the remaining 57 structurally similar target analogues in the category was not performed but observations were gathered to support possible follow-up work.



Workflow 5 Brominated flame retardants (Danish EPA, 2016)

Comparison with procedures described in the OECD GD 194

The grouping of brominated flame retardants led to the formation of a preliminary category of small linear and branched brominated alkyl alcohols with suspected genotoxic and carcinogenic potential. For a comparison with the stepwise procedure recommended for category approaches in the OECD GD 194, each step in the procedure was outlined and assessed in table 7.

Table 7 Analysis of case 5 (Brominated flame retardants) according to the approach recommended in the OECD GD 194

Step	Approach recommended in the OECD GD 194	Analysis of case 5 (Brominated flame retardants)
0	Check whether the substance is a member of an existing category	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", the OECD (Q)SAR Application Toolbox was used to check whether any of the 61 identified category members were associated with an existing category. This approach is in line with the recommendations in the OECD GD 194.
1	Develop category hypothesis and definition and identify category members	The generated group "small linear and branched brominated alkyl alcohols" consisted of 4 substances and was chosen for further analysis due to positive (Q)SAR predictions of genotoxicity and cancer as well as REACH registrations for 2 members and ToxCast data availability for multiple members. A working definition for a preliminary category was made and all theoretical structural members were identified (61 including the 4 members of the original group). Based on the 4 substances from the original group, the boundaries for a preliminary category were defined as: Small linear and branched brominated alkyl alcohols having 3–5 carbons, 2–3 bromine atoms and 1–2 alcohol groups. This approach is in line with the recommendations in the OECD GD 194. Impurity profiles for the substances for which experimental data was found, were not mapped and taken into consideration. This is not in line with the OECD GD 194.
2	Gather data for each category member	In the generated preliminary category: "small linear and branched brominated alkyl alcohols", (Q)SARs predictions were generated for the 61 members of the category and a literature search for experimental data on human health effects was performed for the 25 substances which had a CAS RN. This approach is in line with the recommendations in the OECD GD 194.

Step	Approach recommended in the OECD GD 194	Analysis of case 5 (Brominated flame retardants)
3	Evaluate available data for adequacy	Literature searches were conducted and all retrieved data were checked in order to find relevant data on human health useful for performing a category approach and read-across. This is in line with the recommendations in the OECD GD 194.
4	Construct a matrix of data availability For all category members vs. endpoints, arranged in suitable order to reflect trends or progression across the category. Supporting data may be better represented graphically, e.g. using a heatmap.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", a data matrix was constructed for the 4 source substances, including (Q)SAR predictions and experimental data, including in vitro data. Heat maps for (Q)SAR predictions were constructed for all 61 members. Data on absorption, distribution, metabolism and excretion (ADME) were available for one of the three members of the category identified in the generated preliminary category. This approach is in line with the recommendations in the OECD GD 194.
5	Perform a preliminary evaluation of the category and fill data gaps	The generated preliminary category: "small linear and branched brominated alkyl alcohols" was evaluated based on available experimental toxicological information for three group members and (Q)SAR predictions for all group members. This approach is in line with the recommendations in the OECD GD 194. For the generated preliminary category: "small linear and branched brominated alkyl alcohols", (Q)SAR predictions for all group members were generated for both mechanistic and AO endpoints within the group boundaries. However, data gap filling was prepared for further work but not finalized. Results were communicated in a transparent way. This is in accordance with the recommendations of the OECD GD 194.
6	Perform and/or propose testing	Not performed.
7	Perform a further evaluation of the category	The adequacy of the preliminary category approach was documented in the report with preliminary hypothesis/justification and recommendations for further work. This is in accordance with the recommendations of the OECD GD 194.
8	Document the finalized category and refine the category rationale	The preliminary category approach and proposals for further work to complete read-across was documented in the report in accordance with the recommendations of the OECD GD 194.

Examination according to the ECHA RAAF

In the ECHA RAAF, the methodology in the category approach fits into scenario 6; A category approach with no variation among the category members, different substances having the same type of effects. The assessment elements (AE) specific for this scenario are listed and assessed in table 8. The scenario-specific assessment elements are included in this case example, as a data matrix is constructed, and the relevant data gap(s) is filled by use of read-across. The case example fits into scenario 4 or 6, targeting categories of different substances with qualitatively similar properties. Since it in the case example was not evaluated whether there are variations in properties observed among the source substances, it could not be further evaluated whether the case example fits into scenario 4 or 6. The assessment elements are also similar, so a distinction is not necessary, and Assessment Element (AE) "4.1/6.1" to "4.5/6.5" are included in the table.

Table 8 Analysis of case 5 (Brominated flame retardants) according to the approach recommended in the ECHA Read-across Assessment Framework (RAAF)

Code	Approach recommended in the RAAF	Analysis of case 5 (Brominated flame retardants)
AE C.1	Substance characterization Chemical identity and impurity profile of each category member are sufficiently detailed for assessment of the category approach.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols" this assessment element is judged to be acceptable with medium confidence as impurity profiles for the tested source substances were not assessed. However, chemical identity was reported by CAS RN and EC numbers, names, structures and SMILES for all members for the preliminary category. The preliminary category was defined as having 3–5 carbons, 2–3 bromine atoms and 1–2 alcohol groups. The working definition of the category was made based on the span of the four source substances in the preliminary structural grouping exercise. The final group had 61 members.
AE C.2	Structural similarity and differences within category Structural similarities among all members are identified and structural differences allowed within the category are described.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with medium confidence. Investigation of the relevance of sub-categorization based on further mechanistic analyses was attempted but not finalized but is relevant for further work. Structural substance information was described and assessed in detail throughout the report.

Code	Approach recommended in the RAAF	Analysis of case 5 (Brominated flame retardants)
AE C.3	Link of structural similarities and structural differences with the proposed regular pattern A category hypothesis has been provided and whether it applies to all category members.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with medium confidence, as it was identified that there may be differences in the genotoxic mechanism (direct acting versus active metabolite(s) and this needs further investigation. Structural substance information was described and assessed in detail throughout the report. The identified sources of experimental information could give promise of possible successful read-across and although all three substances did not cluster together in the (Q)SAR based clusterings for genotoxicity, carcinogenicity, manual inspection of the predictions showed good consistency in the (Q)SAR predictions between the members.
AE C.4	Consistency of effects in data matrix Construct a data matrix for all category members vs. existing experimental data, arranged in suitable order to reflect trends or progression across the category.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with medium confidence, as it was identified but not fully investigated that there may be different mechanisms of the genotoxic cancer for different members. Heat maps based on the (Q)SAR predictions were presented in the report as well as experimental data for the 3 substances identified in the initial group identification plus one additional substance. No experimental data for the remaining 21 substances were found in the literature.
AE C.5	Reliability and adequacy of the source study(ies) Study design of source substance(s) fulfills the information requirement and the test material(s) correctly represent source substance(s) in terms of purity and impurities.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with high confidence. All three members were included in the training set for the DTU (Q)SAR Ames model with positive experimental results and 2,3- DBPA was included with positive experimental results in the DTU (QSAR) models for chromosomal aberrations in CHL cells and SHE cell transformation <i>in vitro</i>. All three had positive predicted indications in all included models for carcinogenicity and had harmonized classifications for carcinogenicity.

Code	Approach recommended in the RAAF	Analysis of case 5 (Brominated flame retardants)
AE C.6	Bias that influences the prediction - Is inclusion of other structurally similar substances in the category possible or would they change the prediction of properties for the target substance(s)? - The source substance(s) used for the predictions corresponds to the reliable study(ies) giving rise to the highest concern for the properties under consideration.	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with minor reservations as impurity profiles for the substances for which experimental data was found, were not taken into consideration.
	Scenario 6-specific AEs	
AE 4.1/- 6.1	Substances the test organism is exposed to Have the substances the test organism is exposed to after administration of the source and target substances been identified?	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with medium confidence (minor reservations) as experimental data contained data from REACH registrations, harmonized and notified classifications and regulatory evaluation processes. ADME information was available for one substance.
AE 4.2/- 6.2	Common underlying mechanism, qualitative aspects Has the common underlying mechanism for the observed effects been established and does it allow a prediction of qualitative similar effects?	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with high confidence. Metabolism profilers were run on the category members identifying multiple metabolites. (Q)SAR predictions were run for category members for bioavailability, metabolism/transformation, ED endpoints, repro/developmental, organ and repeated dose toxicity and genotoxicity and carcinogenicity. Several (Q)SAR models were run for genotoxicity and carcinogenicity for the 61 members in the category of small linear and branched brominated alkyl alcohols. All were predicted to be positive for carcinogenic and genotoxic properties indicating that they may have a carcinogenic potential with a possible mutagenic/genotoxic mode of action. The estimated specificities of the models as established by leave-many-out cross-validations are between 85.9% and 95.1%, i.e. the overall false positive rates of the models are around 5%- 14%. No 'one single mechanistic interpretation' in relation to mutagenicity and cancer could be established. The structural alerts indicated that all members shared the same mutagenic/genotoxic MoA with some variations in their possible mechanisms of action.

Code	Approach recommended in the RAAF	Analysis of case 5 (Brominated flame retardants)
	Scenario 6-specific AEs	
AE 4.3/-6.3	Common underlying mechanism, quantitative aspects Has it been established that the common underlying mechanism leads to the same quantitative outcome for the source and target substances?	This assessment element is judged to be acceptable as although the applied (Q)SAR models did not predict potency but rather gave binary positive/negative predictions, quantitative measures may not necessarily be needed for the critical effect of genotoxic carcinogenicity.
AE 4.4/-6.4	Exposure to other substances than those linked to the prediction Has the possibility of other compounds than those linked to the prediction being present (impurities) or formed (intermediates, metabolites) been considered and if so, what their influence on the prediction is?	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with high confidence. Metabolites were predicted for all of the 61 category members in two Toolbox rat metabolism simulators. Profiler-predicted alerts were communicated in heat maps. The OECD toolbox contains information about metabolites of one substance.
AE 4.5/-6.5	Occurrence of other effects than covered by the hypothesis and justification Can other acting mechanisms of the source and/or target substance than the hypothesized one be present and contribute to the observed toxicity and can they impact the prediction?	For the generated preliminary category: "small linear and branched brominated alkyl alcohols", this assessment element is judged to be acceptable with medium confidence. From comprehensive analyses of experimental data for the source substances and (Q)SAR predictions for all category members the category substances seemed to share the same mutagenic/genotoxic mode of action, but with variations in their mechanisms of action. So sub-categorization was found to possibly be relevant but would require further analysis, which was, however, outside the scope of the project.

Discussion of the case example: Brominated flame retardants

The initial (Q)SAR analyses of 85 identified BFRs resulted in 15 groups of BFRs with relatively few members in each. For the initial structurally similar group of small alkyl alcohols in focus (4 substances), the subsequent category definition resulted in a large group of structurally similar substances category members (61 substances) including the 4 from the initial list of BFRs.

Advantages

(Q)SARs were performed in a structured and transparent manner with hypotheses and assessments illustrating the role modelling can have in assessing – also large - categories of structurally similar substances. For the 61 substances in the final group, the structural alerts indicated that all members shared the same mutagenic/genotoxic MoA with some variations in their possible mechanisms of action.

Based on reliable (Q)SAR predictions, the few category members (for which experimental data existed, and which shared comparable toxicological effects), could be used as source substances for data gap filling by read-across to the remaining group members. The substances with experimental data were thus central for data gap filling within the group.

Challenges

With 15 groups formed from the initially identified 85 BFRs, the example shows that despite the same primary use as flame retardants and the presence of similar structural components, including the same halogen, it is not possible to address BFRs homogeneously from a grouping and regulatory point of view.

(Q)SAR predictions for many different toxicological endpoints were obtained. However, as experimental data in general was scarce and available only for a very limited number of group members, the occurrence of these additional structural alerts was not possible to evaluate further.

Uncertainties

Not many uncertainties were identified for this study. It is noted that impurity profiles for the substances for which experimental data was found, were not taken into consideration.

Regulatory perspectives

Following up on this grouping exercise, the brominated flame retardant BMP received a harmonized classification as carcinogenic (Carc. 1B) and mutagenic (Muta 1B) in 2018 based on available experimental data (ECHA 2018). In 2020, the

structurally similar substance TBNPA got a harmonized classification as carcinogenic (Carc. 1B) and mutagenic (Muta 1B) based on read-across from both BMP and 2,3-DBPA, which also is a structurally similar brominated flame retardant with a harmonized classification, due to lack of relevant experimental data for TBNPA (ECHA 2020a).

Further, in 2023 ECHA published a regulatory strategy to identify substances used as flame retardants, assess the needs for regulatory risk management and through grouping aim at faster regulatory action for these substances (ECHA 2023a).

ECHA included in its assessment information on use and exposure of the flame retardants and suggested that information was adequate for the two reactive alcohols (BMP and TBNPA) to initiate a restriction based on their carcinogenic properties.

ECHA further identified aromatic brominated flame retardants as of general concern due to their known or potential PBT/vPvB properties (ECHA 2023a). Aromatic brominated structures were included in several different subcategories in the category approach by DK EPA 2016 and thus suggested a potential for further work on grouping based on other endpoints than carcinogenicity and mutagenicity/genotoxicity. This could be further explored by using (Q)SARs. Several new ED-related QSARs have been developed since the work of the DK EPA 2016 report and could also be included in future follow-up work.

The use of combinations of reliable (Q)SAR predictions and *in vitro* data to support data gap filling of adverse effects by read-across could be applied more extensively in the future to regulate larger groups of chemicals.

Learnings

- It may be valuable to start with broad groups of substances, also including substances not in current use, to have as wide a structural domain and as many potential source substances as possible.
- Structural similarity can be a good starting point for formation of groups
- (Q)SAR predictions can be used to search for similar effect profiles across (sub-)groups
- A data matrix is a useful tool for analysing potential data gaps to possibly be filled by read-across.

Learnings specific for endocrine disruptors

- None.

Discussion of overall results and learnings

In this project, we explored approaches and developed recommendations for formation of (sub-) groups of substances with the ultimate aim of filling data gaps by read-across. Some of the case examples did not aim at data gap filling, but their category formation approaches were still found to provide valuable learnings generally applicable for forming (sub-)groups with this purpose.

It is recommended to use the ECHA RAAF or the OECD GD 194 as a guide in regulatory grouping approaches

The ECHA Read-across Assessment Framework is tailored for the regulatory use of read-across under the REACH Regulation (ECHA 2017). It, therefore, ultimately is designed for regulatory evaluation of applied read-across cases. Examples of use could be for assessing the regulatory needs of a group of registered substances, assessing registration dossier compliance where read-across is used to adapt the standard information requirements or for assessing read-across used in restriction or SVHC proposals.

The OECD GD 194 on Grouping of Chemicals aims to encompass different possibilities and interpretations of predicting properties of chemicals if adequate information is not available. The guidance describes approaches known to have been accepted or developed by regulators and other approaches, used e.g. by registrants under voluntary programs.

In general, all case examples followed the overall grouping principles as described in both the OECD GD 194 and the ECHA RAAF. However, both the scope of case examples, the number of steps they fulfil in accordance with the recommendations and the level of information provided at each step differed widely between the case examples, as the objectives of the case examples were different. For development of (sub-)groups with the aim of data gap filling, it is recommended to use the ECHA RAAF or the OECD GD 194 to guide the process.

Initiation of grouping exercises with broad groups and well-argued inclusion and exclusion criteria is recommended

Optimally, grouping exercises aiming at data gap filling are initiated with broad groups of substances and well-argued inclusion and exclusion criteria to have as wide a structural domain and as many potential source substances as possible. Exclusion of substances based on arguments like use profiles or lack of CLP classification or REACH registration is not recommended since substances may be

valuable source substances for data gap filling even if they are e.g. not registered under REACH. Thus, inclusion of substances in groups should not depend on their use or current regulatory status, as their use may change over time. Highly regulated substances are, furthermore, often data rich and this data may prove valuable in forming the category and in read-across to fill data gaps for other category members.

The case examples show that the decision about which substances to include in the beginning of a grouping exercise is often not well argued/described and in most cases therefore judged to be non-exhaustive. Along the same lines, the reasons for exclusion of substances from a group are in some cases not explained or justified. It is therefore not transparent and does not follow the recommendations. For example, in some cases, substances are excluded because they are not registered under REACH or classified according to CLP, or they have different use profiles than the rest of the substances. Even though some substances are not relevant to the specific regulatory purpose, e.g. if they are currently not registered under REACH, are strictly regulated already, or have another use profile than the rest of the substances, they can still be valuable source substances or they may in the future become important target substances e.g. in "regrettable substitutions". The broader the grouping, the more solid basis for subsequent (sub)category formation.

Substance characterization could be improved

Substance characterization is of high importance and found in the case examples to have room for improvement. Often, reporting of impurities and in some cases also chemical structures and identifiers as CAS RN, are lacking. Mono-constituent substances can have significant impurity profiles as one main constituent has to be present to at least 80%, i.e. up to 20% may be impurities (ECHA 2017). The lack of information on impurities and in some cases chemical structures and identifiers as CAS RN may be a challenge to subsequent data gap filling by read-across.

Multi-constituent substances and UVCBs have more complex compositions and represent a specific challenge which is not covered by this report. Thorough substance characterization is an important element for assuring a solid basis for category formation. The OECD GD 194 has a specific chapter targeting guidance on UVCBs and ECHA has a RAAF specifically targeting UVCBs and ECHA has developed a specific read-across assessment framework for multi-constituent substances, but more detailed consideration of this is out of scope of this report.

Sub-grouping could be improved

Preferably, sub-grouping aimed at data gap filling by read-across should be based on structural similarity considerations relevant to the endpoint under scrutiny.

Ultimately, knowledge is available that substantiates the importance of specific molecular moieties for induction of specific mechanisms that can lead to toxicological effects. The case example on brominated flame retardants demonstrates how (Q)SAR predictions can be used to search for similar effect profiles across (sub-)groups of structurally similar substances and to expand (sub-)groups to include additional relevant substances. Different sub-groups may be relevant to form depending on the endpoint(s) under evaluation and the molecular sub-structures of relevance for the specific underlying mechanisms of action.

Care should be taken when basing (sub-)groups on *in silico* and/or *in vitro* data. If *in silico* and/or *in vitro* information is used as the basis for sub-grouping, ADME/metabolism of the individual substances is important to consider when extrapolating from *in vitro/in silico* to *in vivo*. The resulting (sub-)grouping may be different if *in vivo* data or reliable ADME data are also included/available. For example, some substances may activate the estrogen receptor *in vitro* but not induce a uterotrophic effect *in vivo*, or vice versa, with one possible explanation being metabolic activation or de-activation of the parent compound. The extrapolation between *in vitro* and *in vivo* is thus important to keep in mind in the formation of (sub-)groups.

Specifically for endocrine disruptors

Different substructures of a molecule may be connected to different mechanisms of action as suggested by Kitamura (2005). When developing sub-groups aimed at data gap filling by read-across with focus on ED properties, it is recommended to consider that different molecular moieties may be relevant to different mechanisms and thereby modes of action and adverse effects. Some overlap may occur, but this should ideally be analysed and described. The complexity increases when considering that many endocrine disruptors act through more than one mechanism/mode of action and that several different mechanisms/ modes of actions can lead to the same adverse effects. In a grouping context, this means that different sub-groups may be relevant to form, depending on the mechanism/mode of action in focus. Special care is therefore recommended in (sub-)grouping of endocrine disruptors, taking this complexity into consideration.

EDs may act through many different mechanisms and modes of action. Depending on the substances in question, a broad range of mechanisms of action, which may possibly be connected to different molecular moieties should therefore ideally be considered as important for (sub-)grouping.

Comparative studies are valuable and could aid to provide the mechanistic understanding and foundation for development of (sub)groups of EDs, including bisphenols. (Q)SAR predictions and molecular docking studies (modelling of preferred orientation of one molecule to another when they form a complex) could

be used to substantiate the hypothesis that specific molecular moieties are linked to specific mechanisms or modes of action.

Substances under regulatory scrutiny or registered in high tonnages under REACH may be valuable source substances

The case examples show that valuable source substances for data gap filling by read-across may be identified among substances which are under regulatory scrutiny (e.g. biocides, plant protection products and substances with proposals for SVHC identification or harmonized classification) or registered under REACH in higher tonnage bands (>100 tpa) since they are generally data rich.

Specifically for endocrine disruptors

For grouping of endocrine disruptors aimed at data gap filling by read-across, valuable source substances may be identified among substances which are already identified as endocrine disruptors under REACH, BPR or PPPR and in future, CLP. Including substances already identified as EDs (alternatively under regulatory scrutiny due to other effects or registered in high tonnage bands under REACH) in grouping exercises as source substances may thus be of high regulatory relevance.

The isobutylparaben case example demonstrated that similar structures combined with ADME, *in silico*, *in vitro* (and if available *in vivo*) data on endocrine activity can be used as a basis for data gap filling by read-across of adversity info for successful identification of endocrine disruptors.

Data matrixes are key tools providing the framework for sub-grouping and data gap filling by read-across

Development of a comprehensive data matrix with *in silico*, *in vitro* and *in vivo* data is an important tool, which can provide a robust basis for sub-grouping and data gap filling by read-across. Such a data matrix is developed in some, but not in all cases, depending on their specific objectives. Data availability may determine the subsequent formation of sub-groups.

If reliable mechanistic (*in silico*, *in vitro* or *in vivo*) information is available, read-across from group members with additional information on relevant adverse effects could be applied, as demonstrated in the isobutylparaben case. Considerations about ADME should always be included to the extent possible, e.g. by including ADME and metabolism profilers in the *in silico* modelling. Constructing a data matrix containing both mechanistic, adversity and ADME information is highly recommended for both analogue and category approaches.

Specifically for endocrine disruptors

Non-monotonic dose response curves, low dose effects, different effect profiles depending on exposure windows and times of investigation and several modes of action with different ones being activated at different dose levels are some of the elements to consider when assessing the hazards and risks of endocrine disruptors. As the substances have complex hazard profiles themselves, creating a data matrix in a grouping of endocrine disruptors must be given special attention.

(Q)SARs can be used at various grouping steps

The use of (Q)SAR predictions are recommended to guide, challenge, support and revise groupings and sub-groupings aimed at data gap filling by read-across. (Q)SARs can provide predictions for all potential group members and thereby help to guide already when developing the read-across hypotheses. (Q)SAR predictions may also aid in identifying molecular moieties of relevance for the source and target substances. I.e., (Q)SARs can help point out molecular sub-structures and other properties of relevance for specific mechanisms/modes of action (sub-)groups of substances. (Q)SARs can also be used to identify additional relevant substances to add to the group based on structural similarity of specific relevance for important molecular moieties.

A perspective on the use of (Q)SARs is that today, many ED-related endpoints are modelled in a binary fashion, i.e. not predicting potency, however it may be a priority in the future to develop (Q)SARs for quantitative predictions. Such modelling may for example be based on results of large screening projects such as ToxCast and Tox21, where many substances have been tested by the same test procedure in the same laboratory.

Further discussion - regulatory perspectives

Examples of identifying and regulating endocrine disruptors in groups are starting to emerge. The work conducted by ECHA to assess the regulatory needs of groups of chemical substances and publish the findings, is an important first step in future groupings of chemicals, including for regulatory purposes. In this report, it is exemplified by the bisphenol ARN. The data base of substances registered under REACH is a valuable tool for creating 'pre-assessments' of groupings as it is possible to identify substances for which relevant experimental data is available and use these substances as source chemicals to form groups. (Q)SARs can help provide the basis for including relevant structurally similar substances from a much larger data pool than the REACH registration database.

Adapting the data requirements under the data-generating regulations such as the REACH Regulation, the Plant Protection Products Regulations and the Biocidal Products Regulation could support identification of endocrine disruptors through grouping and read-across. Including an *in vitro* test battery for endocrine activity at all tonnage levels under the REACH regulation would improve the basis for developing groups of endocrine disruptors based on structural similarity and mechanistic information, and subsequently fill data gaps on adversity by use of read-across from high tonnage level substances with relevant *in vivo* adversity information available.

When the common data platform on chemicals under the Commissions 'one substance, one assessment' legal initiative becomes operational, it will further enable use of chemicals data across legislative sectors, which is expected to increase information about effects of endocrine disruptors applicable for use in groupings and read-across exercises.

Mixture assessment, CAGs and PFAS restriction proposal

Similar adversity can be induced through different mechanisms in the same adverse outcome pathway. Several publications have shown dose additivity in the observed adverse effects of endocrine disruptors after combined exposure to different endocrine disruptors with both similar and dissimilar modes of action (Hass et al., 2012; Kortenkamp et al., 2009; Kortenkamp et al., 2012),

If it, within a grouping assessment, is established that members of the category act by different mechanisms but lead to the same adverse effect, instead of separating them, these sub-groups of endocrine disruptors with a Mode of Action (MoA) via e.g. the estrogenic (E), androgenic (A) and steroidogenic (S) modalities should be considered together in a combined exposure assessment to assess the cumulative risks from these substances and restrict them, as appropriate. The regulatory relevance of cumulative risk assessment persists independently of advances to also adopt harmonized classifications of groups of endocrine disruptors.

In 2014, EFSA established cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological profile, including for effects on the thyroid. It was concluded that the developed grouping methodology can be applied even when the underlying biochemical events mediating the effects are not understood (EFSA 2014). Grouping in CAGs had four levels, reflecting an increasing amount of adversity and mechanistic data. Criteria for CAG levels 1–4 were: 1: toxicological target organ, followed by 2: common specific phenomenological effect on the target organ, 3: common mode of action and 4: common mechanism of action. In the assessment of more than 220 pesticide active substances it was evident that CAGs generally could not be refined beyond level 2 as sufficient information on mode- or mechanism of action was available only for a limited number of

chemicals. The report acknowledged that an agreed inventory of mode of actions, as well as a defined set of criteria for ways of characterizing or predicting modes of action in data-poor situations was missing (EFSA 2014). In 2019 EFSA further developed and updated CAGs for two specific effects on the thyroid (EFSA 2019). Work to establish adverse outcome pathways has since accelerated and is prioritized in the scientific community, the OECD and in the EU PARC and research and innovation funding programs such as Horizon 2020 and Horizon Europe.

Forming cumulative assessment groups (CAGs) for endocrine disruptors could be valuable to explore. While doing this, the CAGs could in theory be broadened to include more substances than only those with available adversity information. This could be done by first using each substance included in a preliminary CAG to search for structurally similar substances, secondly develop a data matrix to identify substances for which mechanistic data is available to substantiate data gap filling of adversity info by read-across and finally include these additional substances in the CAG.

In the PFAS restriction proposal, currently under evaluation in ECHA, a common concern for the group of up to 10,000 PFAS is very high persistence due to the strong carbon-fluorine bond (ECHA 2023b). All PFAS are either persistent themselves or degrade to other persistent PFAS, making the carbon-fluorine bond the structural similarity for the group. In the future, specific molecular moieties (like the carbon-fluorine bond for PFAS) may be identified as decisive for ED activity/effects, either within specific groups of substances or more generally applicable. Comparative studies like Kitamura (2005) as well as docking analyses and (Q)SAR modelling are valuable tools for exploring this.

Current practice on cumulative assessment groups and scientific knowledge about endocrine disruptors point to the possibility to develop adverse outcome pathways to be used, among others, for identification of endocrine disruptors in the future. Until then, the review of the case examples shows some general recommendations for future groupings on endocrine disruptors with the aim of data gap filling by read-across.

Conclusions and recommendations

Grouping of endocrine disruptors is possible and can generally be conducted like grouping of substances based on other endpoints. However, endocrine disruption is a complex endpoint which in some respects requires extra consideration. One example is that the occurrence of non-monotonic dose response curves, low dose effects, different effect profiles depending on exposure windows and times of investigation and some substances acting through several mechanisms and modes of action at the same time leads to complex hazard profiles, which should be given special attention when creating a data matrix.

Regulatory identification of endocrine disruptors requires information about both endocrine activity, adversity and a link between the two, and grouping exercises are conducted with different focuses and aims. The main focus of this report was on grouping based on structural similarities and mechanistic information ultimately aiming to read-across adversity information from data rich to data poor substances. Supporting this work, ECHAs assessments of regulatory needs for groups of substances and the continued development and application of (Q)SARs are valuable efforts and tools.

Another approach to grouping is exemplified in the EFSA CAGs, where adversity information is used to group substances for combined exposure assessments, independently of their mechanisms and modes of action (at the first levels). These groups, defined by adversity information, could in future be broadened by including a mechanistic approach based on structural similarities and mechanistic information, leading to read-across of adversity information and thereby inclusion in the relevant CAGs. A third approach to grouping is exemplified by the PFAS proposal, in which a specific molecular moiety (the carbon-fluorine bond) is identified as decisive for the concern. Comparative studies, docking analyses and (Q)SAR modelling are valuable tools for exploring future applications of this approach for endocrine disruptors.

Main findings of the report are that:

- EDs can be grouped like other chemicals
- Complex hazard profiles of EDs require special attention
- Groupings should start with the broadest group possible
- In the grouping process, data-rich substances are valuable source substances and should be included regardless of their regulatory status and whether they are in use
- (Q)SARs should be used to guide, challenge, support and revise the choices made.

Through our analysis, we have identified some recommendations for grouping, which we find of general value, and some which are more specific to grouping of endocrine disruptors. Both the OECD GD 194 and the ECHA RAAF provide valuable recommendations and frameworks for grouping and can be used as the basis for the grouping of endocrine disruptors. We have developed a simplified workflow (Figure 4) based on these frameworks. The workflow can be used as an overall guide for grouping of endocrine disruptors, as it at each step incorporates both the general recommendations and the recommendations specific for endocrine disruptors, we have identified in this project.

Step 1 Define purpose and hypothesis

- Define the regulatory purpose
- Define the grouping hypothesis

Step 2 Include relevant substances

- Start with the broadest group possible. Use (Q)SARs to check if additional substances should be included.
- Include substances under regulatory scrutiny (**For EDs: Regulatorily identified EDs**) or registered in high tonnages under REACH for use as source substances if possible
- Use well-argued inclusion and exclusion criteria, refrain from excluding substances based on current regulatory status or use.
- Make sure that all substances are characterized in detail, including impurity profiles.

Step 3 Produce a data matrix

- Include in silico, in vitro, in vivo data
- Include relevant metabolites and ADME data
- **For EDs: Consider complex hazard profiles, including different effects depending on timing of exposure and investigation, low dose and non-monotonic effects etc.**

Step 4 Redefine the group and define sub-groups, as appropriate

- **For EDs: Consider that different molecular moieties may be relevant for different mechanisms and thereby modes of action and adverse effects. Some overlap may occur, but this should ideally be analyzed and described.**
- **For EDs: Consider that many endocrine disruptors act through more than one mechanism/mode of action and that several different mechanisms/mode of actions can lead to the same adverse effects.**
- Consider that if in vitro data are used to define sub-groups, ADME must be considered and that if ADME/in vivo information is considered, the sub-groups may have different boundaries.
- Use QSARs to check if more substances could be added to the (sub-)group.

Step 5 Read-across to fill data gaps

Step 6 Conclude on results, revisit the hypothesis, as appropriate

Figure 4 Recommended workflow for (sub-)grouping of endocrine disruptors with the ultimate aim of filling data gaps by read-across. General recommendations are provided at relevant steps in the workflow. Recommendations specific for endocrine disruptors are highlighted in bold.

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ANNEX 1: Workflows from the ECHA RAAF

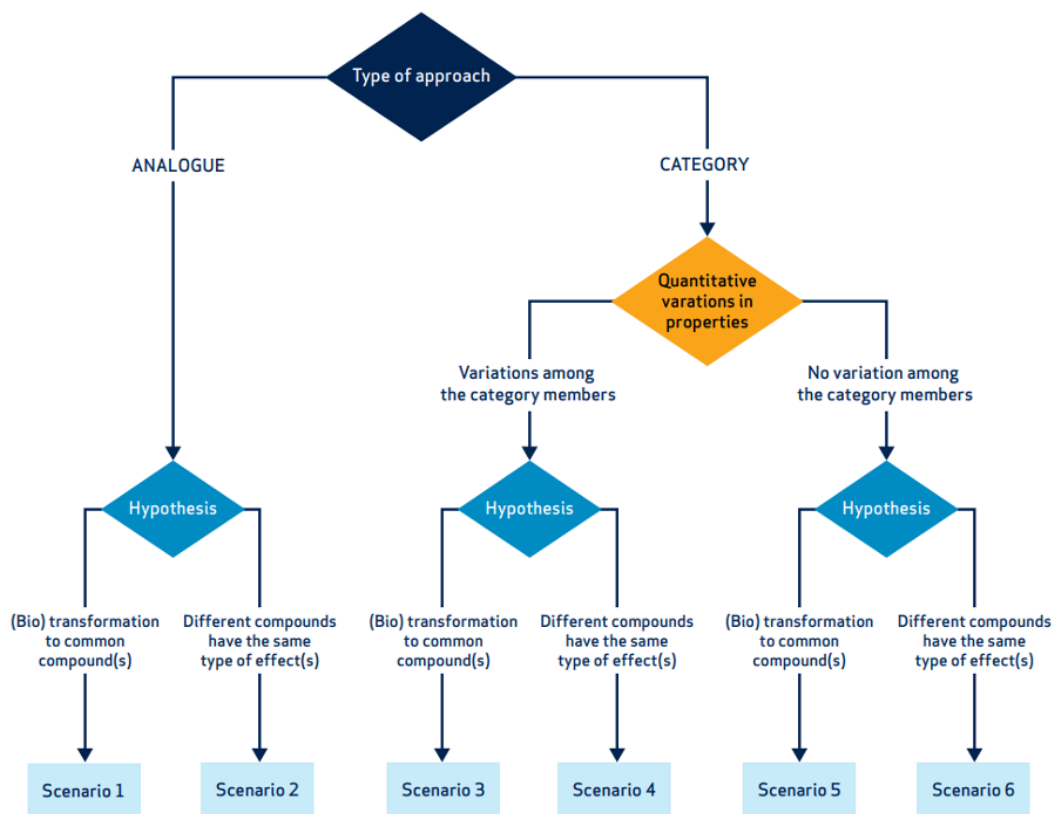


Figure A From the ECHA RAAF: Schematic presentation of scenario selection (ECHA 2017)

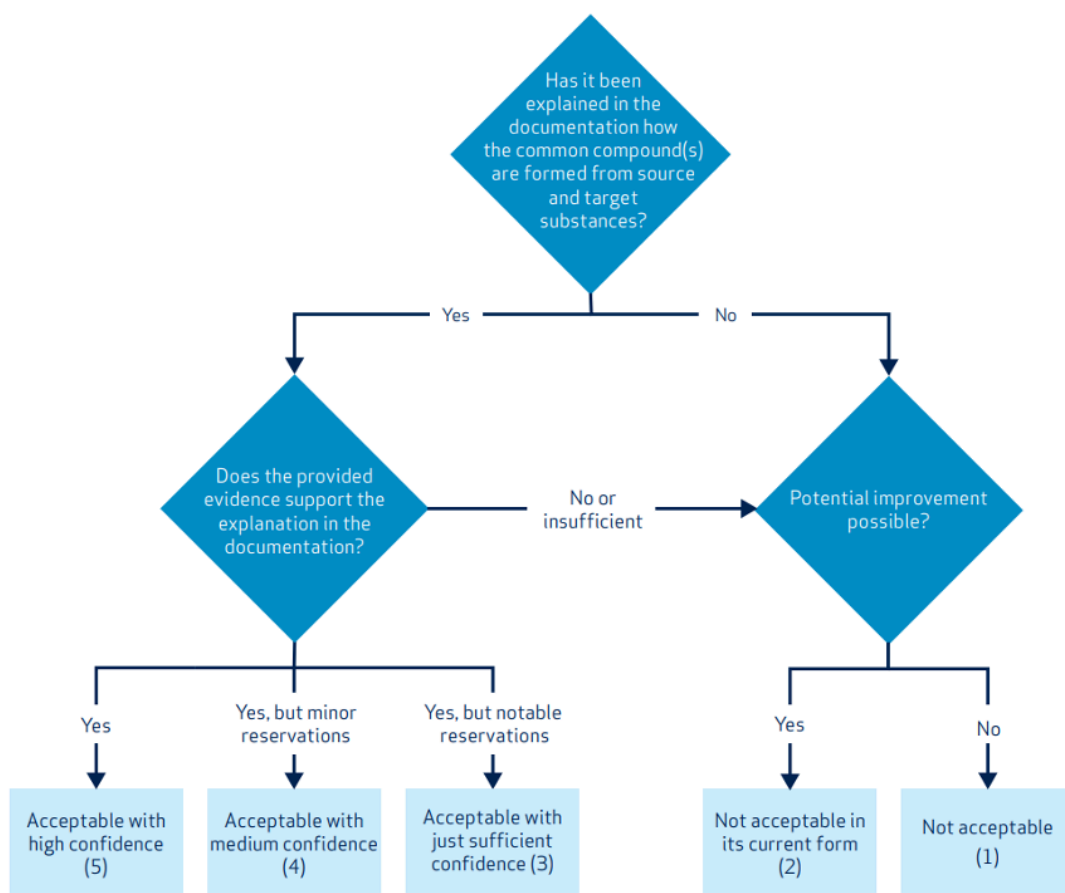


Figure B From the ECHA RAAF: Example of decision logic with an AE – Extracted from AE1.1 Formation of common (identical) substance(s) (ECHA 2017)

About this publication

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Notes about the project

In our evaluation of various projects, please note that some of the included case examples were developed by our own team at DTU Food. Therefore, in certain instances, we assess our own work, while in other cases, we evaluate projects created by external parties.

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