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Case study on the Use of Integrated Approaches for Testing and Assessment (IATA) for Prioritization of chemicals using the Integrated Approaches for Testing and Assessment (IATA)-based Ecological Risk Classification (ERC) approach, Version 2. Tenth Review Cycle (2024)

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

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

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

This case study was developed by Environment and Climate Change Canada (ECCC) for illustrating practical use of IATA and submitted to the 2024 review cycle of the IATA Case Studies Project. This case study was reviewed by the project team.

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

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The authors of this Case Study were Magdalena Jagla[1], Rhyanna Melanson[1], Kayleigh Rick[1], Deborah Bakker[1], Mark Bonnell[1], Ciara Latendresse[1], Bryon Shore[1] and Chris Fraser[1].

The ERC2 IATA case study was formulated based on Science approach document - Ecological risk classification of organic substances version 2.0 (ERC2) which includes acknowledgement of the many individuals who contributed to its development.

[1] Environment and Climate Change Canada (ECCC)

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Abbreviations and acronyms

Legal or regulatory terms

CEPA	Canadian Environmental Protection Act, 1999
CAS RN	Chemical Abstract Services registry number
CoC	Chemical of concern
CMP	Chemicals Management Plan
CRA	Cumulative risk assessment
DSL	Domestic Substances List
ECCC	Environment and Climate Change Canada
GoC	Government of Canada
OFR	Organic Flame Retardants
OECD	Organisation for Economic Co-operation and Development
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration

ERC terms

ADME	Absorption, distribution, metabolism, elimination
CTD	Characteristic travel distance
EA	Actual rate of emission
EC	Critical rate of emission
ERC1	Ecological Risk Classification, Version 1
ERC2	Ecological Risk Classification, Version 2
EAF	Exposure assessment factor
F	Chemical mass fraction
HAF	Hazard assessment factor
MoA	Mode of action
MoE	Margin of exposure
NA	Not available
PBiT	Persistent, Bioaccumulative and inherently Toxic
Pov	Overall persistence

General terms

AC50	Median bioactivity concentrations
AhR	Aryl hydrocarbon receptor
AOP	Adverse outcome pathway
AR	Androgen receptor
B	Bioaccumulation
BER	Bioactivity-exposure ratios
Br	Bromide
Cl	Chlorine
CBR	Critical body residue

CMC	Critical membrane concentrations
ER	Estrogen receptor
IEC	Inherent toxicity
IEC50	Median lethal effects
It	Internal effects concentrations
K_{BW}	Biota-water partition coefficient
K_{ow}	n-Octanol/Water Partition Coefficient
K_{MW}	Membrane-water partition coefficient
K_{SW}	Storage lipid-water partition coefficient
LA	Lethal chemical activity
LC50	Lethal Concentration
LoE	Line of evidence
LOAEL	Lowest Observed Adverse Effect Level
MP	Melting point
MIE	Molecular initiating event
MW	Molecular weight
NAM	New approach methodologies
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
P	Persistence
P	Phosphorus
PBT	Persistent Bioaccumulative and Toxic
PEC	Predicted environmental concentrations
PMT	Persistence, mobility and toxicity
PNEC	Predicted no effect concentration
PPB	Protein Plasma Binding
QA/QC	Quality assurance/quality control
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility
THY	Thyroid receptor
TR	Tissue Residue
TR_A	Aggregate tissue residues
UVCB	Unknown or variable composition, complex reaction products or
Vd	Volume of Distribution
WoE	Weight of evidence
WS	Water solubility

Computer model terms

ACD/Labs®	Advanced Chemistry Development, Inc. (ACD/Labs)
API	Application Programming Interface
ASTER	Assessment Tool for Evaluating Risk
CPDat	Chemical and Products Database
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project
CoMPARA	Collaborative Modeling Project for Androgen Receptor Activity
DART	Developmental and Reproductive Toxicity
EFA	Emissions Fractions Approach Model
EDKB	Endocrine Disruptor Knowledge Base
IATA	Integrated approach to testing and assessment
KNIME	Konstanz Information Miner
MOPAC	Molecular Orbital Package
OPERA	Open (Quantitative) Structure-activity/property Relationship App
QSAR	Quantitative structure–activity relationship models
QSUR	Quantitative structure-use relationship

RAIDAR	Risk Assessment Identification and Ranking
SMILES	Simple Molecular-Input Line-Entry System
TIMES	Tissue Metabolism Simulator
TEST	Toxicity Estimation Software Tool

Executive summary

This report demonstrates the principles, core science and workflow for the Ecological Risk Classification Approach for organic substances, version 2 (ERC2), which is an integrated approach for testing and assessment (IATA) developed to address prioritization needs and used in the Canadian regulatory context under the *Canadian Environmental Protection Act, 1999*. It was published by Environment and Climate Change Canada (ECCC) as ERC2 Science Approach document in 2022. ERC2 builds on the previous version 1 of the approach, released by ECCC in 2016, and also presented in cycle 3 of the OECD IATA Case Study Project and published in 2018.

ERC2 is a high-content IATA method, as it uses many sources of 'alternative data' (also known as new approach methodologies or NAM) such as *in silico*, *in chemico*, and *in vitro* data to complement traditional *in vivo* sources and to provide evidence for risk classification using the weight of evidence approach. The ERC2 approach gathers multiple lines of evidence to profile both hazard (toxicity) and exposure of substances and to determine risk. ERC2 incorporates logic rules to classify hazard, exposure and risk outcomes as 'low', 'moderate' or 'high', to indicate level of concern and to facilitate regulatory decision making. Moreover, classification of hazard, exposure and risk are also put through confidence and severity scoring routines. The confidence scoring was developed using logic rules and workflow processes as a measure of the consensus among data and data availability. The severity scoring, similarly built on logic rules, is applied as a measure of scale for the classifications of hazard, exposure and risk and can be used as a means of weighting various classification outcomes in ERC2 for possible regulatory activities.

The intent of ERC2 is to provide a conceptual workflow for gathering evidence on the hazard, exposure and risk of substances whereby the key elements of classification, confidence and severity can be used individually or combined to address questions posed by regulators when prioritizing chemicals for ecological concern. The weight-based approach of ERC2's workflows are flexible where decision rules can be altered to accommodate a specific regulatory context. For example, the confidence scores assigned to specific data elements of an ERC2 descriptor can be changed to give different weighting to the data elements (e.g., in silico data) such that the maximum confidence score for the descriptor aligns with a member country's position on the relevance and sufficiency of the data for the descriptor. Likewise, the numerical thresholds used by Canada to assign categorical hazard, exposure and/or risk outcomes (e.g., 'low', 'moderate' or 'high') can also be altered depending on context and level of acceptable uncertainty by a member country. These considerations can also be applied to confidence and severity workflows.

Application of ERC2 requires significant computational resources and time. Efforts to automate data generation and compilation from its various models and databases are ongoing at ECCC. Through collaboration, the automation of key aspects of ERC2 hazard profiling within the OECD QSAR Toolbox has been developed which will significantly simplify profiling capabilities.

A group of 24 chemicals of varying profiles, available in ERC2 inventory (published as part of the 2022 ERC2 Science Approach document), is used as a case study to illustrate the functionality of ERC2 and to demonstrate important aspects of the ERC2 approach. An example of a single substance is presented throughout the report to help with explanations of methodology.

1 Introduction

The Ecological Risk Classification version 2 (ERC2), published by Environment and Climate Change Canada as a Science Approach document in 2022 (ECCC 2022), was built on the successful implementation of Canada's system for prioritizing chemicals, its previous version 1 of the approach (ERC1), released in 2016 (ECCC 2016), and lessons learned in the process. ERC1 provided ECCC with a proof of concept and could therefore be used as a template to increase the sophistication of the ERC approach and incorporate new sources of information and tools that had become available since ERC1. ERC1 was also published as an IATA case study in 2017 in cycle 3 of the OECD IATA Case Study Project (OECD 2018). ERC2 builds on the concepts of ERC1, but expands these concepts in specific ways including:

- Refinement of key areas of uncertainty previously identified by ECCC in ERC1 and by OECD members during the OECD IATA 3rd Cycle Case study review
- Better integration and transparency of weight of evidence concepts also identified by OECD members during the OECD IATA 3rd Cycle Case study review
- Expansion of the toxicological and exposure space used for hazard and exposure profiles based on the use of additional empirical data and new *in silico* tools
- Increased consideration of model domain of applicability
- Updating and restructuring of the decision logic governing classification rules based on new tools and lessons learned from ERC1
- The introduction of confidence and severity scoring regimes, to better assess uncertainty and determine the scale of impact with classification outcomes
- Expanded toxicokinetics (i.e., absorption, distribution, metabolism, elimination [ADME]) considerations
- An increased focus on long-term developmental and reproductive toxicity and neurotoxicity in hazard profiling

Like ERC1, ERC2 applies to discrete organics and organic UVCBs (with known structures). ERC2 can also be considered a high content IATA method, as it uses many sources of "alternative data" (also known as new approach methodologies or NAMs) such as *in silico*, *in chemico*, and *in vitro* data to complement traditional *in vivo* sources.

In the Canadian regulatory context, ecological prioritization results from ERC2 provide information for chemicals management priority-setting and planning for organic substances. ERC2 is considered an emerging science as both a data source and a candidate identification mechanism of new assessment priorities. It is noted that as ERC2 is amenable to updates as the science evolves, particularly with respect to *in silico* tools and new data sources (e.g., bioactivity-exposure ratios). Some updates are being considered since the 2022 publication of the Science Approach document (ECCC 2022), but they do not impact this case study and are noted herein where appropriate.

This case study demonstrates methodology for ERC2 approach and explains how the multiple lines of evidence are used to profile and classify both hazard and exposure of substances and to determine risk. In addition, confidence and severity scores, ranging from “very low” to “very high” were developed in the ERC2 approach to apply to classification outcomes to better understand the level of uncertainty that can result if there are data gaps and/or lack of consensus among the lines of evidence, and the scale of impact of the potential adverse outcomes. Built on systematic logic rules, the confidence and severity scoring benefits interpretation of ERC2 results as it provides a way to contextualize the weight of evidence gathered for hazard and exposure profiles, their classification and classification of risk. The confidence and severity scoring can be helpful in both prioritizing chemicals for further regulatory action and/or applying ERC2 as problem formulation to inform assessment or further data gathering in a regulatory context.

Additionally, these methods are flexible allowing decision rules to be altered to potentially accommodate a member country's specific regulatory context. The numerical thresholds for confidence and severity scores can also be updated to better suit the relevant context and level of acceptable uncertainty by a member country.

A group of 24 chemicals, from the ERC2 inventory which was published as part of the ERC2 Science Approach document (ECCC 2022), is used as a case study to illustrate the functionality of ERC2. These chemicals are listed (along with their ERC2 results) in a spreadsheet supplementary to this report, and in Table 7.2 and Table 7.3 in section 7. These chemicals have varying chemical profiles, are either specifically or non-specifically acting, and range in their ERC2 classification, confidence, and severity outcomes. An example of a single substance (CAS RN 632-79-1) is presented throughout the report to help with explanations of methodology. This substance has a complex high hazard profile where there is some lack of consensus between data types, resulting in high hazard risk classification with low confidence and high severity.

2 Purpose and Hypotheses (Problem Formulation)

In the Canadian regulatory context under the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999), the goal of the ERC approach is to help prioritise substances for further regulatory action including assessment, identify substances with the highest potential to cause adverse effects and those of low concern, and to identify data gaps. Overall, ERC2 is a comprehensive system that can provide information useful for a range of activities and concerns related to chemical management that include:

- hazard, exposure and risk profiles for individual chemicals, substance classes or inventories
- a practical tool to inform problem formulations or watch lists
- identification of regrettable alternatives
- potential for endocrine activity
- chemicals with a wide scale potential for harm
- unique chemistries (e.g., permanently charged substances, plasma distributed chemicals such as PFAS)
- cumulative risk prioritization for vulnerable species using tissue residues
- identifying chemical research and monitoring priorities

A group of 24 organic chemicals available in the ERC2 inventory (published as part of the ERC2 Science Approach document (ECCC 2022)) are used to illustrate the functionality of ERC2 for three common regulatory concerns or problems:

1. Making hazard/exposure/risk-based decisions using core ERC2 outputs for further regulatory consideration
    a. Application of ERC2 classification, confidence, and severity outputs
2. Regrettable substitution
        i. Use of a hazard confidence-severity matrix
3. Wide-spread continuous harm
        i. Identifying potential chemical threats to genetic diversity

3 Core Concepts and the Workflow of the ERC2 approach

3.1 Core concepts foundational to ERC2

ERC2 was designed as a consensus-based high content computational Integrated Approach for Testing and Assessment (IATA). The outcome of ERC2 is a problem formulation that addresses the hypothesis as to whether the examined chemical is a chemical of concern (CoC). In the Canadian regulatory context, the ERC approach (versions 1 and 2) has allowed to gain efficiencies in risk assessment work conducted in the third phase of the Chemicals Management Plan (CMP) and has been applied as a prioritization method for thousands of chemicals on the Canadian Domestic Substances List (DSL).

ERC2 allows to separately profile for hazard and exposure of organic chemical substances, while considering both the hazard and exposure profiles together provides evidence for their risk classification. Chemicals can be profiled individually; however, ERC2 has capacity to profile large numbers of chemicals at a time, which can help create efficiencies for examination of chemicals, for example across chemical classes or for inventories. With its problem formulation and chemical profiling capabilities, ERC2 can be applied to identify research and monitoring priorities by examining data gaps and data consensus. Additionally, ERC2 can be helpful to address regrettable substitution by identifying structurally similar substances with high hazard potential as potentially harmful alternatives.

ERC2 generates numerous endpoints which are then subject to a standardized evaluation for consensus, coherence and robustness via sets of rules. This enables a better understanding of the results, and both increases confidence and reduces uncertainties. There are several core concepts that were used in the design of ERC2. These fall in the following areas:

- Application of evidence-based chemical profiling and consideration of uncertainty for confidence scoring and risk scaling (severity)
- The use of computational methods
- Biological extrapolation and the Adverse Outcome Pathway (AOP) concept
- The use of descriptors beyond Persistent, Bioaccumulative and inherently Toxic (PBiT) thresholds to identify CoCs

ERC2 was designed in keeping with the OECD weight of evidence guiding principles and key elements (OECD 2019). As a consensus-based computational IATA, ERC2 allows to collect and generate information for key lines of evidence for chemical profiling and classification in a coherent, transparent, and traceable manner, and employs methodology to infer into the strength of evidence. This includes confidence scoring which allows for evaluation of level of uncertainty and helps to avoid false positive or negative outcomes. As well, ERC2 examines the potential of a substance for simultaneous effects or

adverse outcomes triggered from multiple types of interactions and exposures, via severity scoring, where it is recognized that the potential impact to organisms in the environment from substances capable of acting via more than one toxicity interaction and exposure route can be more severe.

In ERC2, relevant toxicological evidence is collected and generated from different levels of biological organisation, specifically the following as described in OECD (2019):

- *In silico* (computer modelling)
- *In chemico* (chemical reactivity)
- *In vitro* (cell and tissue based)
- *In vivo* (living organism)

Because there is a general lack of experimental data for most industrial chemicals, ERC2 incorporates *in silico* tools to generate data, both to fill gaps for data-poor chemicals and to supplement available empirical *in vitro* and *in vivo* data. A targeted use of computational approaches has proven invaluable in the regulatory context. Both the models' domain of applicability and extreme values of chemical properties are verified for use in ERC2 (i.e., QA/QC check). With respect to characterizing hazard, generally a data preference hierarchy according to the adverse outcome pathway (AOP) concept is used where:

in silico < *in chemico* < *in vitro* < *in vivo*

The above data preference scheme is implemented when there is lack of consensus or there are significant data gaps among the various data types. The above sequence is consequently influential for determining ERC2 hazard outcomes in these situations. Moreover, with the understanding that many biological pathways and xenobiotic interactions are conserved across species (Ankley and Gray 2013; Sapounidou et al. 2021), the biological read-across and cross-species extrapolation are used in ERC2 to fill data gaps and to infer into cross species susceptibility relationships. For example, ERC2 allows to examine for cross-species susceptibility via the use of US EPA's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool (LaLone et al. 2016) and applies the concept of chemical activity, which demonstrates a common susceptibility between mammalian and aquatic receptors to chemical toxicity from various modes of action (Mackay et al. 2009; Mackay et al. 2014). In effect and importantly, ERC2 promotes the notion of "one toxicology" and "one health" (Johnson 1988; Gad 1990; Beasley 2009; Krewski et al. 2010; Pérez and Pierce Wise Sr. 2018; LaLone et al. 2021) and it is noted that advancements in development and application of NAM in the regulatory context can be both accelerated and improved when guided by the "one" concept. Given the need and practicability of extrapolation across species, it is then necessary to establish the relationships between the plausibility (or mechanistic data) and causality (or the adverse effect/outcome). Thus, ERC2 employs the AOP concept (Ankley et al. 2010) to organize toxicological data, and, where possible, to show and build confidence in the plausibility – causality relationships by providing possible linkages between mechanistic data and adverse effects/outcomes. Effectively, ERC2 allows to infer and demonstrate coherence that linked interactions in the toxicological data set across the various biological levels are plausible and therefore the adverse effects/outcomes may be explained. ERC2 can perform these functions for both the target chemical and their metabolites (i.e., products of biotransformation) for some endpoints (e.g., profiling of endocrine activity and genotoxicity).

The potential for persistence (P), bioaccumulation (B) and inherent toxicity (iT) to characterize chemicals has been well recognized and applied in regulatory contexts. These approaches provide one means to

examine chemical hazard potential, however the rigid PBiT thresholds can exclude substances that are expected to have similar behaviour but do not meet the absolute values of PBiT criteria (i.e., close to PBiT thresholds but not over). Canadian experience under CMP shows that substances that meet PBiT or P and B criteria represent only a small fraction across chemical inventories (~2%). This indicates some limitations, particularly for those industrial chemicals that tend to behave more like pharmaceuticals or pesticides which are designed to be highly potent chemicals with wide-ranging modes of toxic action that typically do not meet the P, B, and/or iT criteria. The ERC2 approach reaches beyond PBiT criteria because it incorporates potency-based hazard descriptors, and as it is risk based, it also examines various spatial and temporal scales of environmental exposures, to better identify chemicals with potential for widespread harm. This is akin to high persistence, mobility and toxicity (PMT) considerations at various scales in the environment (McLachlan et al. 2014; Matthies et al. 2016; Reppas-Chrysovitsinos et al. 2017; Rüdel et al. 2020) with an aim to target substances that can have the potential for "irreversible or poorly reversible" effects, where local and global adverse outcomes may continue well beyond the point at which emissions have ceased (Rockström et al. 2009; MacLeod et al. 2014; Diamond et al. 2015; Reppas-Chrysovitsinos et al. 2018).

Establishing the ERC approach on these significant concept areas makes it a comprehensive, systematic, and powerful prioritization method for application in a regulatory context.

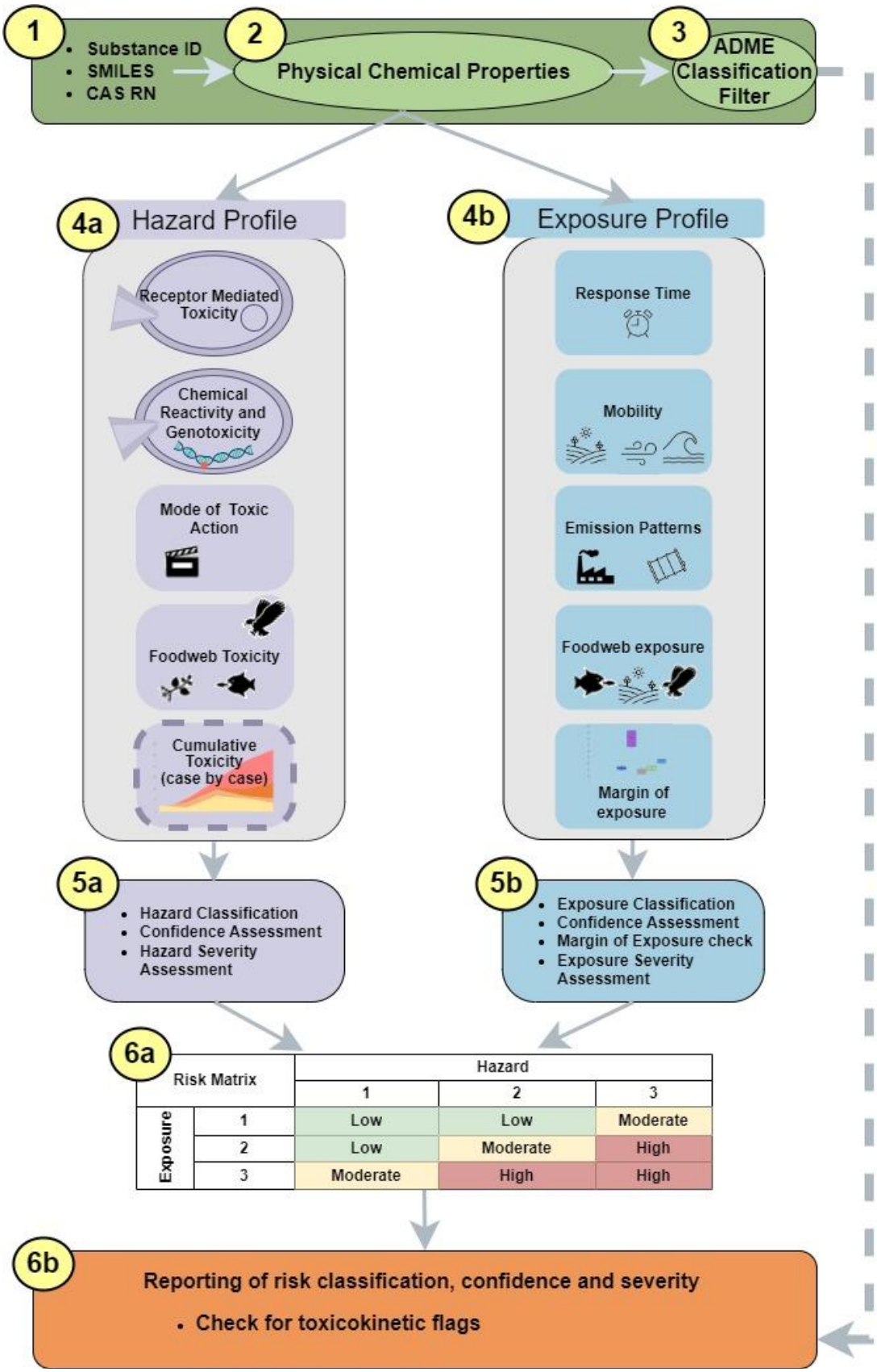
A summary of Core Concepts of ERC2

1. ERC2 uses an evidence-based chemical profiling approach to determine the risk to ecological receptors from exposure to organic chemicals including ionogenics
2. ERC2 examines the degree of consensus within and among in silico, in chemico, in vitro, and in vivo datasets to:
   a. Classify the degree of hazard, exposure and risk
   b. Assess the confidence with the above classifications
   c. Provide a method to weigh the level of severity of the above classifications
3. ERC2 is capable of profiling both a target chemical and its metabolites for toxic effects
4. ERC2 uses cross-species susceptibility concepts (plausible biological extrapolation)
5. Hazard data are organized according to the adverse outcome pathway framework (AOP) to provide plausible mechanistic reasoning to explain adverse outcomes
6. Exposure data are organized at different spatial and temporal scales and used to help understand short vs long-term exposure scenarios using a regional scale multimedia model

3.2 The Workflow of ERC2 approach

The ERC2 workflow is illustrated in Figure 3.1. Modelled and empirical data from *in chemico*, *in silico*, *in vitro* and *in vivo* sources are used to inform steps 2, 3, 4 (from physical-chemical properties and ADME Classification Filter to Hazard and Exposure Profiles) as determined by data availability and suitability.

Figure 3.1. ERC2 workflow



The workflow steps include:

1. Determination of the chemical identity of a substance

It begins with identifying the substance. A discrete organic substance with a valid Chemical Abstract Services registry number (CAS RN) and a chemical structure can undergo profiling in ERC2. UVCBs with acceptable representative structures are treated similarly to discrete organic substances, recognizing that this is a practical solution to address the uncertainty and general lack of UVCB composition data. A manual classification of risk can be performed in ERC2 for those UVCBs without an acceptable representative structure. Similarly, known and targeted mixtures can be investigated on a case-by-case basis when both their structural composition and use patterns are known.

2. Generation and gathering of the physical-chemical property data and half-life estimations

Physical-chemical properties are gathered and generated for discrete organic substance(s) or representative structure(s) of UVCBs. This information is then used as input into:

i. Models for hazard, multimedia fate, behaviour, and exposure
ii. Understand model domain boundaries
iii. Absorption, distribution, metabolism, and elimination (ADME) models

3. Application of the ADME Classification Filter

The Filter is designed to provide alerts for:

i. substances with limited internal and environmental bioavailability or those that become distributed in blood plasma upon uptake
ii. substances with a high molecular fraction occurring in the ionized state at environmentally relevant pH, particularly for permanently charged compounds

Applying the Filter provides toxicokinetic and chemistry flags to consider in evaluation of hazard, exposure and final risk classification outcomes (Figure 3.1, step 6b).

4. Hazard and Exposure profiling

The toxicological hazard profile (Figure 3.1, step 4a) is generated by integrating *in silico*, *in chemico*, *in vitro* and *in vivo* data (mammalian and aquatic) and considers the principal chemical-biological interactions of a substance when in contact with biological material that can lead to toxicity or adverse outcome(s). Key such toxicological linkages include “reactive toxicity” from covalent binding to protein or nucleic acids, often associated with genotoxicity, “specific toxicity” from steric fit interactions associated with nuclear receptor binding (e.g., estrogen receptor) or “non-specific toxicity” (also known as narcosis) from hydrogen bonding or electrostatic interactions with triglyceride-based adipose or cell membrane phospholipids (Nendza et al. 2014). While these and other modes of toxic action and their associated potency can be linked to observed adverse outcomes in the existing toxicity data, they can also be predictive of adverse outcomes if toxicity data are not available or limited, thereby allowing ERC2 to suggest plausible mechanistic reasoning to explain adverse outcomes with increased confidence. ERC2 also integrates PBiT properties into one of its hazard profile descriptors (Foodweb Toxicity) because the influence of P, B or iT properties can vary depending on the specific chemical target (i.e., chemical effects can occur with little bioaccumulation and persistence).

Exposure profiling (Figure 3.1, step 4b) involves using a series of descriptors that define the spatial and temporal scales of exposure and includes a combination of multimedia environmental fate simulations, and

characterization of response time (which is a function of overall persistence, mobility, and mode of entry into the environment as well as substance emission rates (e.g., kilotons per year). Importantly, external concentrations (e.g., mg/L) traditionally used as predicted environmental concentrations (PECs) are not used; rather, internal measures of exposure. i.e., whole body tissue residues (in mmol/kg or ug/kg) are applied to better integrate chemical fate, both external and internal to the organism(s). ERC2 also can aggregate tissue residue concentrations and compare to internal toxicity thresholds for known or targeted mixtures, which is applicable to hazard, exposure and risk profiling.

5. Classification of Hazard and Exposure, and Confidence scoring

Hazard and exposure profiles are examined using set rules to determine the Hazard Classification and Exposure Classification, respectively. Classification of hazard (Figure 3.1, step 5a) relies largely on the potency of a substance and classification scores are generated for each hazard descriptor in the profile, except for cumulative toxicity which cannot be classified at this time. Classification scores can range from 3 to 1, indicating the following levels of hazard concern:

- Class 3 hazard score indicates a high level of concern
- Class 2 hazard score indicates a moderate level of concern
- Class 1 hazard score indicates a low level of concern

Exposure classification (Figure 3.1, step 5b) is driven by the likelihood of an organism being in contact with a contaminant over varying spatial and temporal scales of exposure. It follows set rules for each exposure descriptor in the profile except for margin of exposure which is used as a verification mechanism for low exposure concern outcomes from other exposure descriptors. Classification scores can range from 3 to 1, indicating the following degree of exposure concern:

- Class 3 exposure score indicates a high level of concern
- Class 2 exposure score indicates a moderate level of concern
- Class 1 exposure score indicates a low level of concern

An important aspect associated with determination of the Hazard and Exposure Classes is the understanding of the level of confidence and uncertainty with the Class scores (Figure 3.1, steps 5a and 5b, for hazard and exposure, respectively). This is accomplished by examining for data consensus and data gaps by applying a weighting scheme to the data in each profile descriptor. The confidence weighting scheme was established according to data origin, type and abundance, and where consensus between all data sources results in higher descriptor confidence scores. Lower confidence scores can result from lack of consensus between descriptor classification outcomes as well as significant data gaps.

ERC2 incorporates the concept of severity by recognizing possible simultaneous effects via more than one toxicological mechanism/pathway and exposures at different temporal and spatial scales that may lead to greater adverse impacts on organisms. This is a means of scaling of ERC2 outcomes. Rules are set up to enable scoring of severity for both Hazard and Exposure Profiles.

6. Risk Matrix and scoring confidence and severity for hazard, exposure, and risk

The ecological risk classification outcomes are determined using a risk matrix which combines the classification for Hazard and Exposure Profiles, i.e., Class 1 – 3 (Figure 3.1, step 6a). The categorical designation of risk outcomes in the matrix (i.e., high, moderate, low) reflects a weight of evidence scoring concept (OECD 2019). The risk classification will have ranging confidence scores based on the sum of hazard and exposure classification confidences. The risk confidence scores reveal the degree of data consensus as well as data gaps.

Finally, because moderate and high-risk outcomes can be triggered by any one hazard and exposure descriptor, severity is used as a method to weight the possible risk outcomes in the risk matrix and can be regarded as a measure of the scale of risk. The weighting is performed using severity scores, first calculated separately for hazard and exposure, and for risk, it is determined by summing the hazard and exposure severity scores (Figure 3.1, step 6b).

In summary, in ERC2, confidence is a measure of certainty with the classification outcomes whereas severity is a weighted measure of risk scale.

4 ERC2 Methodology

4.1 Substance Identity and physical chemical properties

ERC2 generates results for organic chemicals identified by CAS RN, chemical name and SMILES (Simple Molecular-Input Line-Entry System) (Figure 3.1, step 1). ERC2 can also accommodate organic counterions from metal salts but under the assumption that complete dissociation of the metal salt occurs in the environment, thus exposing organisms to the organic moiety.

The representative structure approach is a suitable and practical method for less complex UVCBs. For ERC2, ECCC selected as SMILES a "worst-case" representative from a PBT (persistent, bioaccumulative and toxic) perspective. This strategy is based on the "representative chemical constituent-based approach" (Salvito et al. 2020) under the assumption that the structure used represents a worse case from a risk classification perspective. It is noted that some UVCBs may require manual risk classification even if a representative structure were available to primarily ensure current ERC2 outcomes are not resulting in false negative conclusion. Consequently, a level of uncertainty is recognized with ERC2 results for UVCBs based on a representative chemical structure and very complex organic UVCBs with no available SMILES cannot be characterized by ERC2.

The OECD QSAR Toolbox allows for cross-validation of the CAS RN-SMILES relationship and provides canonical (unique) SMILES as output. The Toolbox canonical SMILES were also used for further ERC2 *in silico* data generation including physical-chemical properties (Figure 3.1, step 2). Model domain of applicability and extreme chemical property flags are described in the *in silico* classification and confidence rules for individual descriptors in ERC2 Science Approach Document in Appendices II-IV (ECCC 2022).

Table 4.1. Chemical Identity for CAS RN 632-79-1

1

CHEMICAL IDENTITY

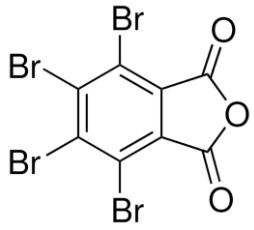
CAS RN	Substance Name	Chemical Structure	SMILES
632-79-1	tetrabromophthalic anhydride;4,5,6,7-tetrabromo-1,3-isobenzofurandione;1,3-isobenzofurandione, 4,5,6,7-tetrabromo-;4,5,6,7-tetrabromo-2-benzofuran-1,3-dione;phthalic anhydride, tetrabromo-		O=C2c1c([Br])c([Br])c([Br])c([Br])c1C(=O)O2

Table 4.2. Examples of phys-chem properties for CAS RN 632-79-1

2

CHEMICAL PROPERTIES – Physical Chemical Properties

CAS RN	Molecular Weight (g/mol)	pKa (0=neutral)	Chemical Species Fraction	Kow (neutral species)	Koa (neutral species)	Kaw (neutral species)	Koc (neutral species)	Water Solubility (mg/L)
632-79-1	463.7	0	1	4.27E+05	1.60E+10	2.66E-05	1.49E+05	241

4.2 ADME Classification Filter

The ADME Classification Filter (Figure 3.1, step 3) provides alerts for substances that present a likely outcome of very low internal and environmental bioavailability or that upon uptake, become distributed in the blood plasma of organisms (protein plasma binding). Substances with a high molecular fraction occurring in the ionized state at environmentally relevant pH are also identified at this stage. In such cases, these chemicals may be outside of the capability of ERC2 to provide confident classification.

The degree of bioavailability, both internally in organisms and externally in the environment, and the confidence associated with the results are determined by the model consensus on indicators of low permeability (see Figure 4.1 [2] for list of indicator/properties, all models and data sources are listed in the References section). See Appendix A for a breakdown of the process for the ADME Classification Filter.

The degree of plasma distribution of substances and the confidence associated with the results are determined using certain key *in silico* indicators and by the model consensus (Appendix A). The ADME filter identifies substances that are not expected to partition to lipids, but instead partition to blood proteins such as albumin in blood plasma. Flags are given to these substances during final classification to determine the reliability of the risk classification outcome.

[2] (MOPAC 2016; RAIDAR 2019; Percepta c2021)

Figure 4.1. ADME indicators and models

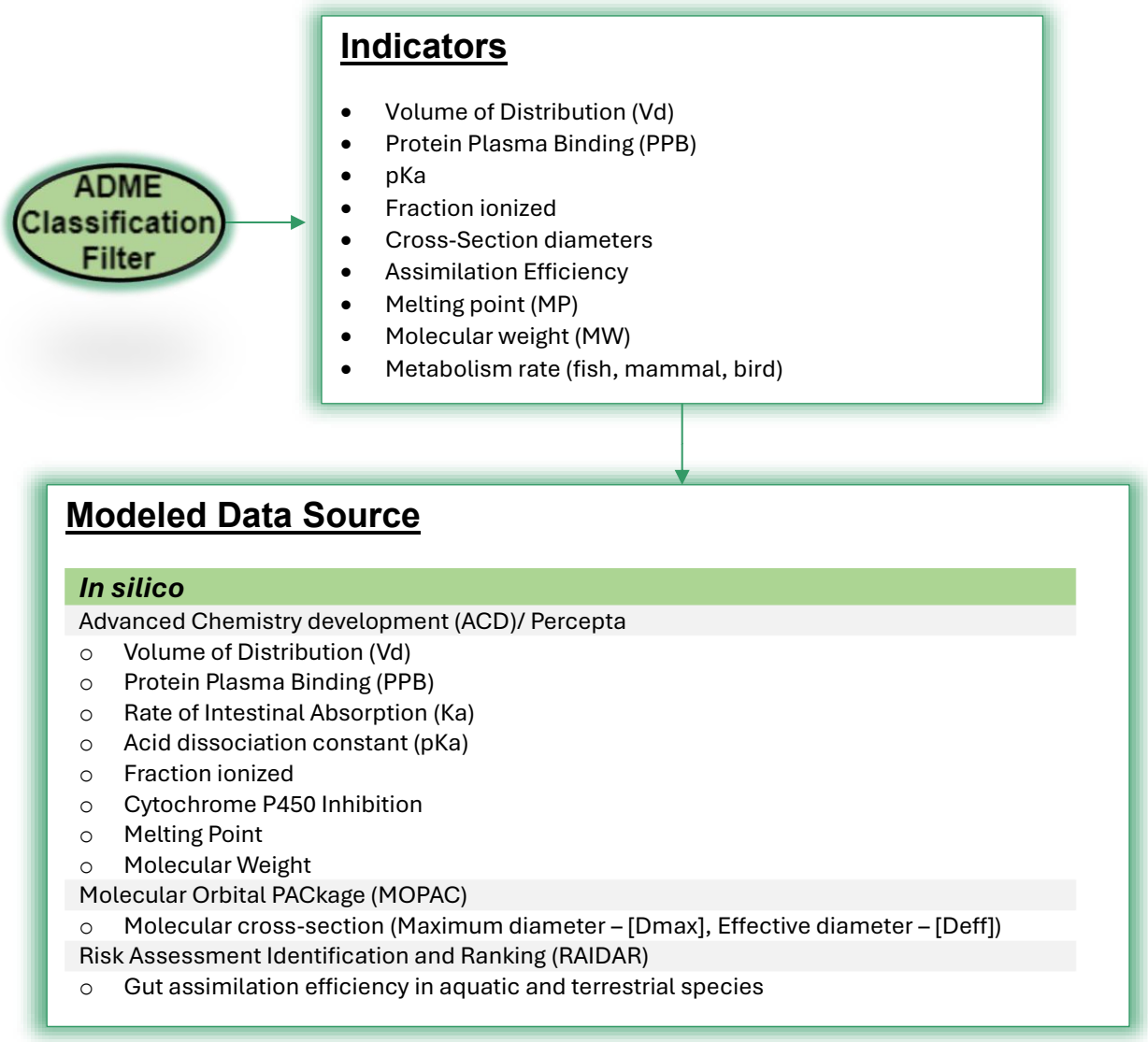


Table 4.3. Example of ADME flags for CAS RN 632-79-1

3

ADME - Bioavailability

CAS RN	Is Permeable ?	Permeability Confidence Category	Is Plasma Distributed ?	Plasma Confidence Category
632-79-1	TRUE	HIGH	FALSE	HIGH

4.3 Hazard Profiling

In ERC2, a substance can be profiled for toxicological hazard using 5 specific descriptors designed to consider its principal interactions (i.e., high level molecular initiating events or MIEs) with various biological tissues. The interaction(s) and their potency can be linked to observed outcomes in the available toxicity dataset. If toxicity data are absent, these interactions or events can be predictive of adverse outcomes.

The five hazard descriptors account for the toxicological space and types of data used to create a substance Hazard Profile in ERC2. These are Receptor Mediated Toxicity, Chemical Reactivity and Genotoxicity, Mode of Toxic Action, Foodweb Toxicity, and Cumulative Toxicity (on a case-by-case basis) (Figure 3.1, step 4a). ERC2 integrates *in silico, in chemico, in vitro* and *in vivo data* (mammalian and aquatic) into the hazard profile to arrive at consensus AOP-organized conclusions for hazard classification.

A data preference hierarchy is used for hazard classification and hazard confidence scoring according to AOP concepts: *in silico* < *in chemico* < *in vitro* < *in vivo*. It is implemented when there is lack of consensus or there are significant data gaps among the various data types. The above sequence is consequently influential for determining ERC2 hazard classification in these situations as well as for confidence scoring (because higher scores are given to *in vivo* data).

The final hazard classification is driven by the highest scoring descriptor(s). For the purpose of binning large sets of chemicals or chemical inventories (e.g., ERC2 examined approximately 12, 000 chemicals for regulatory purposes under CEPA), considering the whole hazard profile is useful to address questions or concerns posed by problem formulations and can provide a powerful and comprehensive aid to chemical hazard evaluation.

4.3.1 Receptor Mediated Toxicity

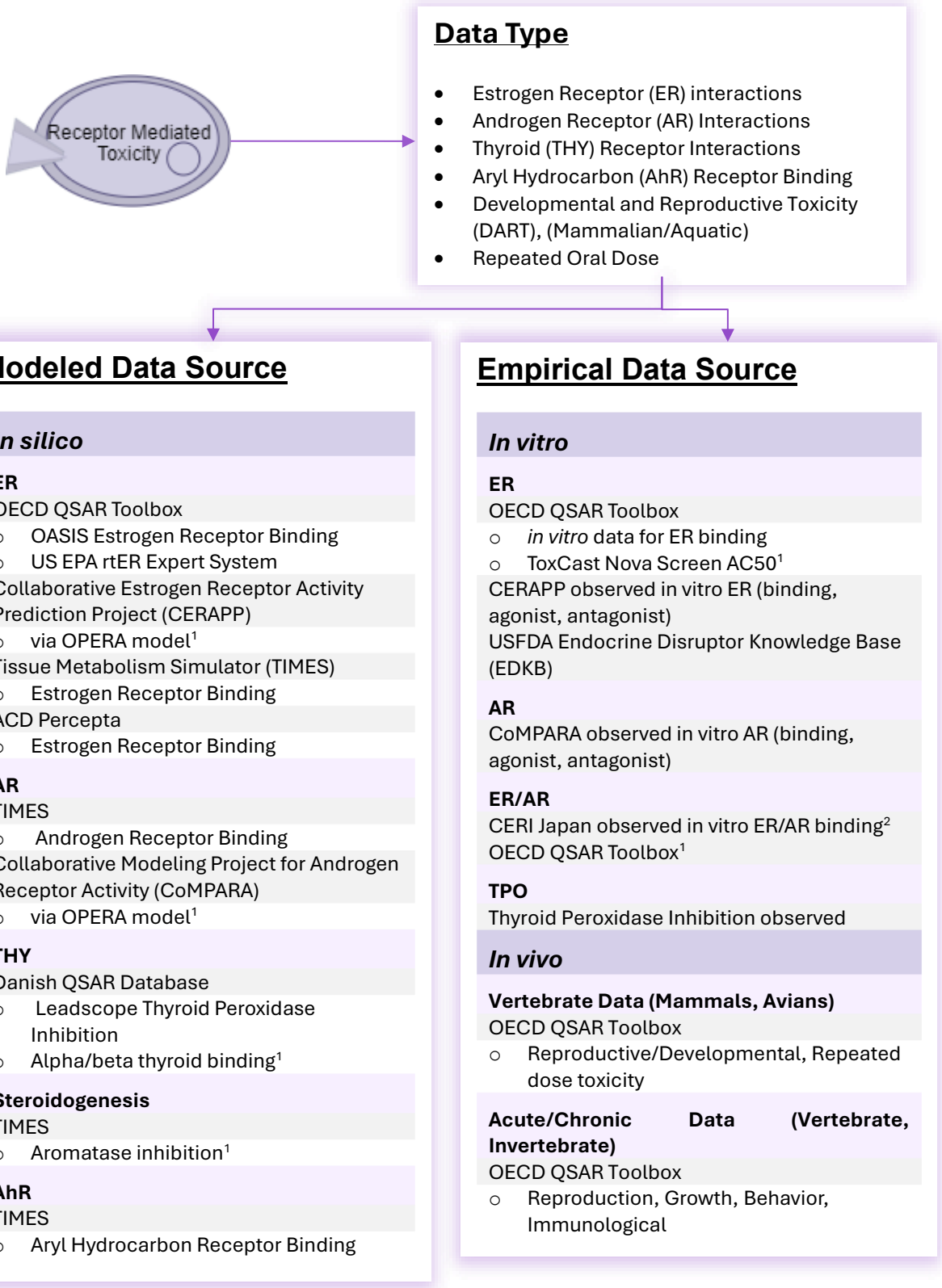
The Receptor Mediated Toxicity descriptor examines the potential for endocrine activity of substances via the ability to bind with certain intracellular/nuclear receptors. This descriptor considers interactions with estrogen (ER), androgen (AR), and thyroid (THY) receptors, in addition to the aryl hydrocarbon receptor (AhR). Endocrine interactions may or may not lead to adverse outcomes associated with, for example, reproduction or development, but the interaction can suggest a plausible mechanism for adverse effects in species not yet tested or observed. Interference with steroidogenesis was not considered in this version of the ERC, as too few data and few viable *in silico* tools currently exist to account for steroidogenesis[3].

[3] Steroidogenesis has been added for version 2.3

In silico, *in vitro* and *in vivo* data for both mammalian and aquatic species were organized along the AOP concept (Ankley et al. 2010; also see section 3.1) to plausibly explain adverse developmental and reproductive effects associated with endocrine and AhR interactions. Figure 4.2[4] outlines the tools and databases used for receptor-mediated effects (all models and data sources are listed in the References section).

[4] (EDKB 1990; Danish (Q)SAR Database 2015; Mansouri et al. 2018; TIMES 2018; CERAPP 2020; CoMPARA 2020; OECD QSAR Toolbox 2020; Percepta c2021)

Figure 4.2. Data sources for Receptor Mediated Toxicity



[1] Added in version 2.3 [2] Removed in version 2.3

Table 4.4. Data sources for Receptor Mediated Toxicity

4a

HAZARD – Receptor Mediated Toxicity

CAS RN	Interaction Type
632-79-1	unconfirmed

The hazard profile for ERC2 also provides target interactions (see Appendix D Excel® spreadsheet of example chemicals for this IATA). The target interaction is given regardless of which data type drives classification of hazard (e.g., protein/DNA) unless there is "no target" as suggested by mechanistic data. When no target is known and there are in vivo data available, the interaction is marked as "unconfirmed" (see Table 4.4). The in vivo data are retained and are still used for hazard classification (i.e., to account for other potential toxicity pathways not covered by the mechanistic profiling).

4.3.2 Chemical Reactivity and Genotoxicity

ERC2 examines the potential for developmental and reproductive effects associated with general chemical reactivity and genotoxicity. Both the genotoxic endpoint-specific information (e.g., DNA damage, chromosomal aberrations) and endpoint-agnostic information (e.g., DNA or protein binding) are used to cover the toxicological space associated with interactions of chemicals with nucleic acids and proteins in or at the surface of organism tissues (e.g., sensitization). Exposure to chemicals that can interact with genetic material, even at very low concentrations, may result in developmental and reproductive toxicity. When coupled with chemical properties that lead to wide environmental distribution and long residence time in the environment, adverse effects may be transferred beyond the time frames of actual exposure. Mutagenic responses are known to be genetically transferred to subsequent generations not exposed to the chemical agent.

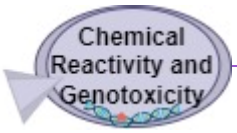
ERC2 does not pursue the carcinogenic aspects of genotoxicity. For the purposes of profiling genotoxicity for non-human organisms, ERC2 examines "eco-genotoxic" responses related to developmental and reproductive effects and thus mainly mutagenic responses (Kirkland 1998). Cancerous outcomes are not typically considered as endpoints in the ecological assessment context at this point in time.

Like receptor-mediated interactions, *in silico*, *in chemico*, *in vitro*, and *in vivo* data for both mammalian and aquatic species were organized along the AOP concept to plausibly explain mutagenic, developmental and reproductive effects associated with chemical reactivity and genotoxicity. *In silico* and *in vitro* profiling examined various DNA and protein interactions that could be plausibly linked mechanistically to *in vivo* developmental and reproductive effects. The mechanistic profiling can be linked to *in vivo* genotoxic outcomes such as DNA and chromosomal damage. While i*n silico* data can be both endpoint agnostic (e.g., protein binding) and endpoint based (e.g., DNA adduct formation, DNA strand-breaks), i*n vitro* data collected for this interaction are endpoint-based only. Quantitative developmental and reproductive *in vivo* toxicity data collected for receptor-mediated interactions are again used for genotoxicity with the understanding that any or all of these interactions could be plausibly linked to an observed adverse *in vivo* outcome.

Figure 4.3[5] lists the *in silico*, *in chemico*, *in vitro* and *in vivo* data types and sources for this descriptor (all models and data sources are listed in the References section).

[5] (TIMES 2018; DART 2020; OECD QSAR Toolbox 2020)

Figure 4.3. Data sources for Chemical Reactivity and Genotoxicity



Data Type

- Chromosome aberration
- Gene mutation I, II
- DNA and protein damage
- DNA damage and repair
- *in vitro/in vivo* Micronucleus I, II
- Ames
- DNA and Protein Binding
- Protein Binding Potency
- Glutathione depletion assay (GHS) RC50
- Developmental and Reproductive Toxicity (DART), (Mammalian/Aquatic)
- Repeated Oral Dose

Model Data Source

In silico

Chemical reactivity (endpoint agnostic)

OECD QSAR Toolbox

- DNA binding structural alert from OECD
- Protein binding structural alert from OASIS
- Protein binding alerts for skin sensitization according to GSH
- Protein binding alerts for skin sensitization by OASIS
- Protein binding by OASIS
- Protein binding by OECD[2]
- Protein binding potency Cys (DPRA 13%)
- Protein binding potency Lys (DPRA 13%)
- Protein binding potency GSH

Mutagenicity

OECD QSAR Toolbox

- DNA binding structural alert for CA from OASIS
- *in vitro* mutagenicity (Ames) by ISS
- DNA Alerts for AMES from OASIS
- OASIS Micronucleus test (MNT)
- *in vivo* mutagenicity (Micronucleus) alerts by ISS

TIMES

- Transgenic Rodent (TGR) Mutation Assay
- Comet Assay
- Chromosomal aberration
- Micronucleus test (MNT)

Development and Reproductive Toxicity (DART)

- DART Scheme

Empirical Data Source

In chemico

OECD QSAR Toolbox

- RC50 database
- DPRA profiler

In vitro

Empirical *in vitro* databases

OECD QSAR Toolbox

- ECHA REACH
- Genotoxicity OASIS
- Bacterial mutagenicity ISSSTY
- Toxicity Japan MHLW
- Genotoxicity & Carcinogenicity ECVAM
- Genotoxicity pesticides EFSA

In vivo (categorical)

OECD QSAR Toolbox

- T143 (strain)
- Chromosome aberration V (ECVAM)
- Micronucleus I
- Micronucleus II
- Chromosome aberration IV (EFSA)
- Gene mutation II
- Gene mutation I
- Mutagenicity I (ECVAM)

In vivo (numerical (NOAEL/LOAEL))

Developmental and Reproductive Toxicity (DART), (Mammalian/Aquatic)

- Repeated Oral Dose

[1] Added in version 2.3 [2] Removed in version 2.3

Table 4.5. Example of results for Chemical Reactivity and Genotoxicity descriptor for CAS RN 632-79-1

4a

HAZARD – Genotoxicity

CAS RN	Interaction Type
632-79-1	DNA + Protein

In Table 4.5 above, the target interaction is assigned because potent mechanisms have been identified. However, there is lack of hazard potency classification consensus with the *in vivo* data for the example CAS RN 632-79-1. Classification for this CAS RN triggers the data hierarchy rules and is based on *in vivo* data.

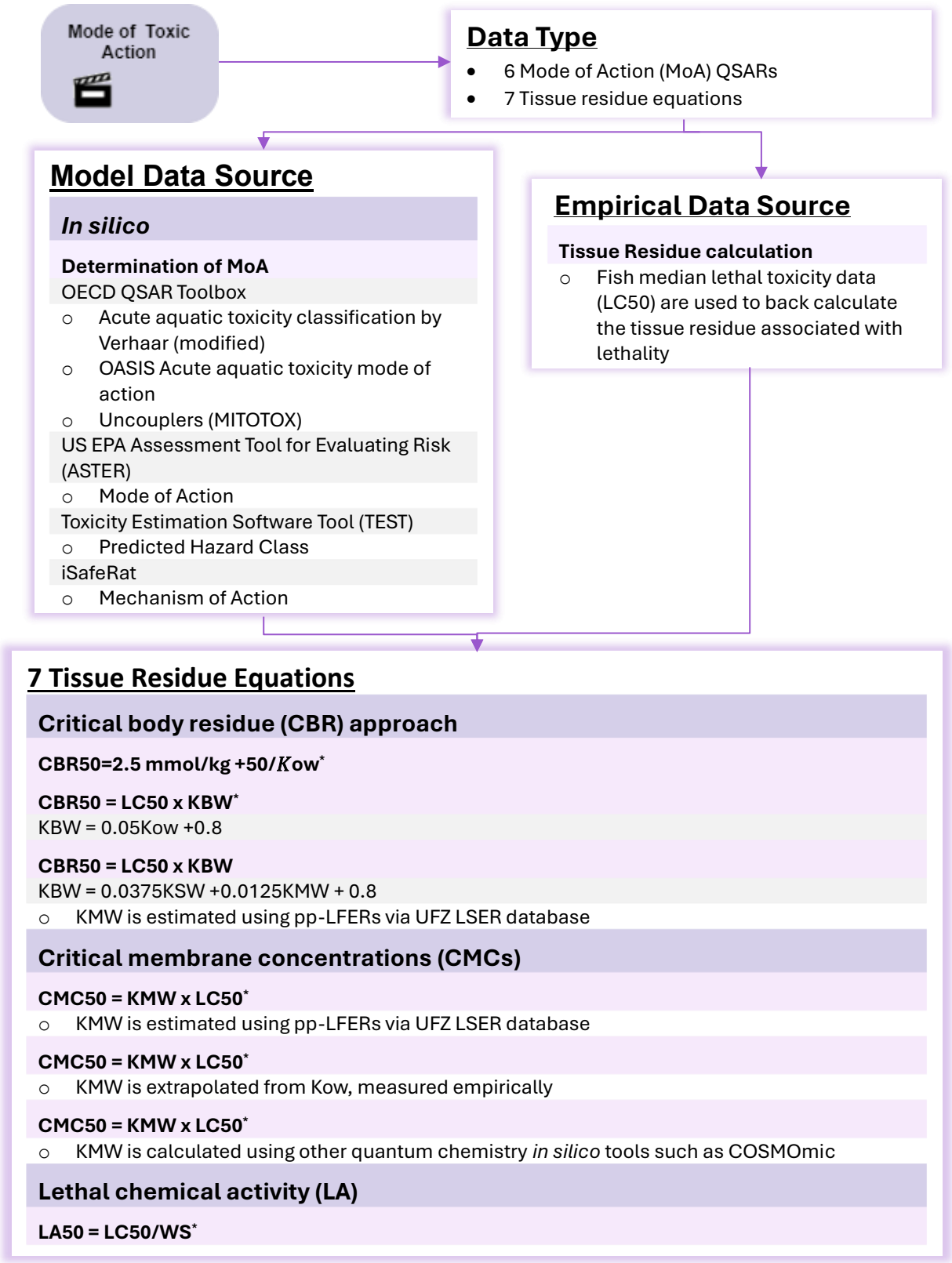
4.3.3 Mode of Toxic Action

The mode of action (MoA) is the functional change (e.g., adverse outcome) resulting from exposure to organic chemicals interacting with biological tissues at the cellular level (Kienzler et al. 2019). The Mode of Toxic Action is an important descriptor in ERC2 because it acts as a cell-level diagnostic for adverse outcomes in addition to descriptors for Receptor Mediated Toxicity and Chemical Reactivity/Genotoxicity. Essentially it contributes a mechanistic classification outcome for other modes of toxic action not covered by the first two hazard descriptors (e.g., AChE inhibition, uncoupling of oxidative phosphorylation).

In ERC2, MoA is determined using a consensus of *in silico* tools (QSARs) and a consensus of MoAs determined using tissues residue-based toxicity ratios (outlined Figure 4.4[6], all models and data sources are listed in the References section).

[6] (ASTER 2012; TEST 2016; iSafeRat 2019; OECD QSAR Toolbox 2020)

Figure 4.4. Data sources and equations for Mode of Toxic Action



*Removed in version 2.3

Tissue residue refers to a concentration of a chemical measured in an organism, typically on a whole-body wet weight basis (mmol/kg) or lipid basis (mmol/kg lipid). Different approaches can be used to calculate tissue residues, resulting in seven methods in ERC2 for tissue residue estimation[7] (see Figure 4.4). Once tissue residue values have been calculated, toxicity ratios are then estimated to determine narcotic versus specifically-acting substances (i.e., broad modes of toxic action). Toxicity ratios refer to the difference in concentration between a baseline narcotic and a chemical exerting a more specific MoA by comparing the tissue residue estimated using anyone or all of the above approaches and the known internal effects concentration associated with median lethal effects (IEC50).

Toxicity Ratio = Tissue Residue /IEC50

With this formulation, values greater than one indicate specific modes of action whereas values equal to or less than one indicate baseline toxicity for acute exposures. An acute to chronic ratio of 10 was applied to the lower bound of the baseline toxicity (1 mmol/kg) to account for the extrapolation to chronic lethal toxicity (0.1 mmol/kg). Thus, in principle, tissue residue values of <0.1 mmol/kg indicate specific modes of action and have a toxicity ratio >10 (e.g., Table 4.6). Consensus is therefore reached between *in silico* and *in vivo* approaches for the CAS RN in the table below and confidence scores for each method are thus summed.

Table 4.6. Example of results for Mode of Action descriptor for CAS RN 632-79-1

4a **HAZARD – Mode of Toxic Action**

CAS RN	Selected Consensus QSAR MoA	Consensus Tissue Residue (mmol/kg)
632-79-1	Acylation based reactivity	0.01 (specific)

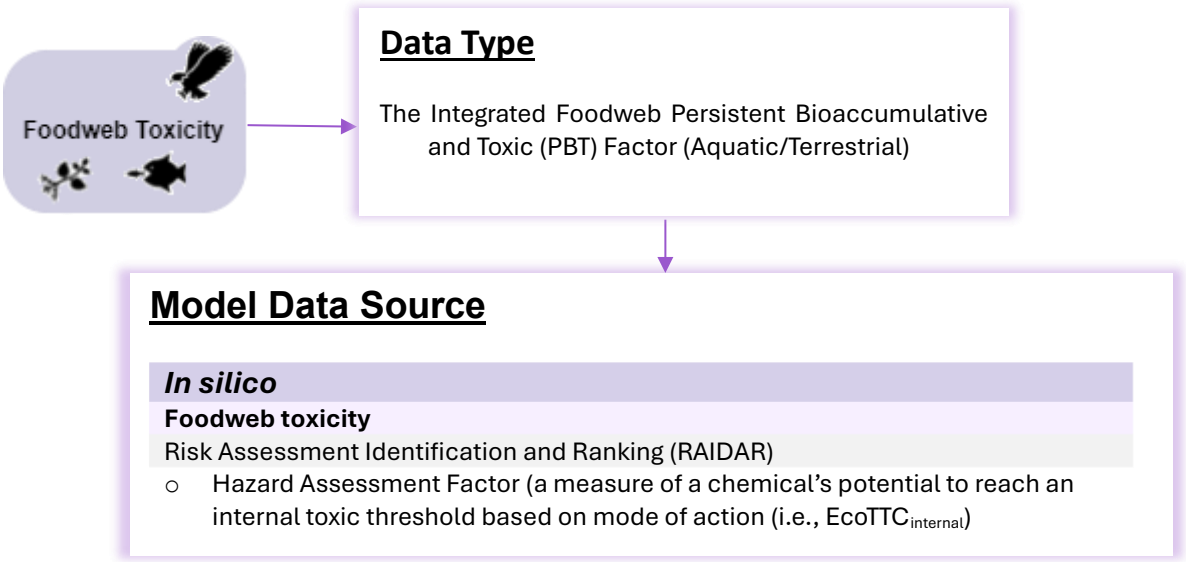
4.3.4 Foodweb Toxicity

The Foodweb Toxicity descriptor integrates the properties of persistence, bioaccumulation and mode of action-based IEC50 (mmol/kg) for internal toxicity. It determines the potential for food web effects by tracking the fate of an organic chemical using the connected environmental fate and food web mass balance model known as the Risk Assessment Identification And Ranking (RAIDAR 2019) model (See Figure 4.5[8], all models and data sources are listed in the References section). In essence, this descriptor ranks a chemical's potential to reach an IEC50 for one or more sensitive species (most exposed) based on its chemical fate in the RAIDAR model foodweb.

[7] In updates considered since ERC version 2, for version 2.3, only one equation is being used because it was found that tissue residue equations provided very similar results.

[8] (RAIDAR 2019)

Figure 4.5. Data sources for Foodweb Toxicity



RAIDAR quantifies chemical transport from diffuse sources in an evaluative, regional scale environment to representative ecological receptors and humans (Arnot et al. 2006; Arnot Research & Consulting (ARC) 2018). To determine the potential for acute toxicity in both aquatic and terrestrial food webs, ERC2 uses the hazard assessment factor (HAF) from version 3.0 of RAIDAR.

RAIDAR simulates food web exposures using bioaccumulation sub-models to estimate concentrations in representative species given a default and actual rate of emission to the environment.

Representative species include:

- Vegetation, and aquatic and terrestrial organisms comprising ecological and agricultural food webs (e.g. fish, wildlife, agricultural crops and livestock, and humans)

The mode of entry to the environment used for model simulations was "mostly water", where air-water partitioning of the substance was used to adjust the fraction emitted to water. It is expected that the classification of hazard using an aquatic emission scenario in RAIDAR as well as other hazard descriptors described previously will be protective of terrestrial species given cross-species susceptibility. However, releases to soil were also included in ERC2 to account for potential terrestrial exposures and effects.

The HAF is a ratio of the tissue residue concentration estimated in the most sensitive species in the RAIDAR aquatic or terrestrial food web, based on a default emission and the MOA-based IEC50 also known as an internal ecological threshold of toxicological concern (eco-$TTC_{internal}$). HAF values of 1 or higher suggests that acute effects in sensitive species are expected using the default rate of emission. Selected HAF thresholds for ERC2 classification are given in the Appendix B workflow for this descriptor.

4.3.5 Cumulative Toxicity (case by case)

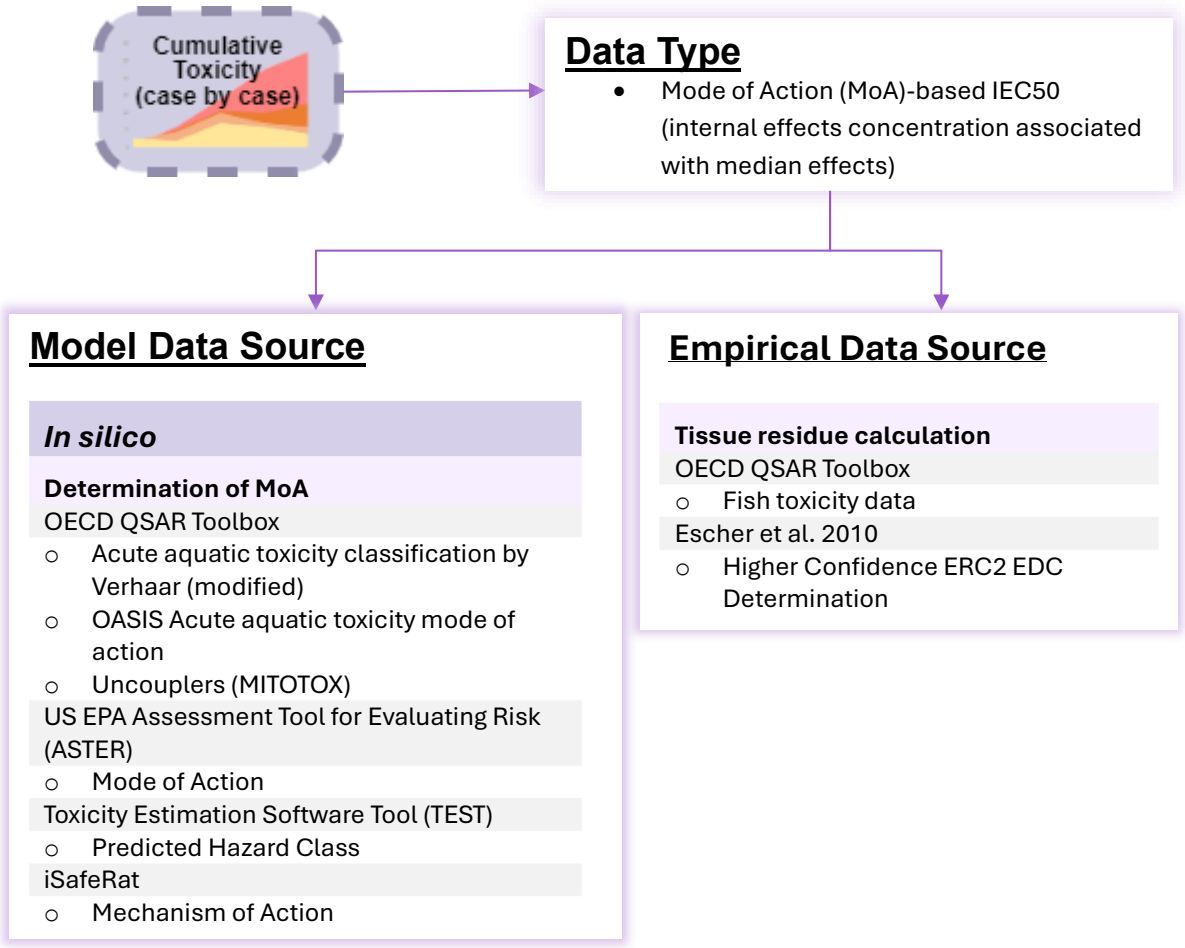
ERC2 can be used to identify candidates for cumulative risk assessment (CRA). Use pattern information provides a rationale for forming a functional chemical group (category) for targeted mixtures during assessment using ERC2 output.

Target mixture toxicity is accomplished in ERC2 by using an internal tissue residue toxic unit approach. ERC2 uses the IEC50 for various modes and mechanisms of action (i.e., an eco-$TTC_{internal}$ per MoA). The IEC50 represents the threshold of effects to compare to the total tissue burden from aggregated exposures in targeted chemical mixtures. The IEC50 is dependent on MoA and therefore can address chemical class cumulative risk assessment (CRA) endpoint or mechanism-based CRA (e.g., endocrine effects), and combinations of both.

Cumulative risk profiling can be performed in ERC2 by summing the tissue residues (mmol/kg) for targeted mixtures (i.e., of known composition) estimated using the RAIDAR model or data from other sources such as monitoring data (Figure 4.6[9], all models and data sources are listed in the References section).

[9] (ASTER 2012; TEST 2016; iSafeRat 2019; OECD QSAR Toolbox 2020)

Figure 4.6. Data sources for Cumulative Toxicity



Tissue residue NOEC (TR_{NOEC}) is derived from IEC50 according to the mode of action (see Appendix B) and applying appropriate assessment factor. The summed internal mixture concentration or aggregate tissue residue (TR_A) can be estimated for a single or multiple sensitive species for representative aquatic and terrestrial food web species from the RAIDAR model output. A tissue residue based CRA profile for a target mixture is then derived as follows:

$$CRA_{TARGETED} = TR_{NOEC} / TR_A$$

Profiling of cumulative toxicity is only conducted when information on co-occurrence in the environment is available and thus acts only as an early indicator of the potential for future cumulative assessment activities. Consequently, this descriptor does not impact the classification of hazard or risk in ERC2 as it is not presently used in the consensus model for hazard classification.

Table 4.7. Example of results for Cumulative Toxicity descriptor for CAS RN 632-79-1

4a

HAZARD – Cumulative Toxicity

CAS RN	Aquatic Internal Effects Concentration (mmol/kg)	Selected MoA
632-79-1	0.01	Acylation based reactivity

An example of cumulative risk profiling for 19 chemicals with a fragrance use pattern is available in ERC2 Science Approach document (ECCC 2022; see section 6.2.3 of the Science Approach document). In the example, environmental co-occurrence was assumed for these 19 chemicals for illustrative purpose.

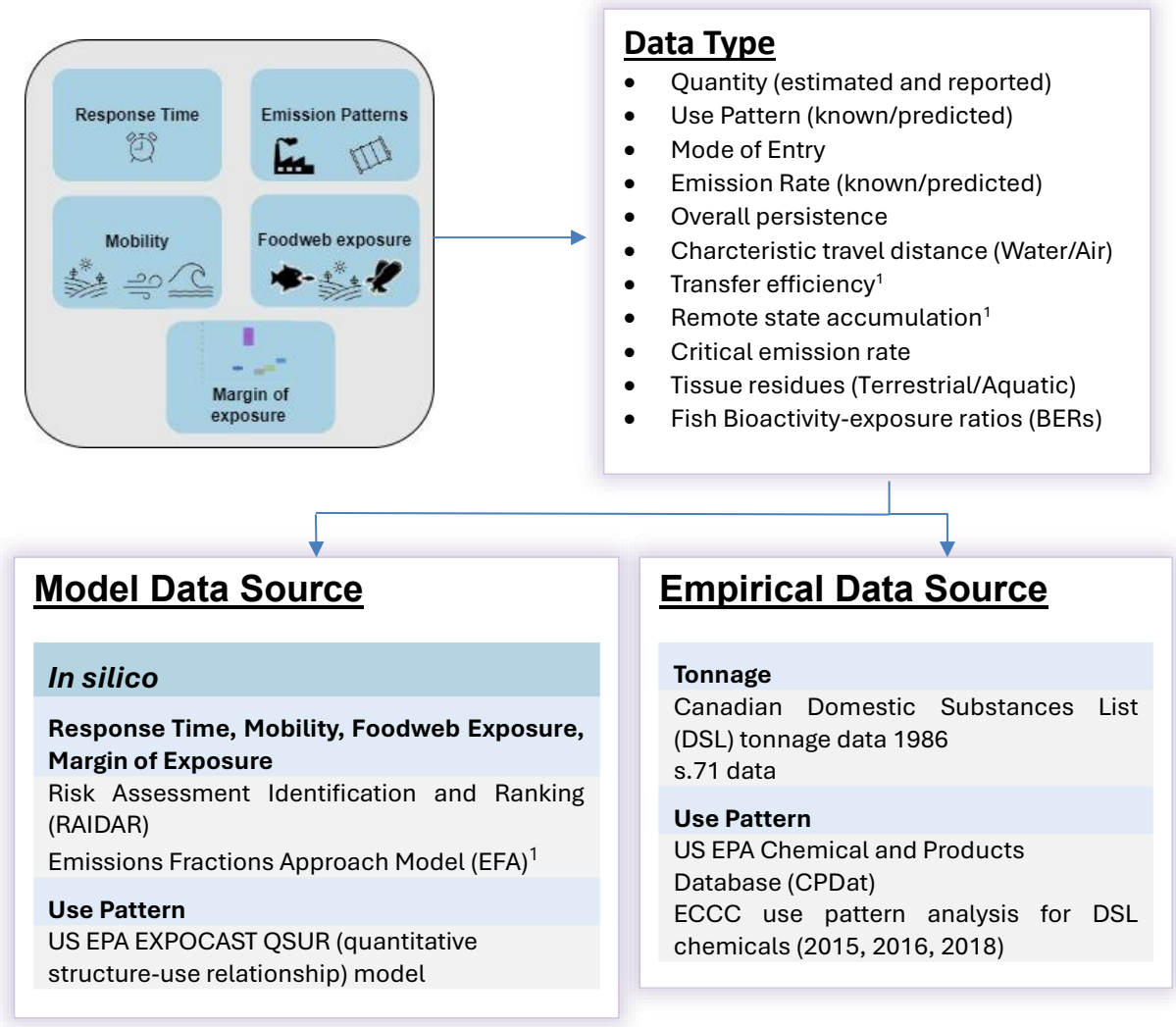
4.4 Exposure Profiling

Classification of exposure is based on the likelihood of contact of an organism with a contaminant, primarily driven by the persistence and distribution of a substance in the environment. In ERC2, a series of 5 descriptors is used to define the spatial and temporal scale of exposure (Figure 3.1, step 4b). Exposure profiling is achieved by using a combination of multimedia environmental fate simulations, mode of entry in the environment, and emission rate (estimated) data. Most descriptor values were generated using the RAIDAR v3.0 model (see section 4.3.4), except chemical quantity (mass) and use pattern information. The use pattern information is not used in ERC2 to classify the exposure concern; it is used as supporting information when forming chemical groups based on functional use (e.g., colorants, antioxidants, fragrance). Use pattern is also helpful for understanding the expected emission pattern. See Appendix C for a breakdown of the various processes used for exposure profiling.

Five exposure descriptors are used to classify exposure concern in ERC2 and include the Response Time, Emission Patterns, Mobility, Foodweb exposure, and Margin of exposure (see Figure 4.7[10])

[10] (US EPA EXPOCAST QSUR 2016; CPDat 2018; RAIDAR 2019; EFA date unknown)

Figure 4.7. Data sources for exposure profiling



4.4.1 Response Time

Response time (or lag time/clearance time) is a measure of how long a chemical can reside in any one or more environmental media from the time global emissions to the environment have ceased (e.g., due to regulatory action, product lifespan, economic viability).

RAIDAR calculates overall persistence (Pov)[11], which is often used synonymously with "residence time" and does not account for losses from "advection" (e.g., sediment burial, transport out of the model environment) nor dilution. The value of Pov is governed by the amount of substance residing in a medium (based on a default emission to the environment) determined by fugacity relationships and the reaction rate (i.e., degradation rate) of a chemical in a specific medium.

[11] Here, we define Pov as the sum of all medium-specific half-lives (hours) weighted by the mass fraction of the chemical in each medium in which it resides

Response time in ERC2 is calculated as the Pov multiplied by five and can also be regarded as an approximate measure of the full lifetime of the chemical in the total environment.

Table 4.8. Example of results for Response Time descriptor for CAS RN 632-79-1

4b

EXPOSURE - Response Time

CAS RN	97% Response Time (yr)
632-79-1	25.55

4.4.2 Mobility

In ERC2, the ability of a chemical to persist in a mobile medium such as air or water and be transported over long distances via that medium is a key factor for determining the spatial extent of exposure. Mobility[12] in ERC2 is determined using a multimedia model where "characteristic travel distance" (CTD) is calculated using the RAIDAR model. CTD is a "transport-oriented" metric, meaning there is no specific remote environment considered and calculations are based on a travel distance from source emissions (e.g., in kilometers). The chemical mass fraction (F) in air and water are determined according to the mass-balance outcome of the RAIDAR model.

RAIDAR v3.0 uses the CTD method for air or water first proposed by Beyer et al. (Beyer et al. 2000):

$$CTD_{air} = Pov \times F_{air} \times \text{wind speed}$$

$$CTD_{water} = Pov \times F_{water} \times \text{water speed}$$

Table 4.9. Example of results for Mobility descriptor for CAS RN 632-79-1

4b

EXPOSURE - Mobility

CAS RN	Travel Distance (Km) (Highest Value Reported)	Transport Medium	Scale of Exposure
632-79-1	521	Water/Air	Local to Regional

4.4.3 Emission Patterns

In addition to persistence, the quantity (mass) of chemical entering the environment at any given time, the rate at which it enters (emission rate) and how it enters (mode of entry) are critical parameters for determining the likelihood of contact of an organism with a contaminant in the environment. Chemical quantity is determined using empirical data sources that describe annual tonnage data (kilotonnes per year) from import and manufacturing survey data for the pure substance (i.e., not the quantity of products

[12] Both transfer efficiency and accumulation potential in a receiving medium are implemented in ERC2.3 update in addition to CTD

or articles in which it is contained). Canadian empirical data sources used by ERC2 are listed in Figure 4.8 above (all models and data sources are listed in the References section).

Since rates of emission (e.g., kg/h) for substances are generally not well characterised, for relative chemical-by-chemical comparisons in ERC2 it is assumed that 100% of the chemical mass is emitted to the regional environment (i.e., homogeneously distributed over 100 000 km/sq.). This is a conservative regional scale emission pattern because it does not consider any potential loss of the total chemical mass during substance use (e.g., formulation in products) or removal by sewage treatment before entering surface waters. Exposure classification of Emission Patterns descriptor relies on chemical quantity and Pov for water or soil, depending on mode of entry to the environment. In this descriptor, Pov is used as a metric to weigh the relative importance of chemical tonnage. For example, some chemicals with very high tonnage can have very quick response times (i.e., response time being an approximate measure of the full lifetime of the chemical in the total environment) in the environment while lower tonnage chemicals can have slow response times.

Local point source emission patterns are not directly included in Emission Patterns descriptor. However, the Margin of Exposure descriptor was designed to address this difference in scale of emission pattern.

Table 4.10. Example results for Emission Patterns descriptor for CAS RN 632-79-1

4b **EXPOSURE – Emission Patterns**

CAS RN	Reported or Estimated Quantity (kt/yr)
632-79-1	0.3

4.4.4 Foodweb Exposure

Foodweb exposure is determined by integrating the properties of persistence and bioaccumulation. This descriptor estimates the degree of exposure from direct contact and food web transfer of contaminants in vulnerable food web species. This is accomplished using the RAIDAR exposure assessment factor (EAF), which is the concentration in sensitive food web species based on a default or unit emission rate to the default environment of the model (e.g., 1 kg/h). This descriptor may appear to be an overlap with the Foodweb Toxicity descriptor but is employed here to account for potential foodweb exposures regardless of toxicity or for protection from unknown toxic potential (e.g., given data gaps in toxicological data) and for estimating aggregate tissue residues (TRA).

4.4.5 Margin of Exposure

Margin of exposure (MoE) refers to the difference between a critical level of effect, such as a predicted no effect concentration (PNEC), and the measured or predicted concentration in ecological receptors (MEC or PEC). In ERC2, the MoE is calculated as the ratio of the critical rate of emission (EC) in kg/hr and the actual rate of emission (EA) kg/hr. EC refers to the amount of chemical that must enter the environment for effects to be expected for the most vulnerable species in aquatic or terrestrial food web. EA refers to the calculated rate of emission based on known or estimated tonnage data collected by ECCC from Canadian inventory survey data per CAS RN. As MoE is calculated using a regional fate simulation (100,00

km^2), it does not capture concerns at the local scale. A MoE of less than 1000 is used to flag substances that may present this concern[13].

It is also noted that the bioactivity-exposure ratios (BERs), which are the ratios between an estimated internal effect concentration (IEC) using median bioactivity concentrations (AC50) compared to a whole-body internal tissue residue, can offer another method to determine MoE based *on in vitro* bioactivity data. However, BERs are not used for MoE determination in ERC2 at this time but can be included in future updates to ERC2 as they become available and could be applied in a similar manner to MoEs calculated using the RAIDAR food web scenario.

Table 4.11. Example of results for Margin of Exposure descriptor for CAS RN 632-79-1

4b

EXPOSURE – Margin of Exposure

CAS RN	Critical Emission Rate (kg/h)	Actual Emission Rate (estimated) (kg/h)	MOE >1000 ?
632-79-1	27.0742023	34.24657534	FALSE

In the above table, the example CAS RN triggers the MoE descriptor rule. If the exposure classification is low (i.e., Class 1), this CAS RN would receive an adjustment to Class 2 to account for potential local scale impacts.

4.5 Hazard and Exposure Classification, Confidence and Severity Scoring

The classification and scoring methodology used in ERC2 is based on numerical values, the magnitude of which has been established based on existing empirical knowledge (e.g., potency scales, travel distances), where possible. ERC2 has established a numerical scoring scheme which was translated to categorical outcomes (e.g., very high, high, moderate, low, very low) for interpretation in a regulatory context. This scoring scheme is applied at each individual hazard and exposure profiling step, and to determine confidence and severity levels, with details outlined in sections 4.5.1 and 4.5.2 below. Scoring schemes for risk are determined by combining these outcomes as 'risk' (a function of both hazard and exposure). These scoring schemes are described in section 5.

4.5.1 Hazard Classification, confidence scoring and severity

Classification scores are generated for each hazard descriptor in the Hazard Profile (except for cumulative toxicity which cannot be classified at this time) (see section 4.3.5).

Each hazard descriptor is classified as either low (Class 1), moderate (Class 2) or high (Class 3) hazard according to classification logic rules and associated workflows (Appendix B).

[13] See Science Approach Document: - Canada.ca for further rationale

Hazard profiling is potency-based, meaning the classification rules are driven by potency of chemical interactions or observed effects (where potency of chemical interactions refers to the "strength" of the interaction or exposure concentration associated with an adverse effect or a scaled measure of plausible or observed adversity. For example, in receptor binding results the relative binding affinity is scaled from very weak to strong binding). A Class 3 or Class 2 descriptor level outcome requires no more than one set of hazard descriptor rules to be met, otherwise hazard classification defaults to Class 1. All hazard descriptors are accepted to be equally significant for prioritization purposes.

Hazard classification for each descriptor is also evaluated for the confidence level. Confidence can be regarded as a measure of certainty with classification outcomes. The confidence scoring for each descriptor is weighted according to the data source (i.e., level of biological organization) and abundance of data type. Table 4.12 summarizes the maximum confidence scores that can be awarded to each hazard descriptor (more details are available in the 2022 ERC2 Science Approach document). For example, for Chemical Reactivity and Genotoxicity, five data sources are used (*in silico, in chemico, in vitro*, and *in vivo* mammalian and aquatic data), and where many sources of data are used to parameterize each data type, a greater consensus must result for higher descriptor confidence scores. In contrast, for Food Web Toxicity, a single *in silico* data source is used and so no consensus is considered within the descriptor. To avoid low confidence assignments to *in vivo* data used as the only source of evidence for hazard classification due to lack of data consensus or data gaps, a default confidence score of 26 is assigned when multiple data are available or 21 when only single values are available. These values were selected to ensure a moderate level of confidence is given when the total hazard confidence score is summed and only *in vivo* data are used to classify hazard (i.e., due to lack of consensus with other data). In such cases, plausible mechanistic-causality relationships are “unconfirmed” and noted as such in final results along with all other plausible target interactions.

Table 4.12. Descriptor level confidence assignments for hazard

Descriptor	Maximum Descriptor Confidence Score
Receptor-Mediated Toxicity	88
Chemical Reactivity/Genotoxicity	93
Mode of Toxic Action	15
Food Web Toxicity	10
Cumulative Toxicity	0[a]
Total score	206

[a] Not included in total hazard confidence score

Table 4.13 shows how the ranges of confidence scores are partitioned to assign a confidence category. These category thresholds were assigned based on examination of the relative distribution of substances in the ERC2 inventory (approximately 12, 000 substances; (ECCC 2022)) within each category and considering the maximum possible confidence score of 206.

Table 4.13. Categorical assignment of total confidence score for the Hazard Profile

Hazard Confidence Score	Confidence Category	Visual representation
1-10	Very Low	
11-25	Low	
26-60	Moderate	
61-100	High	
>100	Very High	

Final classifications of hazard are based on the highest score for one or more hazard descriptors as outlined above and shown in Figure 4.8. To calculate the total hazard confidence score, the confidence scores of hazards whose descriptor classification matches the final hazard classification (i.e., are the same value) are added together.

The scoring routine to assign a level of severity is weighted such that a higher severity score is achieved when multiple Class 3 (high) hazard descriptor classifications result for a substance (e.g., see rule no 5 in Table 4.14).

Table 4.14. Determination of severity for the Hazard Profile

	Hazard Severity		
Rule No.	**Rule Description**	**Score**	**Severity Assessment**
1	Class 1 (low) hazard or exposure outcomes	0	Very low
2	Class 2 (moderate) hazard or exposure outcome	1	Low
3	A single Class 3 (high) hazard or exposure outcome	2	Moderate
4	Two or three Class 3 (high) hazard or exposure outcomes	3	High
5	Greater than three Class 3 (high) hazard or exposure outcomes	4	Very high

Severity can generally correlate with confidence, where higher confidence scores often can result in higher severity scores and vice versa. However, this is not always the case because a chemical can have a low confidence score (e.g., relies mainly on in silico results) yet achieve a higher severity score due to hazard (and/or exposure) descriptor classification consensus. Table 4.14 summarizes how severity is determined for the Hazard Profile (see also step 4 in Figure 4.8 and Figure 4.9).

Figure 4.8 illustrates the logic workflow for hazard classification and confidence scoring. Figure 4.9 demonstrates the classification of Hazard, and confidence and severity outcomes for the example substance CAS RN 632-79-1. It is noted that, while the final confidence score considers the highest classification determined for the substance (i.e., for example substance CAS RN 632-79-1, it is Class 3), and is especially useful for the purpose of prioritization when working with large numbers of substances, when analysing the hazard profile of a substance it is also important to consider classification of other hazard descriptors and their associated confidence scores. These classifications provide valuable information and a more fulsome understanding of a substance's potential to cause adverse effects. For example, substance CAS RN 632-79-1 has Class 2 outcomes for both Receptor Mediated Toxicity and Chemical Reactivity/Genotoxicity descriptors, with moderate level of confidence.

Appendix B demonstrates a breakdown of the various workflows or processes used for classification of each hazard descriptor and associated general logic rules for confidence scoring. More detailed information on confidence scoring and logic rules is available in ERC2 Science Approach document (ECCC 2022).

Figure 4.8. Logic workflow for exposure classification and confidence scoring

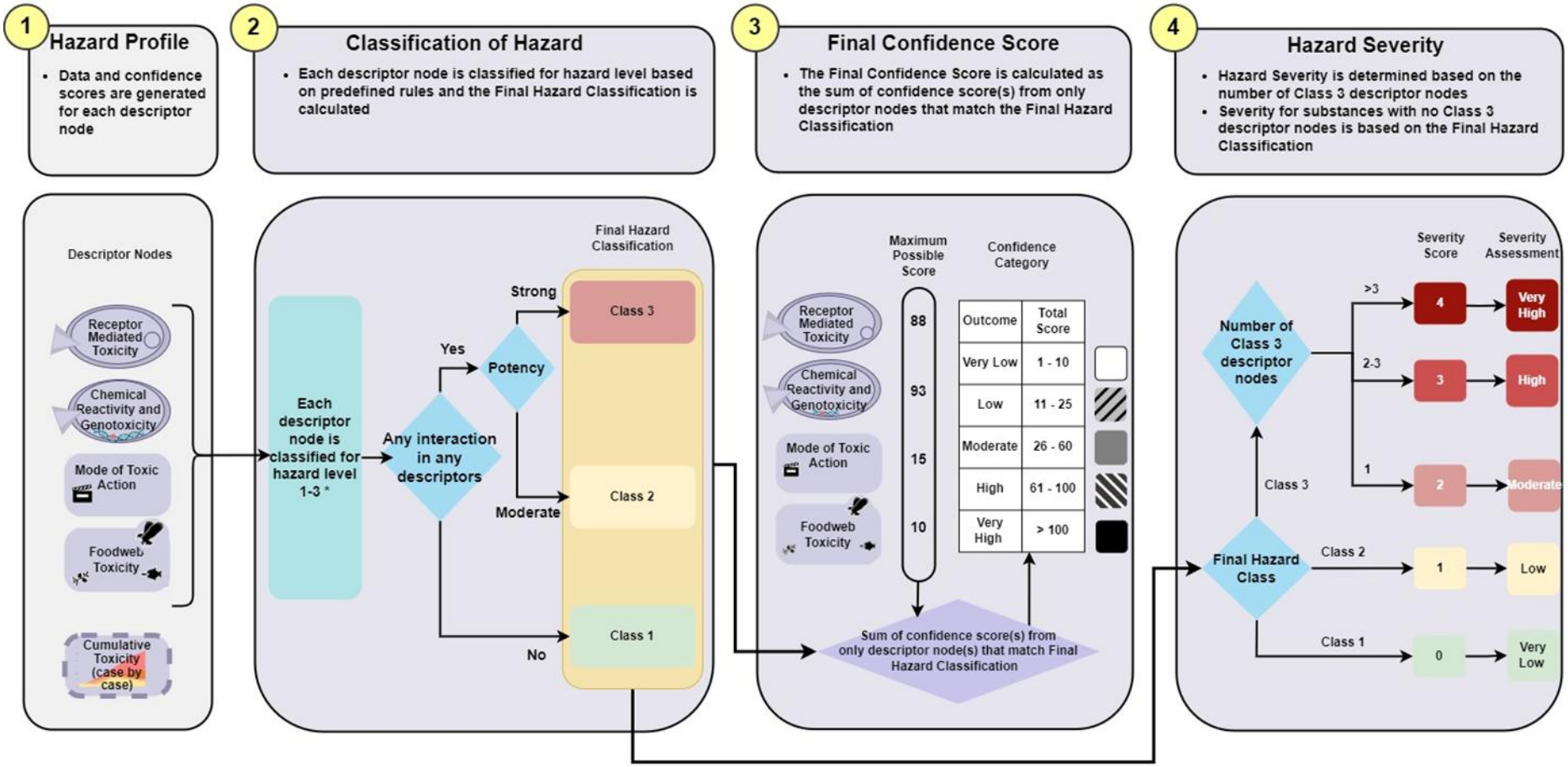
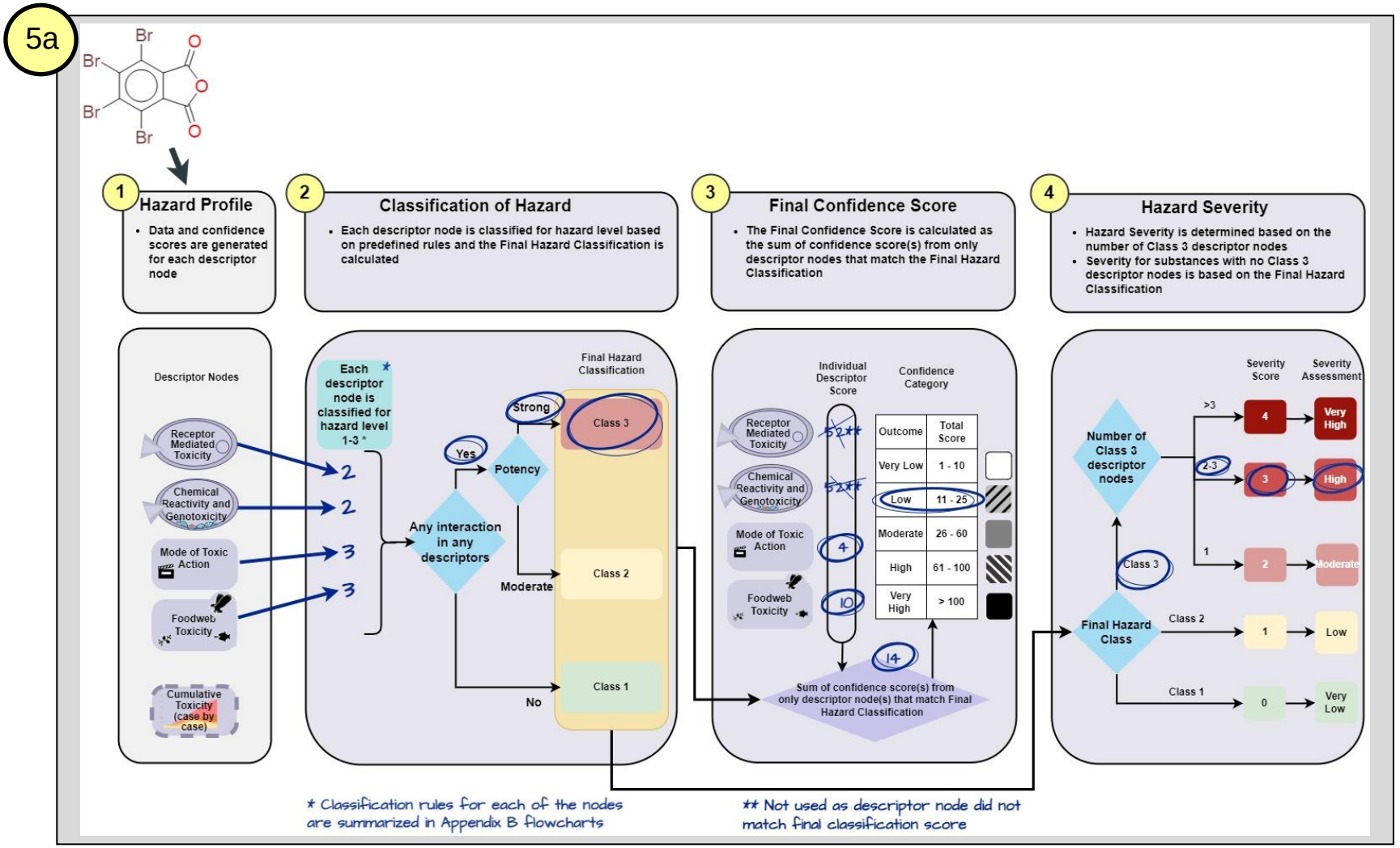


Figure 4.9. Example logic workflow for hazard classification and confidence scoring



4.5.2 Exposure Classification, confidence scoring and severity

Classification of Exposure is driven by the likelihood of organism contact with a contaminant over varying spatial and temporal scales of exposure according to the classification rules and considering the exposure space defined for ERC2 (Figure 3.1, step 5b).

Exposure classification is conducted in a very similar manner to that described above for Hazard, with Class 3 (high), Class 2 (moderate) or Class 1 (low) outcomes (see Appendix C for exposure classification logic rules and associated workflows). Classification scores are generated for each exposure descriptor, except for margin of exposure which is used as a verification mechanism for low exposure concern outcomes. Emission pattern differences and fate in the environment can influence classification scores. A Class 3 (high) or Class 2 (moderate) descriptor level outcome requires no more than one set of exposure descriptor rules (i.e., “any” in Figure 4.10, step 2) to be met, otherwise exposure classification defaults to Class 1 (low) exposure. Identical to hazard, the final classifications of exposure are based on the highest score for one or more exposure descriptors (Figure 4.10).

An exposure confidence score is calculated for each exposure descriptor according to confidence logic rules (see Appendix C), similarly to how it is performed for hazard classification and confidence scoring. Similarly, confidence can be regarded as a measure of certainty with exposure classification outcomes. However, in the Exposure Profile, the Margin of Exposure descriptor is used in a verification step to confirm low exposure concern in the environment and contributes to the total exposure confidence score only when triggered (Figure 4.10, step 2). Lower confidence scores can result from lack of consensus between exposure descriptor classification outcomes as well as significant data gaps.

A maximum total exposure confidence score of 50 can be achieved using the confidence weighting scheme in Table 4.15. The maximum score of 50 reflects an equal weighting of exposure descriptors because all descriptors make use of *in silico* data only for exposure classification and chemical quantity data (for Canada) is currently extrapolated for 97% of ERC2 substances.

Table 4.15. Descriptor level confidence assignments for exposure

Descriptor	Maximum Descriptor Confidence Score
Response Time	10
Mobility	10
Emission Pattern	10
Food Web Exposure	10
Margin of Exposure	10[a]
Total score	50

[a] Only used when triggered

Using the maximum total confidence score for exposure, the categorical confidence category assignments are determined based on equal distribution across the range of exposure confidence scores (Table 4.16).

Table 4.16. Categorical assignment of total confidence for the Exposure Profile

Exposure Confidence Score	Confidence Category	Visual representation
1-10	Very Low	
11-20	Low	
21-30	Moderate	
31-40	High	
41-50	Very High	

Severity determination for the Exposure Profile is conducted in the manner identical to that for the Hazard Profile (see section 4.5.1). Severity rules described in Table 4.14 are the same for severity assessment of the Exposure Profile descriptors, i.e., a higher severity score is achieved when multiple Class 3 (high) exposure descriptor classifications result for a substance. See also step 4 in Figure 4.10 and Figure 4.11, which also demonstrate severity determination.

Figure 4.10 illustrates the logic workflow for exposure classification, confidence scoring and severity. Figure 4.11 demonstrates the classification of exposure, and confidence and severity outcomes for the example substance CAS RN 632-79-1.

Figure 4.10. Logic workflow for exposure classification and confidence scoring

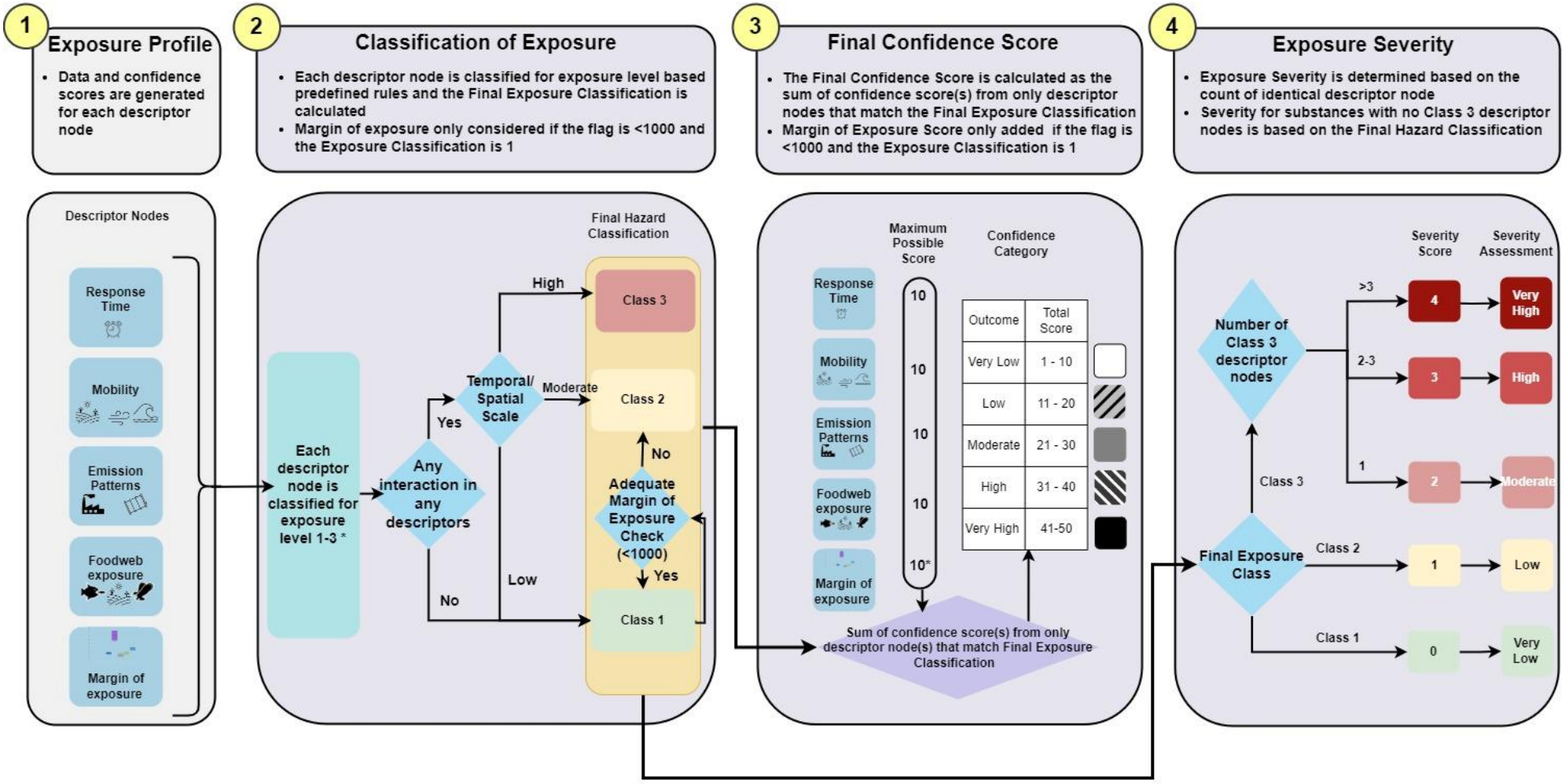
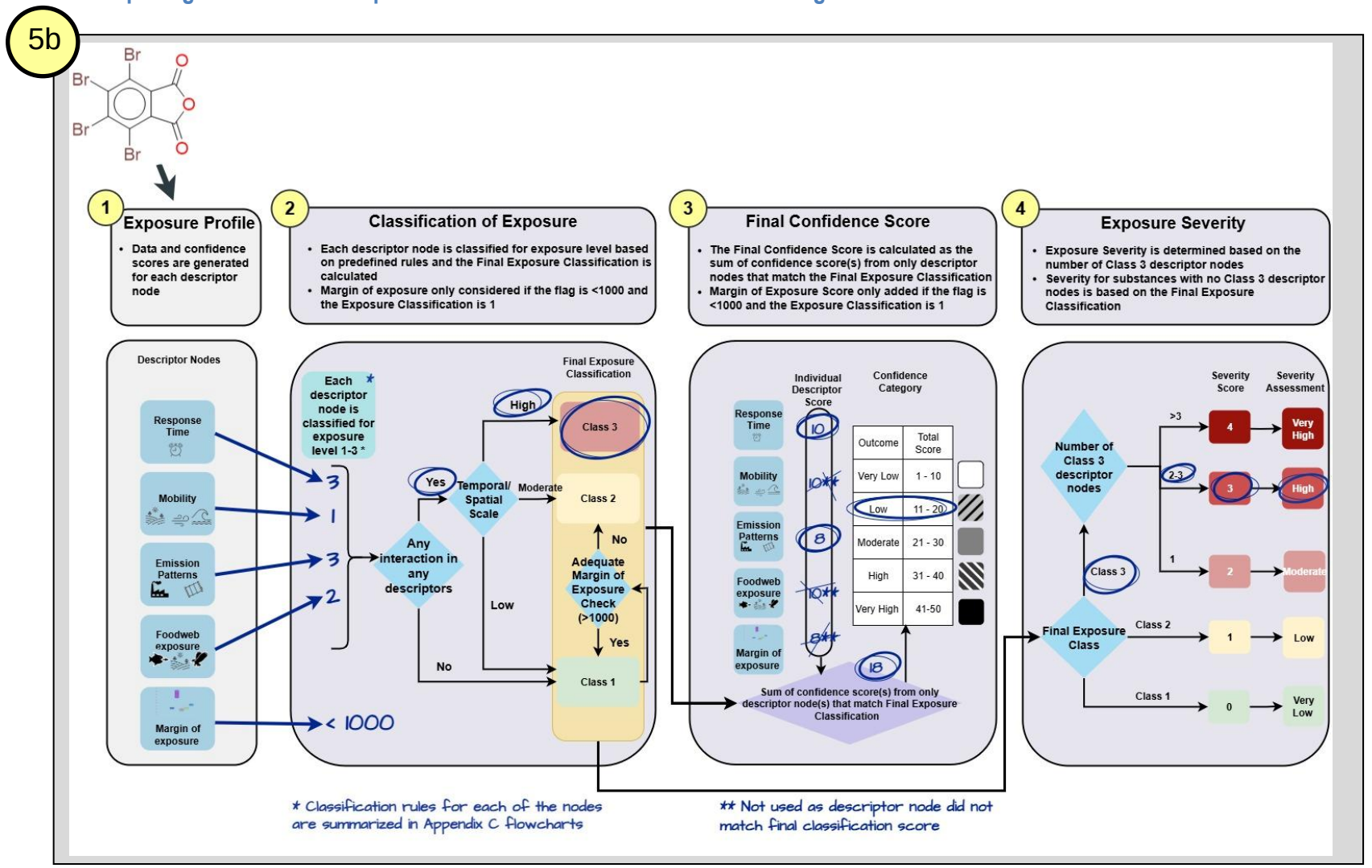


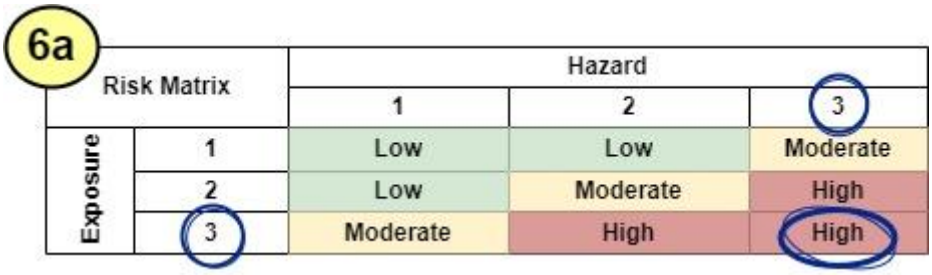
Figure 4.11. Example logic workflow for exposure classification and confidence scoring



5 The Risk Matrix and Reporting on Risk Classification and Risk Confidence and Severity

Similarly to ERC1 (ECCC 2016; OECD 2018), the ecological risk classification outcomes in ERC2 are determined using a risk matrix by combining the classification scores for hazard and exposure (Figure 3.1, step 6a).

Table 5.1. The risk matrix and risk classification for CAS RN 632-79-1



However, in ERC2, risk classification is also scored for confidence to reveal the degree of data consensus as well as data gaps. Confidence scores for risk classification are produced by adding hazard confidence scores and exposure confidence scores.

Table 5.2 summarizes the scoring routines used for categorical risk classification and risk confidence outcomes.

Table 5.2. Risk Classification and confidence scoring

Risk Classification Score	Risk Classification	Visual representation
5-6	High	
4	Moderate	
1-3	Low	
Risk Confidence Score	**Risk Confidence Category**	**Visual representation**
>150	Very High	
101-150	High	
51-100	Moderate	
26-50	Low	
1-25	Very Low	

Table 5.3 Final classification of risk and confidence for CAS RN 632-79-1

6b

FINAL CLASSIFICATION – Risk and Confidence

CAS RN	Risk Description	Risk Score	Confidence Description	Confidence Score
632-79-1	High	6	Low	32

In addition to risk confidence scoring, the severity of risk outcome is also considered and evaluated given that chemicals can simultaneously cause effects via more than one mechanism or pathway and organisms can be exposed at different temporal and spatial scales, from different routes of exposure and different emission patterns. In situations where higher volume substances are capable of more than one toxicity interaction and more than one route of exposure at different spatial and temporal scales, the potential impact to organisms in the environment from such substances can be said to be more “severe”. Because moderate and high-risk outcomes can be triggered by any one hazard and exposure descriptor, severity is used as a method to weight the possible risk outcomes in the risk matrix and can be regarded as a measure of the scale of risk. Severity scores are first calculated separately for hazard and exposure (Figure 3.1, step 5a and 5b). Risk severity is then a function of summing the hazard and exposure severity scores (Figure 3.1, step 6b).

Table 5.4. Hazard, Exposure and Risk Severity Scoring

Hazard and Exposure Severity		
Rule Description	**Score**	**Severity Assessment**
Class 1 (low) hazard or exposure outcomes	0	Very low
Class 2 (moderate) hazard or exposure outcome	1	Low
A single Class 3 (high) hazard or exposure outcome	2	Moderate
Two or three Class 3 (high) hazard or exposure outcomes	3	High
Greater than three Class 3 (high) hazard or exposure outcomes	4	Very high
Risk Severity		
Rule Description	**Score**	**Severity Assessment**
Sum of hazard and exposure severity scores	0 to 8	Hazard / Exposure

Table 5.5. Risk Severity reporting for CAS RN 632-79-1

6b

FINAL CLASSIFICATION – Severity

CAS RN	Severity Description (Hazard / Exposure)	Severity Score	Risk Scale	Flags for Evaluation Consideration
632-79-1	High / High	6	Local to Regional	Margin of Exposure Flag

6 Automation of ERC2 IATA

Generation of data and results for prioritization of substances in ERC2 Science Approach document (ECCC 2022) required significant computational power, resources and time. In effort to streamline the generation of ERC2 information for substances not currently in ERC2, automated workflows are being developed at ECCC. Additionally, automation will allow for accelerated updates to the framework with, for example, incorporation of new information, new models or changes to the approach and will reduce introduction of errors due to manual data manipulation.

Presently, workflows are being developed by ECCC using the free/Open Source Konstanz Information Miner software (KNIME) (Berthold et al. 2007). KNIME uses a modular 'building block' approach to create workflows. This approach was adapted at ECCC as it is user-friendly as only moderate programming experience is required. KNIME also addressed limitations regarding Microsoft Excel and large datasets, as well as ongoing issues with unwanted automatic manipulation of chemical identifiers (CAS RN). KNIME has also been used by Health Canada in the Science Approach document for Health Canada's automated workflow for prioritization (HAWPr) (Health Canada 2024).

For ERC2 automation, KNIME is used to parse, aggregate and classify the required information (for example, QSAR model and database outputs). Then, where feasible, it is used to run the required QSAR models (e.g. via command line inputs) or gather data automatically (e.g. via OECD Toolbox Application Programming Interface (API)). Appendix E illustrates several examples of approaches to automation using the Receptor Mediated Toxicity descriptor (described in section 4.3.1).

Aspects of the ERC2 hazard workflows are also undergoing automation using the OECD QSAR Application Toolbox (2020). An exact copy of the hazard workflows cannot be adopted for the Toolbox given ECCC's use of proprietary models and agreements with model developers on the public dissemination of model predictions for vast numbers of substances. Supported by ECHA and ECCC, work has commenced via the Toolbox developer in the fall of 2024, with details of the workplan discussed by the OECD QSAR Toolbox Management Group in November of 2024 and implementation completed in spring of 2025. Addition of ERC2-based hazard profiling is expected to also greatly simplify the profiling of chemicals outside of ERC2's domain of substances.

7 Application of ERC2 IATA

A group of 15 organic flame retardants (OFRs) and 9 non-specific acting chemicals are used as a case study to illustrate the generation of ERC2 data and the practical application of ERC2 outcomes for describing hazard, exposure, and risk.

These 15 OFR substances were selected from ERC2 inventory (published as part of the ERC2 Science Approach document (ECCC 2022)) because they trigger various hazard and exposure descriptors and exhibit diverse hazard and exposure profiles, they are also on Canada's DSL, have a flame-retardant use code and were confirmed as being used as flame retardants. For comparative purposes, a set of 9 non-specific acting substances was also selected representing substances having little or no concern for hazard or exposure classification and thus classified as low risk.

The following sections (7.1 to 7.5) outline what ERC2 data for the 15 OFRs and 9 non-specific acting chemicals can reveal about the three common regulatory concerns identified in the Purpose and Hypotheses (Problem Formulation) section 2.

Table 7.1 summarizes the substance identity for the 15 OFRs used in the illustrative case study.

Table 7.1. Substance identity information of OFRs used in the illustrative case study

CAS RN	Substance name	SMILES used for modelling
79-27-6[1]	1,1,2,2-tetrabromoethane,;1,1,2,2-tetrabromoethane;ethane, 1,1,2,2-tetrabromo-;tetrabromoethane	[Br]C([Br])C([Br])[Br]
118-79-6[1]	2,4,6-tribromophenol;phenol, 2,4,6-tribromo-;2,4,6-bromophenol;tribromophenol, 2,4,6-;2,4,6-tribromo-phenol	Oc1c([Br])cc([Br])cc1[Br]
126-71-6[1]	phosphoric acid, tris(2-methylpropyl) ester;triisobutyl phosphate;tris(2-methylpropyl) phosphate;phosphoric acid, triisobutyl ester;isobutyl phosphate, ((c4h9o)3po)	CC(C)COP(=O)(OCC(C)C)OCC(C)C
632-79-1[1,3]	tetrabromophthalic anhydride;4,5,6,7-tetrabromo-1,3-isobenzofurandione;1,3-isobenzofurandione, 4,5,6,7-tetrabromo-;4,5,6,7-tetrabromo-2-benzofuran-1,3-dione;phthalic anhydride, tetrabromo-	O=C2c1c([Br])c([Br])c([Br])c([Br])c1C(=O)O2
1241-94-7[1]	2-ethylhexyldiphenyl phosphate;ethyl hexyl diphenyl phosphate;2-ethylhexyl diphenyl phosphate;phosphoric acid, 2-ethylhexyl diphenyl ester;diphenyl 2-ethylhexyl phosphate;o=p(o-phenyl)2 o-ethexyl;2-ethylhexyl diphenyl;2ehdpp;diphenyl-2-ethylhexyl phosphate;2-ethylhexyl diphenyl phosphate (octicizer)	CCCCC(CC)COP(=O)(Oc1ccccc1)Oc1ccccc1
2524-04-1[2]	o,o-diethyl chlorothiophosphate;phosphorochloridothioic acid, o,o-diethyl ester;o,o-diethyl phosphorochloridothioate;ethyl phosphorochloridothioate, ((eto)2clps);o,o'-diethyl chlorothiophosphate	CCOP(=S)([Cl])OCC
3234-02-4[1, 2]	trans-2,3-dibromo-2-butene-1,4-diol;2-butene-1,4-diol, 2,3-dibromo-;2,3-dibromo-2-butene-1,4-diol;dibromobutenediol;2,3-dibromobut-2-ene-1,4-diol	OC\C([Br])=C(\[Br])CO
3322-93-8[1]	1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane;cyclohexane, 1,2-dibromo-4-(1,2-dibromoethyl)-;1-(1,2-dibromoethyl)-3,4-	[Br]CC([Br])C1CCC([Br])C([Br])C1

	dibromocyclohexane	
20566-35-2[1]	1,2-benzenedicarboxylic acid, 3,4,5,6-tetrabromo-, 2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl ester;2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetrabromophthalate;2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetrabromobenzene-1,2-dicarboxylate	CC(O)COC(=O)c1c([Br])c([Br])c([Br])c([Br])c1C(=O)OCCOCCO
26444-49-5[1]	phosphoric acid, methylphenyl diphenyl ester;cresyl diphenyl phosphate;diphenyl cresyl phosphate;diphenyl tolyl phosphate;methylphenyl diphenyl ester, phosphoric acid	Cc2ccc(OP(=O)(Oc1ccccc1)Oc1ccccc1)cc2
30554-72-4[1]	cyclohexane, tetrabromodichloro-;tetrabromodichlorocyclohexane;1,1,2,2-tetrabromo-3,3-dichlorocyclohexane	[Cl]C1([Cl])CCCC([Br])([Br])C1([Br])[Br]
30554-73-5[1]	cyclohexane, tribromotrichloro-;tribromotrichlorocyclohexane;1,1,2-tribromo-2,3,3-trichlorocyclohexane	[Cl]C1([Cl])CCCC([Br])([Br])C1([Cl])[Br]
36483-57-5[1]	1-propanol, 2,2-dimethyl-, tribromo deriv.;2,2-dimethylpropan-1-ol, tribromo derivative;1-propanol, 2,2-dimethyl-, tribromo derivative	CC(C)(C[Br])C(O)([Br])[Br]
42757-55-1[2]	benzene, 1,1'-sulfonylbis[3,5-dibromo-4-(2,3-dibromopropoxy)-;bis[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl] sulphone;1,1'-sulfonylbis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene];benzene, 1,1 -sulfonylbis[3,5-dibromo-4-(2,3-dibromopropoxy)-;benzene, 1,1'-sulfonylbis[3,5-dibromo-4-(2,3-dibromopropoxy)-;bis [3,5-dibromo-4-(2,3-dibromopropoxy) phenyl] sulfone	O=S(=O)(c1cc([Br])c(OCC([Br])C[Br])c([Br])c1)c1cc([Br])c(OCC([Br])C[Br])c([Br])c1
52907-07-0[2]	4,7-methano-1h-isoindole-1,3(2h)-dione, 2,2'-(1,2-ethanediyl)bis[5,6-dibromohexahydro-;n,n'-(ethylene)bis[4,5-dibromohexahydro-3,6-methanophthalimide];2,2'-ethane-1,2-diylbis(5,6-dibromohexahydro-1h-4,7-methanoisoindole-1,3(2h)-dione);4,7-methano-1h-isoindole-1,3(2h)-dione, 2,2'-(1,2-ethanediyl)bis[5,6-dibromohexahydro-;saytex bn 451	O=C2C1C4CC(C1C(=O)N2CCN2C(=O)C1C3CC(C1C2=O)C([Br])C3[Br])C([Br])C4[Br]

[1]12 OFR chosen for further investigation (section 7); [2]4 substances with ADME flags (section 7.2); [3]Substance used as an example throughout sections 4 and 5

Table 7.2 summarizes the substance identities for 9 non-specific acting chemicals.

Table 7.2. Substance identity information of non-specific acting substances used in the illustrative case study

CAS RN	Substance name	SMILES used for modelling
96-22-0	3-pentanone;pentanone, 3-;pentan-3-one	CCC(=O)CC
106-98-9	1-butene;but-1-ene	CCC=C
109-79-5	butane-1-thiol;butanethiol;1-butanethiol;n-butanethiol;n-butyl mercaptan	CCCCS
118-55-8	phenyl salicylate;phenyl 2-hydroxybenzoate;phenylsalicylate;benzoic acid, 2-hydroxy-, phenyl ester;salicylic acid, phenyl ester	Oc1ccccc1C(=O)Oc1ccccc1
137-32-6	2-methyl-1-butanol;1-butanol, 2-methyl-;1-butanol, 2-methyl-, (s)-;2-methylbutan-1-ol	CCC(C)CO
1559-34-8	tetra ethylene glycol butyl ether;3,6,9,12-tetraoxahexadecan-1-ol	CCCCOCCOCCOCCOCCO
17741-63-8	benzamide, n,n'-[6,13-bis(acetylamino)-2,9-diethoxy-3,10-triphenodioxazinediyl]bis-;n,n'-[6,13-diacetamido-2,9-diethoxy-3,10-triphenodioxazinediyl]bis(benzamide);n,n'-[6,13-bis(acetylamino)-2,9-diethoxy[1,4]benzoxazino[2,3-b]phenoxazine-3,10-diyl]dibenzamide;benzamide, n,n'-[6,13-bis(acetylamino)-2,9-diethoxy-3,10-triphenodioxazinediyl]bis-	CCOC1=CC2=C(OC3=C(NC(C)=O)C4=NC5=CC(OCC)=C(NC(=O)C6=CC=CC=C6)C=C5OC4=C(NC(C)=O)C3=N2)C=C1NC(=O)C1=CC=CC=C1
25551-13-7	1,2,3-trimethylbenzene;trimethylbenzenes;benzene, trimethyl-;trimethylbenzene	Cc1cc(C)cc(C)c1
51566-62-2	6-octenenitrile, 3,7-dimethyl-;3,7-dimethyloct-6-enenitrile	CC(CCC=C(C)C)CC#N

ERC 2 results for the 15 OFRs and 9 non-specific acting chemicals are summarized in a spreadsheet provided as supplement to this document (noted as Appendix D).

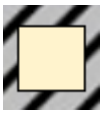
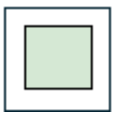
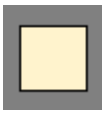
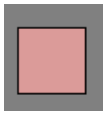
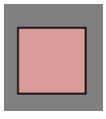
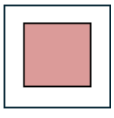
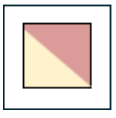
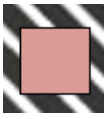
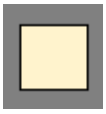
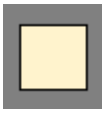
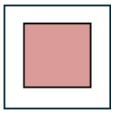
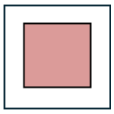
7.1 Hazard profiling and mode(s) of action

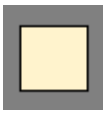
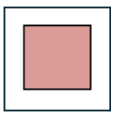
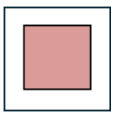
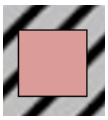
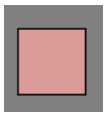
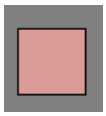
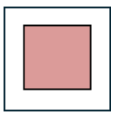
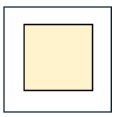
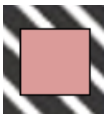
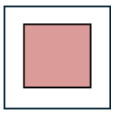
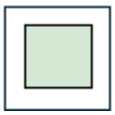
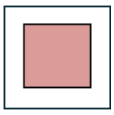
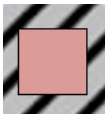
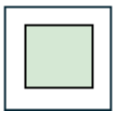
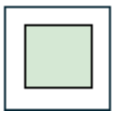
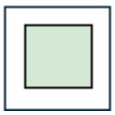
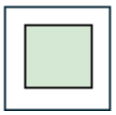
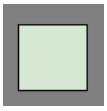
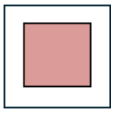
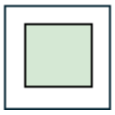
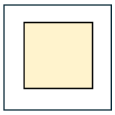
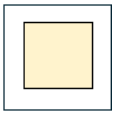
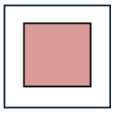
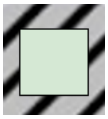
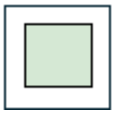
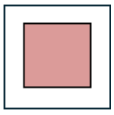
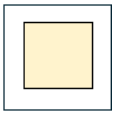
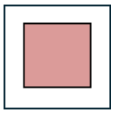
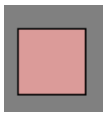
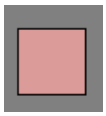
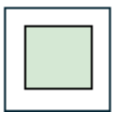
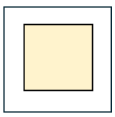
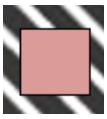
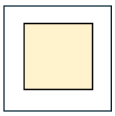
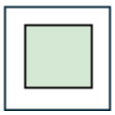
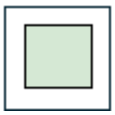
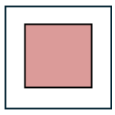
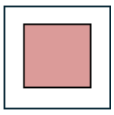
Chemicals can elicit adverse effects simultaneously via numerous modes of action (e.g., narcosis, endocrine, neurotoxicity). Hence, from the toxicological and ecological perspectives, it is recognized that certain modes of action are important to identify and better understand how sensitive these endpoints can be as compared to others, e.g. neurotoxicity vs narcosis. ERC2 hazard descriptors in the hazard profile cover a large toxicological space and allow to evaluate endpoints from the various modes of action in a systematic way. Consequently, chemicals that exhibit high potency and unique modalities can be effectively identified.

Table 7.3 and Table 7.4 present hazard profile classifications and associated confidence scoring for four profile descriptors and the final hazard profile classification and confidence score, for the OFRs and non-specific acting chemicals, respectively. When examining substances in Table 7.3, it is apparent that these substances trigger multiple descriptors with high classification scores, but varying confidence levels (reflective of some data gaps and/or consensus among data), indicating both high potency and ability to act via numerous modes of action. In contrast, chemicals in Table 7.4, do not trigger a variety of effects in each of the descriptors, demonstrating lower potency as they are typically acting via narcosis.

Legend for Table 7.3 and Table 7.4:

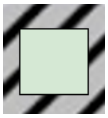
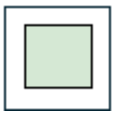
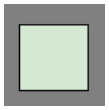
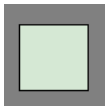
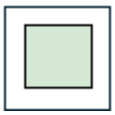
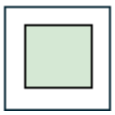
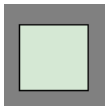
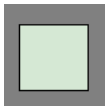
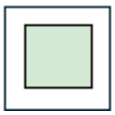
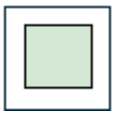
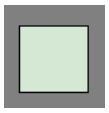
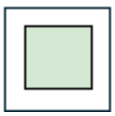
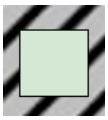
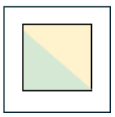
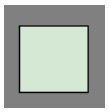
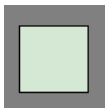
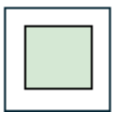
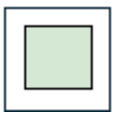
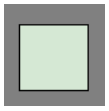
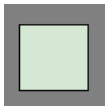
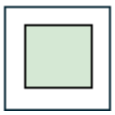
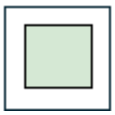
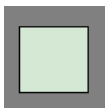
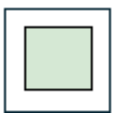
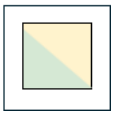
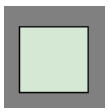
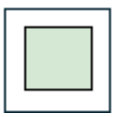
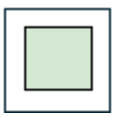
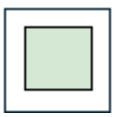
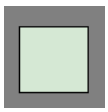
Table 7.3. Hazard descriptor results for the OFRs used in the illustrative case study

CAS RN	Chemical Reactivity and Genotoxicity	Receptor Mediated Toxicity	Mode of Toxic Action	Foodweb Toxicity[1]	Final Hazard Classification
79-27-6					
118-79-6					
126-71-6					

632-79-1					
1241-94-7					
2524-04-1					
3234-02-4					
3322-93-8					
20566-35-2					
26444-49-5					
30554-72-4					
30554-73-5					
36483-57-5					
42757-55-1					
52907-07-0					

[1]Bottom triangle denotes hazard classification (aquatic) and top triangle is hazard classification (terrestrial); for purpose of ERC2 prioritization the higher class is the outcome

Table 7.4. Hazard descriptor results for the non-specific acting chemicals used in the illustrative case study

CAS RN	Chemical Reactivity and Genotoxicity	Receptor Mediated Toxicity	Mode of Toxic Action	Foodweb Toxicity[1]	Final Hazard Classification
96-22-0					
106-98-9					
109-79-5					
118-55-8					
137-32-6					
1559-34-8					
17741-63-8					
25551-13-7					
51566-62-2					

[1]Bottom triangle denotes hazard classification (aquatic) and top triangle is hazard classification (terrestrial); for purpose of ERC2 prioritization the higher class is the outcome

7.1.1 Specific MoAs and the Mode of Toxic Action descriptor

Examining specific MoAs in the hazard profile can be helpful to a more comprehensive understanding of the chemicals' potential to cause adverse effects, particularly at sensitive life stages, and can help to avoid under-estimation of predicted no effect levels and to inform approaches to regrettable substitution. Another benefit of profiling MoA is to gain an understanding of cross-species susceptibility to potential adverse

effects suggested by a broad mode or mechanism of action (e.g. molecular level interactions). Sapounidou et al. (2021) have complied the most extensive taxonomic domain of species associated with specific modes, mechanisms and MIEs currently in the literature. For example, chemical reactivity (i.e., mainly molecular level covalent reactions and oxygen radical damage) can lead to various adverse outcomes including acute lethality, genotoxicity and oxidative stress. This mechanism of action has a very wide range of taxonomic applicability which in some cases includes all eukaryotes.

To provide more detail on specific modes and mechanisms of action and to capture ones not covered by the first two descriptors in the hazard profile, i.e., the Receptor Mediated Toxicity and Chemical Reactivity/Genotoxicity, the results from the Mode of Toxic Action descriptor are analysed for the small group of 12 halogenated (Br, P or BrCl) OFRs in Table 7.3. The Mode of Toxic Action descriptor is important in the hazard profile because it acts as a cellular-level diagnostic for adverse effects in addition to those cellular-level MIEs identified via the Receptor-mediated Toxicity and toxicity due to Chemical Reactivity/Genotoxicity descriptors. It involves *in silico* profiling using 6 MoA QSARs and tissue residue-based approaches (Figure 5), where greater confidence is assigned when there is consensus with both, to determine specific or non-specific interactions. Those chemicals with specific interactions tend to be more potent, i.e., can be associated with more sensitive endpoints, with adverse effects elicited at lower exposure concentrations.

Figure 7.1. In silico (QSAR) MoAs of 12 halogenated OFRs determined through ERC2 Mode of Toxic Action descriptor.

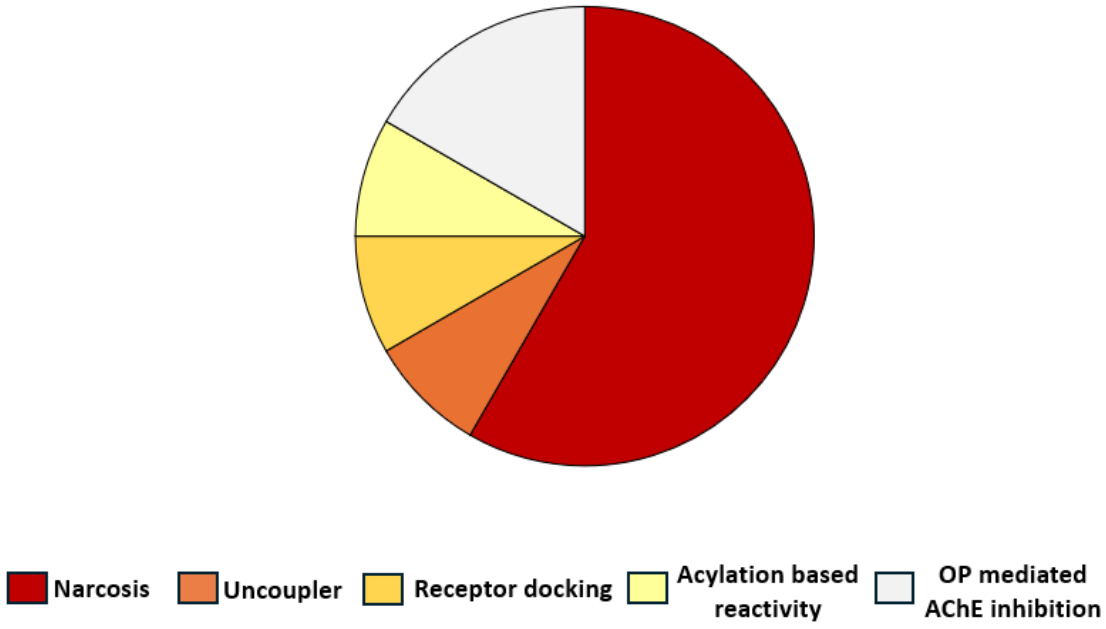


Figure 7.1 illustrates the QSAR MoAs predicted for the group of 12 OFRs. Most of these substances act through narcosis, which has a median Internal Effect Concentrations (IEC) at 3 mmol/kg. CAS RNs with narcosis as a MoA include: 79-27-6, 3234-02-4, 3322-93-8, 30554-72-4, 30554-73-5, 36483-57-5, 26444-49-5. Another 20% of these substances act through organophosphate (OP)-mediated acetylcholinesterase (AChE) inhibition (CAS RNs 126-71-6 and 1241-94-7), which have the smallest IEC (at 0.00001 mmol/kg) out of all the MoAs. The rest of the substances fall under either a mitochondrial uncoupler MoA (CAS RN

118-79-6), acylation-based reactivity (CAS RN 632-79-1) or receptor docking (CAS RN 20566-35-2) in equal percentages. These three MoAs also have the same IEC, at 0.01 mmol/kg. Most of the MoA predictions have low confidence score (max=15) suggesting a lack of consensus between the QSAR and tissue residue approaches, but some OFRs do show consensus MoA outcomes, particularly for a narcosis MoA and the set of non-specific acting chemicals which act via narcosis (see also Appendix D).

In summary, if multiple modes/mechanisms are present, overall confidence with the likelihood of adversity is gained even in the absence of *in vivo* data. This is an important consideration for any ecological assessment activity given the great paucity of *in vivo* data for the multitude of species in the environment. The MoA profile in ERC2 can be quite valuable as consideration in a regulatory context and helpful for a wide range of related questions.

7.2 Potential for Widespread Continuous Harm

One of the benefits of the ERC approach (version 1 and 2) is that it is risk-based, thus allowing both hazard and exposure potential to be evaluated at the prioritization step. The ERC approach incorporates descriptors to identify substances that have the potential for widespread continuous harm.

Substances that are mobile in air and/or water and have a long response time can be said to have “poor exposure reversibility”. That is, once emissions to the environment have ceased (e.g., because of regulatory or voluntary actions), these substances remain in the environment for years to decades or longer and can be widely distributed. Substances meeting this profile, i.e., having “poor effects reversibility”, for example causing heritable mutations or endocrine effects or those with a wide species susceptibility, present a widespread potential for continuous harm in the environment. Such types of effects can continue and even proliferate after exposure is eliminated. This is a concerning chemical profile because it could result in “regime shift” in populations (MacLeod et al. 2014). Widespread damage of this nature has obvious implication as threats to genetic diversity and ecosystem functioning, and one of the planetary boundaries that define the “safe operating space for humanity” (Rockström et al. 2009; Steffen et al. 2015; Richardson et al. 2023).

In ERC2, by combining higher confidence hazard descriptor results for Chemical Reactivity/Genotoxicity and higher confidence results for Response Time and Mobility exposure descriptors, chemicals can be identified that meet this profile and where exposure can range over various scales depending on the extent of transport in air or water.

Figure 7.2 brings together the hazard classification, confidence and severity scores for 12 OFRs as well as their predicted mobility in air and water using the CTD (km). By examining Figure 7.2 and the hazard profile details (provided in Appendix D), one can determine that all but three of the 12 OFRs trigger protein or DNA interactions or both (see column AQ in Appendix D supplementary spreadsheet) and are classified as moderate or high hazard (Appendix D, column AO). Confidence scores for these 12 OFRs vary from very low (1-10) to moderate (26-60) (Appendix D, column AP). Almost half of the 12 OFRs also have predicted MoAs associated with neurotoxicity (via OP-mediated AChE inhibition) or growth inhibition (via mitochondrial uncoupling) and receptor docking (i.e., endocrine activity) (Appendix D, column AY). The specific modes of action identified by the first three hazard profile descriptors, i.e., Receptor Mediated Toxicity, Chemical Reactivity/Genotoxicity and Mode of Toxic Action, do not require significant exposure to commence a cascade of biological effects across species and thus are regarded to have a very narrow

margin of exposure. In contrast, the non-specific acting chemicals (summarized in Appendix D, Tab 2) trigger no other modes of action than narcosis in ERC2 and thus are considered of a lower priority in this particular problem for potential of widespread continuous harm.

Response time in the environment varies significantly for the 12 OFRs ranging from less than one year to over two decades (see column BN in Appendix D), but almost all would be considered to be persistent or very persistent if regulatory criteria existed for overall persistence (e.g., >60 days). Eight of the 12 OFRs received moderate or higher response time classifications as a result. The selected narcotics all have short response times, all less than 1 year, resulting in a lower classification for Response Time descriptor.

In Figure 7.2, 6 out of 12 OFRs displayed have air as the predominant medium, whereas the remaining 6 have water/air as their primary medium. For the 10 non-specific acting substances, shown in Figure 7.3, all but one of the nine substances have water/air as the primary medium. Generally, the substances that travelled through air had greater mobility (travelling farther distances than those that travelled in water). The scale of exposure for the OFRs in Figure 7.2 reaches up to the continental level suggesting a wide range of exposure.

The overall impact of combining these key ERC2 descriptors suggests that among the group of 12 OFRs, several have the potential to elicit widespread effects, and possibly include effects that cannot be reversed and may even proliferate (e.g., genetic damage). In other words, the adverse effect can also be mobile and continuous over a range of ecological receptors. These chemicals might then be regarded as potential threats to genetic diversity and ecosystem functioning because of the potential for widespread continuous harm. Regulatory actions can include prioritization for risk assessment or for addressing loss of biodiversity under Target 7 of the Kunming-Montreal Global Biodiversity Framework (CBD 2022). In addition, the results discussed here can also be used to better target environmental media monitoring and surveillance activities and biomonitoring.

Figure 7.2. Risk, risk severity, and risk confidence as well as air and water ( ) characteristic travel distance of halogenated (Br, P, or BrCl) OFRs

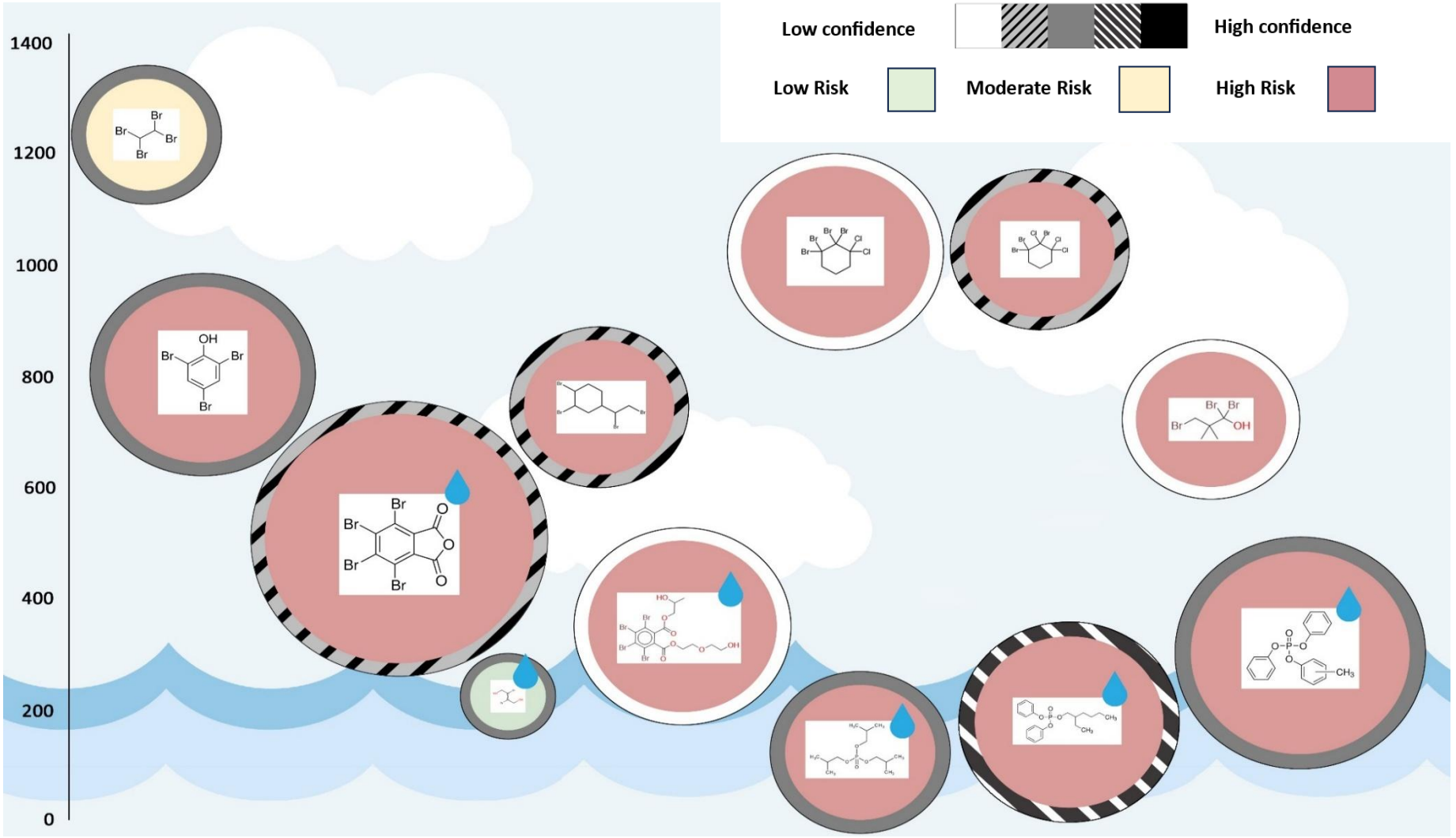
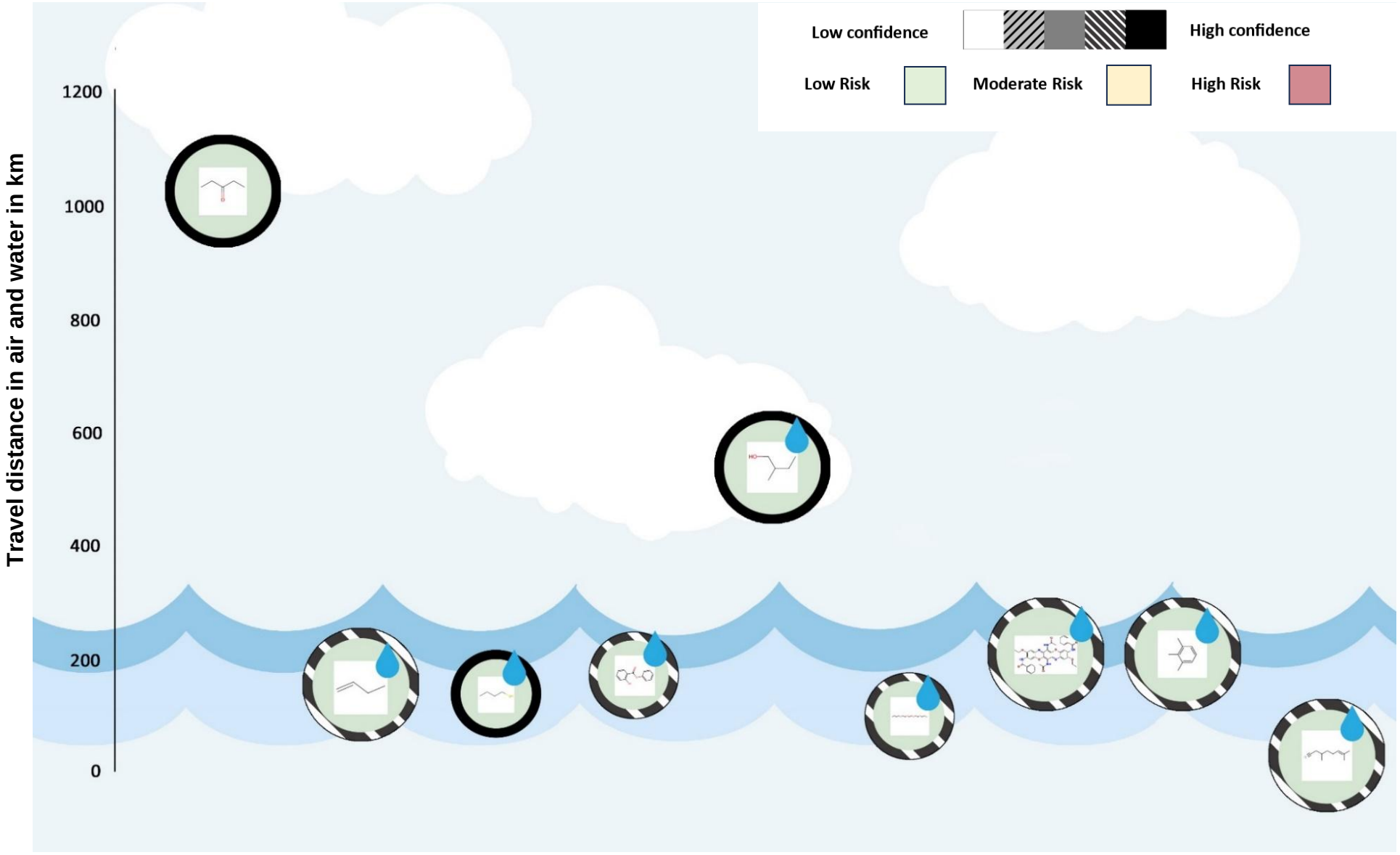


Figure 7.3. Risk, risk severity, and risk confidence as well as air and water ( ) characteristic travel distance of non-specific acting substances



Figures 7.2 and 7.3 show the risk, risk severity, and risk confidence, as well as the air and water ( ) characteristic travel distance (CTD) of 12 Halogenated (Br, P or BrCl) ORFs and 9 non-specific acting substances, respectively. Substances are placed along the x-axis (with no specific importance to the order) and the CTD (Km) in both air and water is plotted on the y-axis. Substances that travel in both air and water are denoted with a water droplet. Risk severity scores correspond to circle sizes, i.e., the larger the circle, the higher the score. Risk confidence is shown with the circle border. Figure 7.2 presents the chemical structures from left to right; CAS RNs of substances that travel in air: 79-27-6, 118-79-6, 3322-93-8, 30554-72-4, 30554-73-5, 36483-57-5; CAS RNs of substances that travel in water and air: 632-79-1, 3234-02-4 (ADME flag), 20566-35-2, 126-71-6, 1241-94-7, 26444-49-5. In Figure 7.3, the CAS RN of chemical structures are presented from right to left: 96-22-0, 106-98-9, 109-79-5, 118-55-8, 137-32-6, 1559-34-8, 17741-63-8, 25551-13-7, 51566-62-2.

7.3 Regrettable substitution

The Government of Canada is exploring ways to advance responsible replacement of chemicals of concern (CoCs), including ways to apply informed substitution to support chemical management.

A “regrettable substitution” occurs when an alternative substance is equally or more harmful to humans and/or the environment than the substance being replaced, e.g., BPA vs BPS. ERC2 can identify substances with high hazard potential as potentially harmful alternatives. In Figure 7.2, high risk substances are identified, along with both confidence and severity associated with the determination of risk. Of the 12 flame retardants depicted, only two were not high risk (Class 3) according to ERC2, one being low (Class 1) and the other moderate (Class 2). Indeed, across all of the 15 OFRs summarized in Table 7.3, 2 substances were classified as low hazard (Class 1), one as moderate hazard (Class 2) and 12 were high hazard (Class 3). There was also a wider range in confidence and severity scores indicating some data gaps or less concordance across available data, compared to the 9 narcotics, which were consistently all low risk and low severity and high confidence.

When a classification of moderate risk and confidence is used as the primary method for identifying substances that can be regarded as regrettable substitutes, 4 of the 12 (33%) OFRs from Figure 7.2 can be flagged as regrettable substitutes. These four have both moderate or high hazard classifications and confidence scores. If a low confidence is included as a precautionary approach, 7 substances (58%) are potentially harmful substitutes. However, a more useful approach considers a severity-confidence matrix by focusing on substances with a higher probability of adverse effects occurring from one or more toxicological triggers. Table 7.5 conceptualizes this idea where various decisions can be made along both the severity and confidence axes of the matrix to determine candidates for regrettable substitution as well as other regulatory considerations, including data gathering, depending on regulatory context and tolerance for uncertainty.

Candidate substances assigned moderate and high severity scores and categories (or descriptions) (see Appendix D, column CL and CM) and low and greater confidence scores and categories (or descriptions) (Appendix D, columns CI and CJ), account for 6 of 15 substances (40%) and can be considered as more probable to be regrettable substitutes. If moderate or high severity scores alone are used (i.e., without considering confidence scores), almost all, 14 out of 15, of the OFRs are implicated as regrettable substitutes. The lower confidence OFRs in this case could be candidates for further data collection (Table 7.5).

Table 7.5. Conceptual decision matrix for identifying candidates for regrettable substitution

		Hazard Confidence Score[1]				
		Very Low	**Low**	**Moderate**	**High**	**Very High**
		1-10	11-25	26-60	61-100	>100
Hazard Severity Score[1]	**0**	No further action	No further action	No further action	No further action	No further action
	1	Data collection	Data collection	Data collection	No further action	No further action
	2	Data collection	Candidate	Candidate	Candidate	Candidate
	3	Data collection	Candidate	Candidate	Candidate	Candidate
	4	Data collection	Candidate	Candidate	Candidate	Candidate

[1] Scoring routines hazard confidence and hazard severity are summarized in Table 4.5 and Table 4.6, respectively (section 4).

7.4 Consideration of possible risk-based regulatory activities with ERC2 outcomes

The ERC2 approach was designed to prioritize many organic chemicals on the Canadian DSL for consideration in future risk assessment activities under CEPA. However, it can be viewed as a comprehensive tool to inform several activities in a regulatory context and is also very useful as a problem formulation tool for risk assessment (i.e., can simply be used as a database of knowledge by CAS RN guiding the risk assessment). For example, severity and confidence outcomes associated with ERC2 risk classification of substances can be examined together to inform some typical regulatory steps for a substance or substance groups and inventories (see Appendix D, column CY and DB). Table 7.6 is a conceptual example of a decision matrix which highlights potential suitable activities, including assessment, data gathering or no further action.

- Assessment (red): severity and confidence range from moderate to very high, suggesting that substances with these outcomes could be priorities for regulatory action such as assessment based on the risk classification. If not recently obtained, chemical quantity data should be collected as a priority for these substances to confirm/ increase confidence in their exposure profile.
- Data Collection (orange): severity and confidence range in scale from low to very high, suggesting additional hazard and/or exposure data should be collected to fill data gaps (e.g., via research and monitoring or surveys) to refine ERC2 findings. Because ERC2 is based on the weight of evidence approach, it allows to better identify key data gaps or uncertainties in the dataset.
- No Further Action (green): severity is very low in all cases, suggesting none of the classification rules for moderate or high concern have been activated for these substances. Confidence ranges from very low to very high.

Table 7.6. A confidence-severity matrix for risk

	Risk Confidence Score[1]				
Risk Severity Score[1]	**Very Low**	**Low**	**Moderate**	**High**	**Very High**
0	No further action	No further action	No further action	No further action	No further action
1	No further action	No further action	No further action	No further action	No further action
2	Data collection	Data collection	Data collection	Data collection	Data collection
3	Data collection	Data collection	Data collection	Data collection	Data collection
4	Data collection	Data collection	Assessment	Assessment	Assessment
5	Data collection	Data collection	Assessment	Assessment	Assessment
6	Data collection	Data collection	Assessment	Assessment	Assessment
7	Data collection	Data collection	Assessment	Assessment	Assessment

[1] Scoring routines risk confidence and risk severity are summarized in Table 5.2 and Table 5.4, respectively (section 5).

Using Table 7.6 above as a guide, since the 9 non-specific acting chemicals seen in Figure 7.3 all have either severity levels of 0 or 1, therefore, they would fall under “no further action”. In contrast, the 12 OFR substances in Figure 7.2 have a larger range of severity and confidence scores, and as a result individual substances have different possible recommendations for future regulatory assessments. Of these OFRs, only CAS RN 3234-02-4 would fall under “no further action” (using Table 7.6 as a guide) due to having a severity score of 0. All others would fall under either “data collection” or “assessment”, often depending on their risk confidence scores. When confidence is lower, data collection and/or data generation are recommended to help address data gaps. For instance, while the substances of CAS RN 30554-72-4 and 1241-94-7 have the same severity score of 4, the latter has a “high” confidence score compared to the confidence score of “very low” for the former. According to Table 7.6, the “high” confidence score would put the substance under “assessment” while a “very low” confidence score puts the substances under “data collection”.

7.5 ADME and other flags in ERC2

Toxicokinetic and chemical property flags are an important consideration when examining ERC2 outcomes (Figure 1, step 6b). These flags provide additional information for an evaluator to consider when interpreting data from ERC2. Considering that often ecological models assume 100% bioavailability, or that for the more poorly soluble substances laboratory test protocols typically include artificial enhancement with solvents to facilitate testing, ADME toxicokinetic flags help to reduce over-estimation of risk outcomes. ADME flags improve reliability for the risk classification outcome, either to reduce over-estimation or to adjust for the fact that many *in silico* results for these substances may not apply or will have a high degree of uncertainty.

As described in section 4.2, ADME provides toxicokinetic alerts for substances that may present limited internal bioavailability or that upon uptake, become distributed in the blood plasma of organisms (protein plasma binding). Of the 15 OFRs, 4 substances (CAS RN, 2524-04-1, 3234-02-4, 42757-55-1, 52907-07-0) were identified by ERC2 as not fully permeable/bioavailable by the ADME Classification Filter (Appendix D, column AG and DD) (Table 7.6). This ADME flag can be used to better understand toxicokinetic constraints when examining toxicological data and may help explain variation in adverse effects. In addition, substances with a high molecular fraction occurring in the ionized state or permanently charged compounds at environmentally relevant pH are also identified at this stage (chemical property flags). Such

chemicals may be outside of the capability of ERC2 to provide confident classification, but none of the OFRs or non-specific acting chemicals triggered this flag. Finally, 7 OFRs and 5 non-specific acting chemicals received margin of exposure (MoE) flags, suggesting verification of exposure classification, where initial classification as low (Class 1) exposure was elevated to moderate classification (Class 2) for the reasons stated in Section 4.4.5, namely lack of sufficient MoE to rule out with certainty no potential for adverse effects in environment due to exposure.

Table 7.7. ERC2 Classification outcomes for 4 OFR substances with ADME flags

CAS RN	Hazard Profile Classification	Exposure Profile Classification	Risk Classification	Risk Confidence	Risk Severity Description (Hazard/ Exposure)	ADME flag (Is permeable?; Is plasma distributed?)[1]
2524-04-1	High	Low	Moderate	Moderate	High / V. Low	False, False
3234-02-4	Low	Low	Low	Moderate	V. Low / V. Low	False, False
42757-55-1	High	High	High	V. Low	Moderate / Moderate	False, False
52907-07-0	High	Moderate	High	V. Low	Moderate / Low	False, False

[1]FLASE = not permeable or not plasma distributed (i.e., likely will not be bioavailable in organisms, not able to penetrate cell membranes or distribute in plasma)

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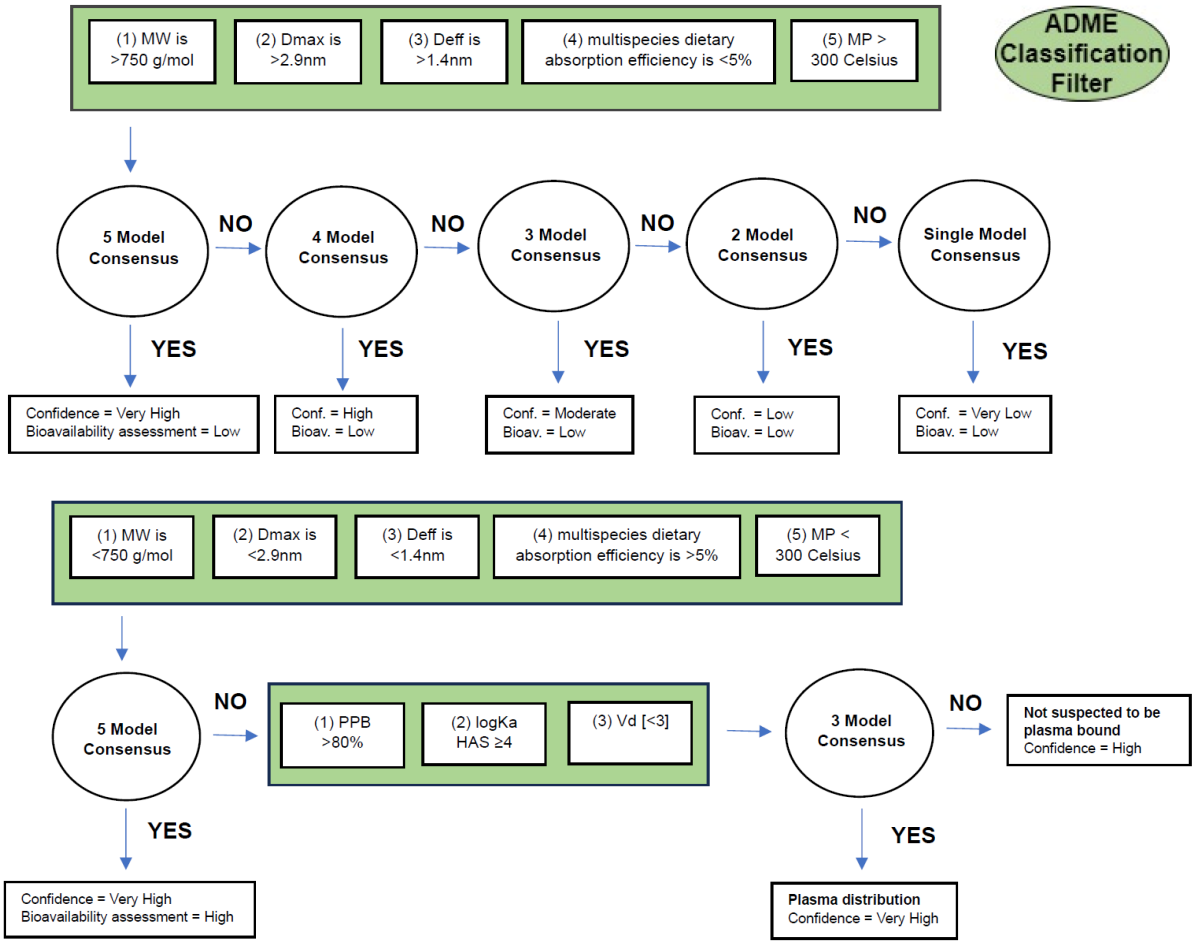
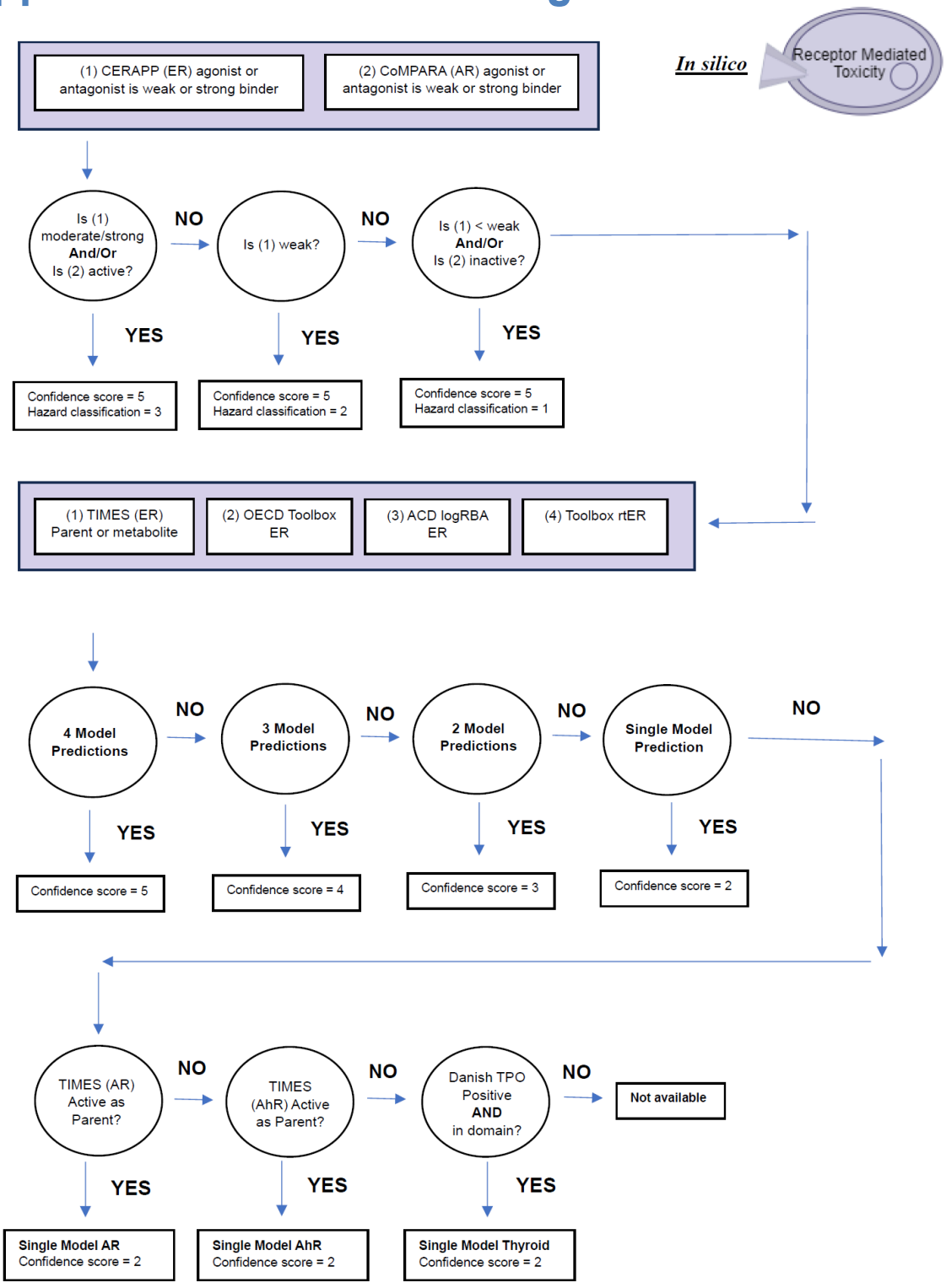
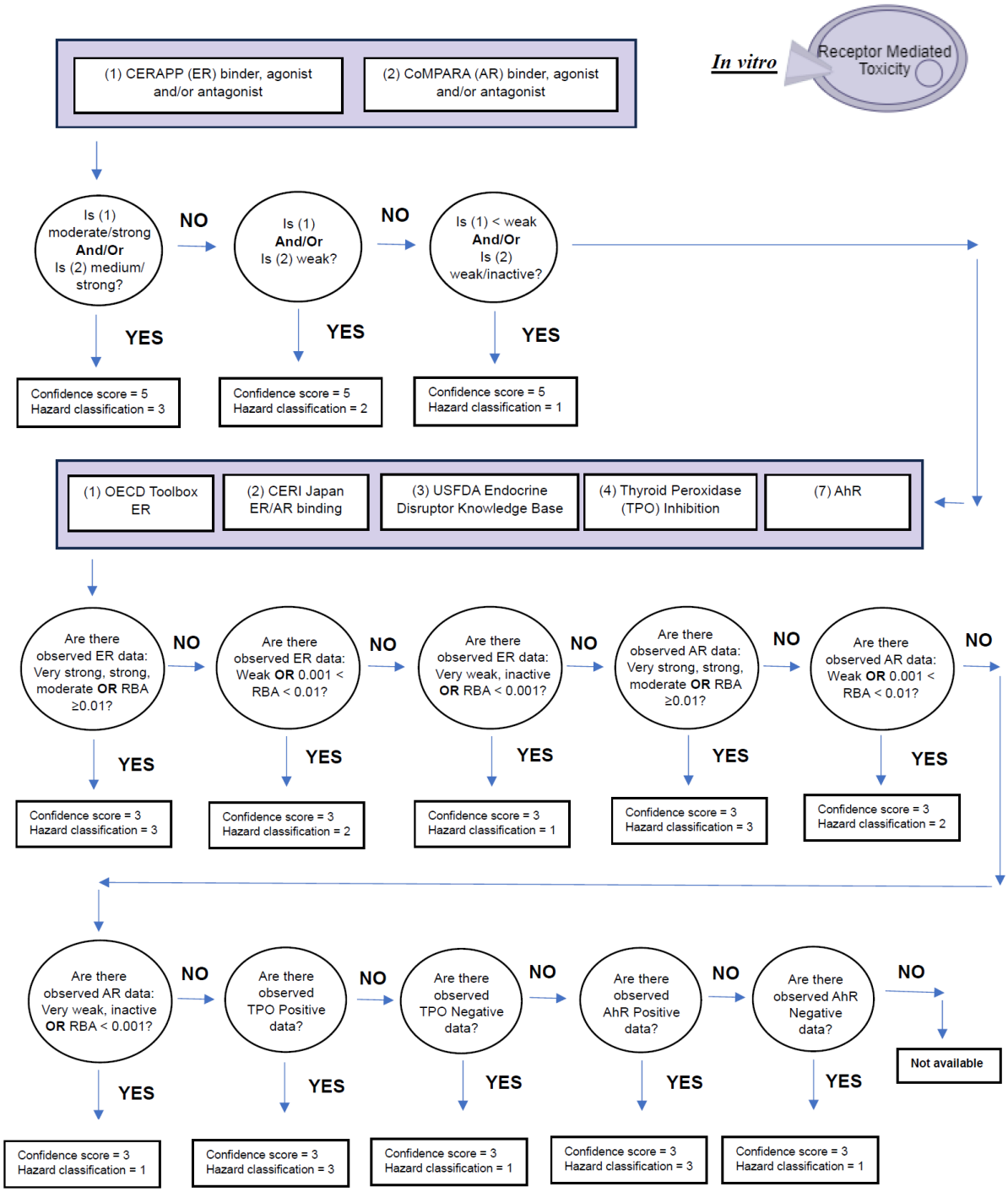
Appendix A: ADME Classification Filter Process

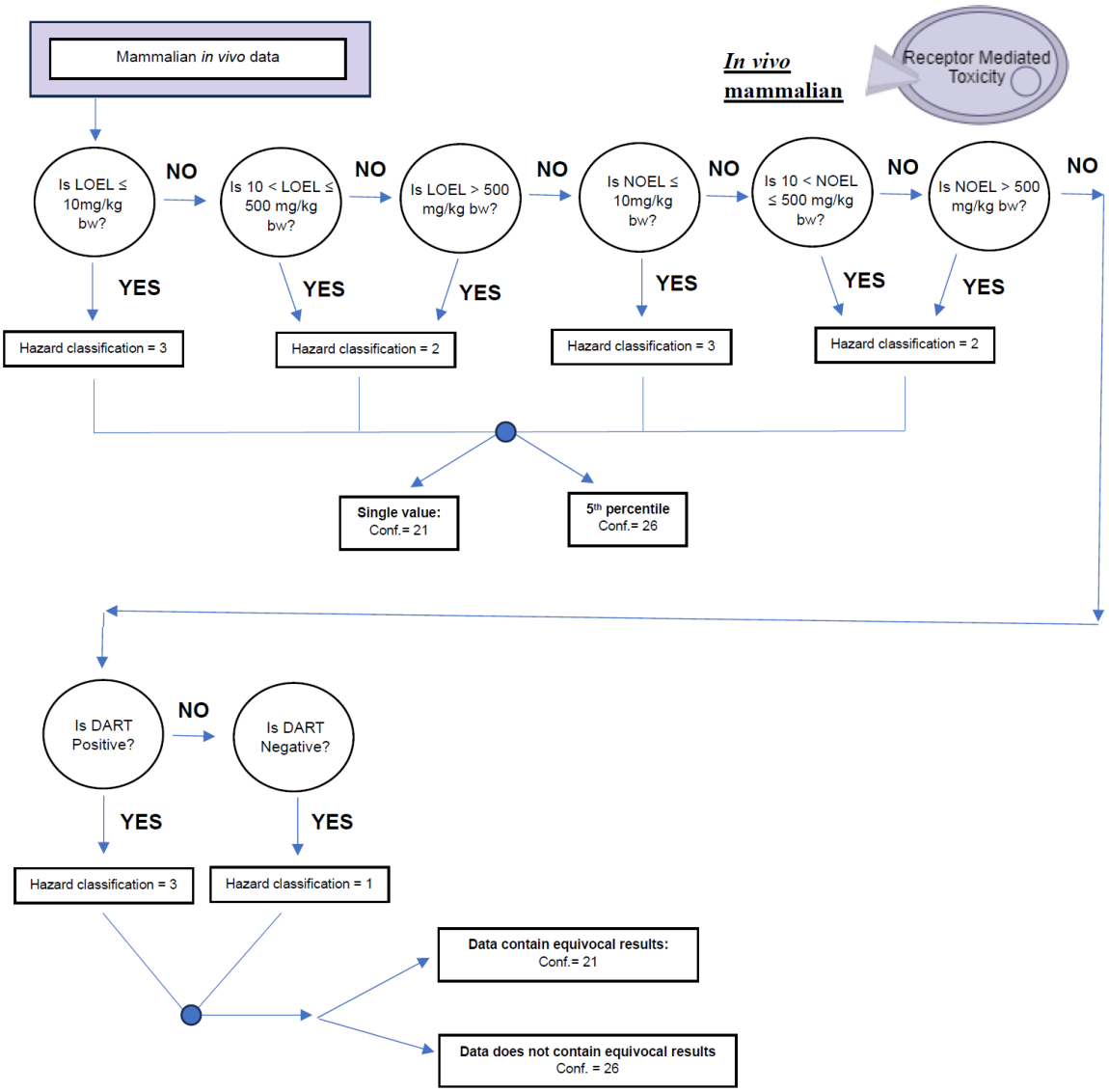


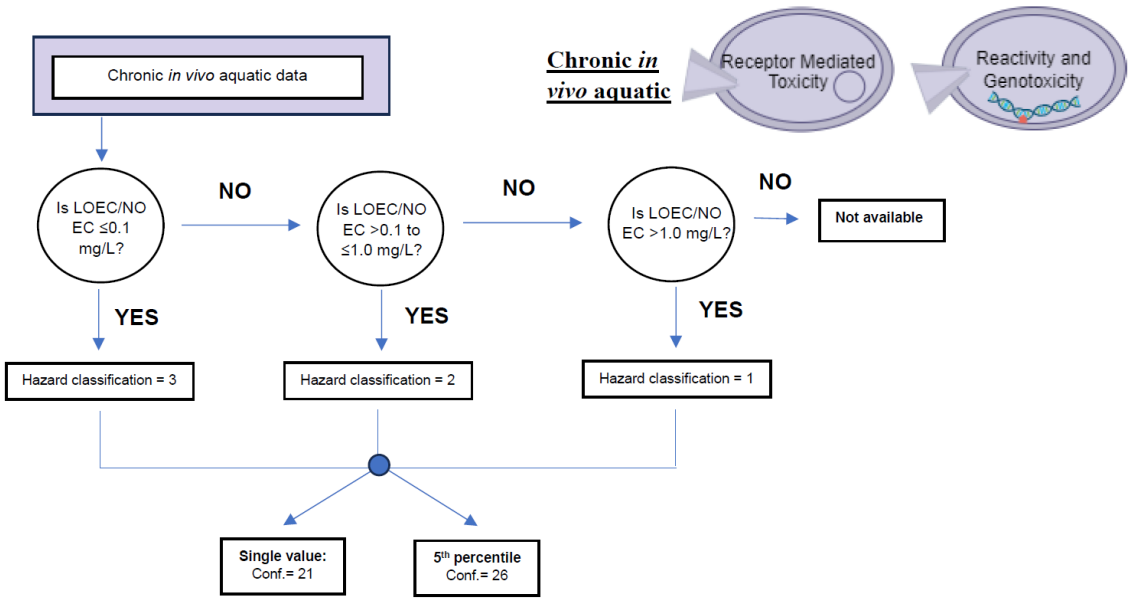
The range selection for the molecular cross-section diamater (Dmax) and effective diameter (Deff) is based on the published ranges and expert experience with the 3D conformational analysis from Molecular Orbital PACage (MOPAC)(MOPAC 2016) calculations and applications. Further details on thresholds are provied in 2022 ERC2 Science Appraoch document (ECCC 2022) and its Appendix for ADME.

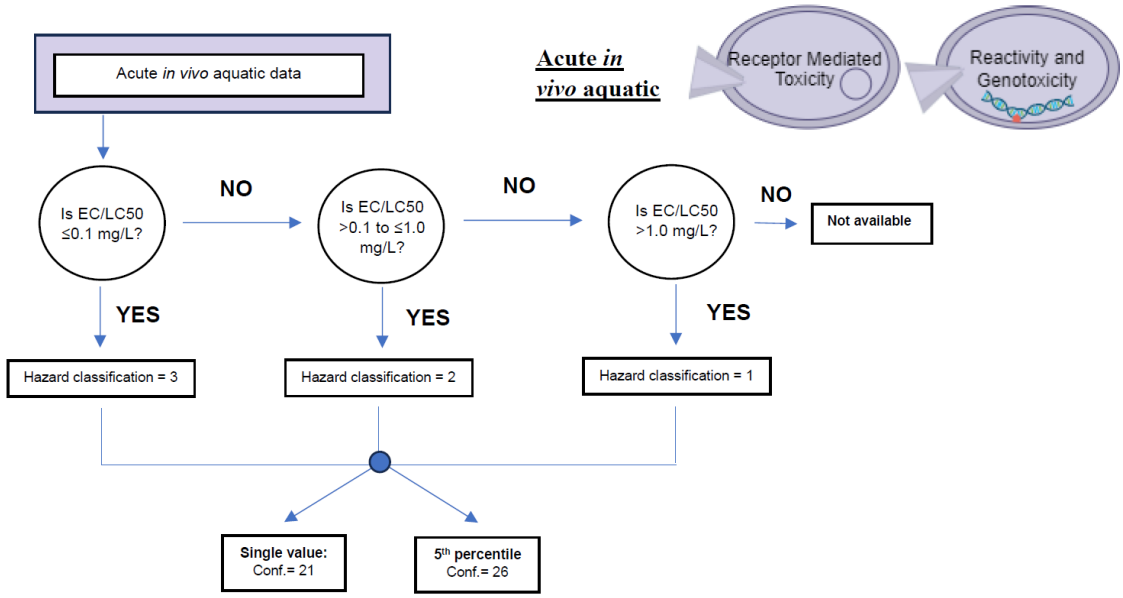
Appendix B: Hazard Profiling

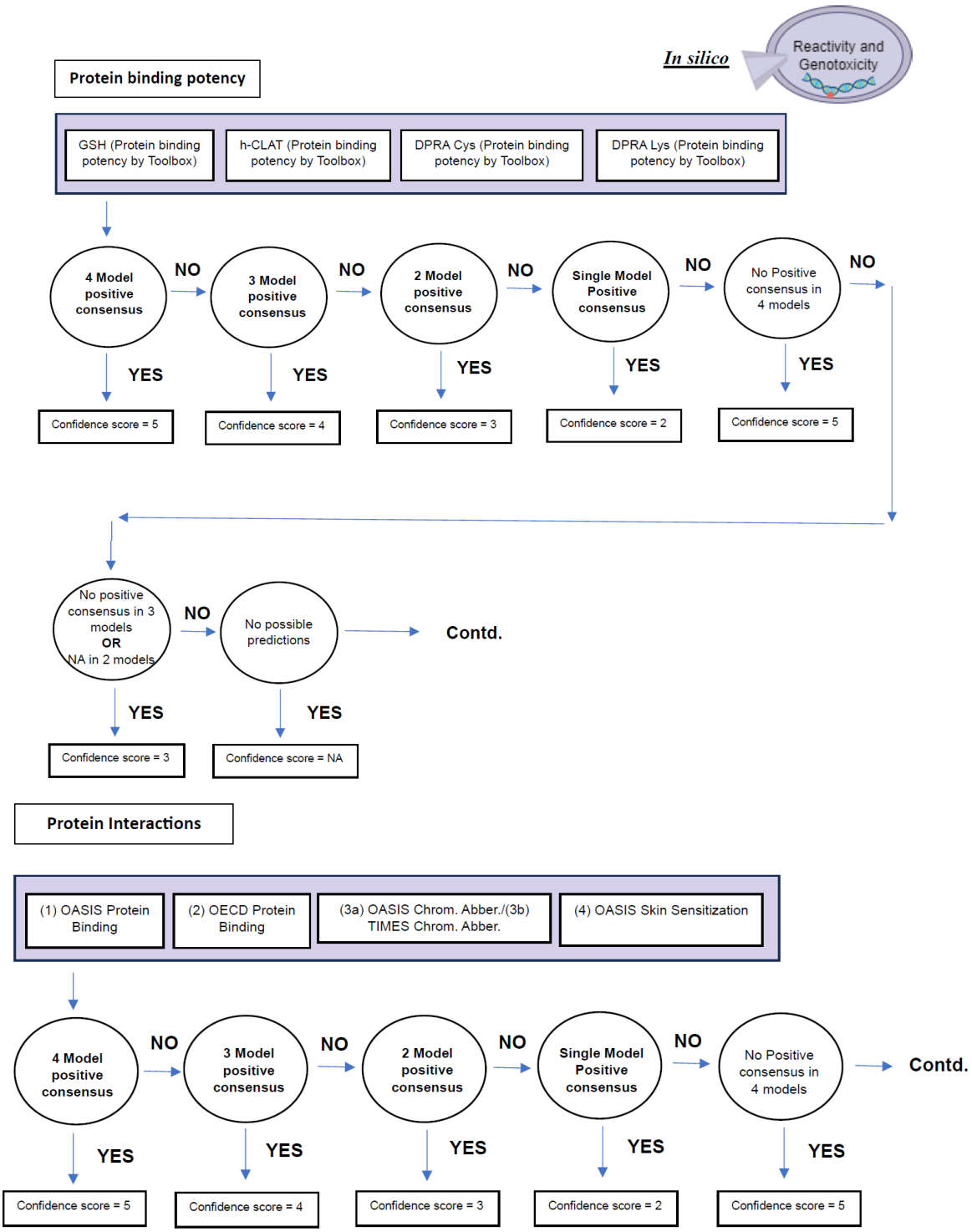


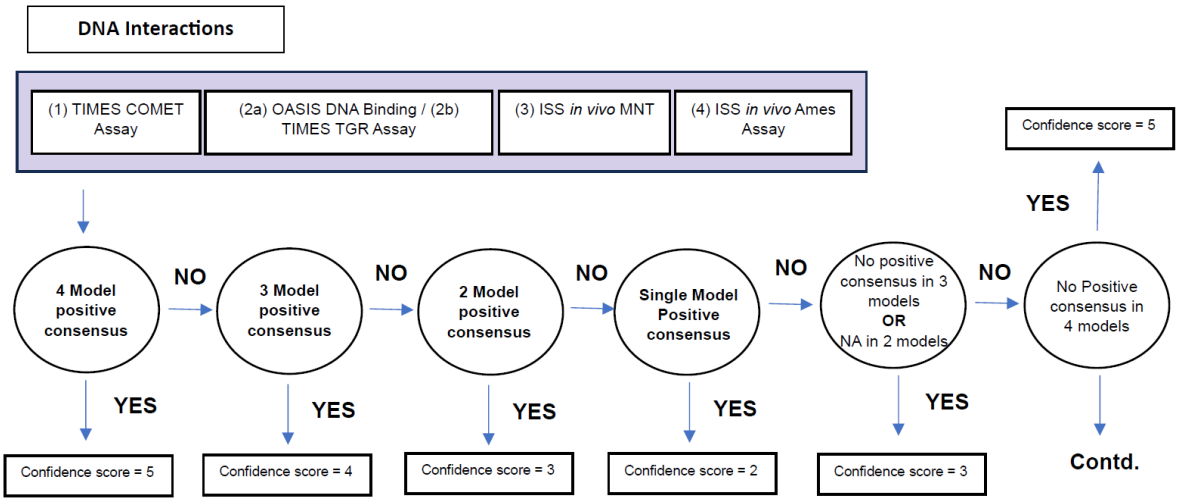

In vitro
Receptor Mediated Toxicity
(1) CERAPP (ER) binder, agonist and/or antagonist
(2) CoMPARA (AR) binder, agonist and/or antagonist
Is (1) moderate/strong And/Or Is (2) medium/ strong?
NO
Is (1) And/Or Is (2) weak?
NO
Is (1) < weak And/Or Is (2) weak/inactive?
YES
Confidence score = 5 Hazard classification = 3
YES
Confidence score = 5 Hazard classification = 2
YES
Confidence score = 5 Hazard classification = 1
(1) OECD Toolbox ER
(2) CERI Japan ER/AR binding
(3) USFDA Endocrine Disruptor Knowledge Base
(4) Thyroid Peroxidase (TPO) Inhibition
(7) AhR
Are there observed ER data: Very strong, strong, moderate OR RBA ≥0.01?
NO
Are there observed ER data: Weak OR 0.001 < RBA < 0.01?
NO
Are there observed ER data: Very weak, inactive OR RBA < 0.001?
NO
Are there observed AR data: Very strong, strong, moderate OR RBA ≥0.01?
NO
Are there observed AR data: Weak OR 0.001 < RBA < 0.01?
NO
YES
Confidence score = 3 Hazard classification = 3
YES
Confidence score = 3 Hazard classification = 2
YES
Confidence score = 3 Hazard classification = 1
YES
Confidence score = 3 Hazard classification = 3
YES
Confidence score = 3 Hazard classification = 2
Are there observed AR data: Very weak, inactive OR RBA < 0.001?
NO
Are there observed TPO Positive data?
NO
Are there observed TPO Negative data?
NO
Are there observed AhR Positive data?
NO
Are there observed AhR Negative data?
NO
YES
Confidence score = 3 Hazard classification = 1
YES
Confidence score = 3 Hazard classification = 3
YES
Confidence score = 3 Hazard classification = 1
YES
Confidence score = 3 Hazard classification = 3
YES
Confidence score = 3 Hazard classification = 1
Not available

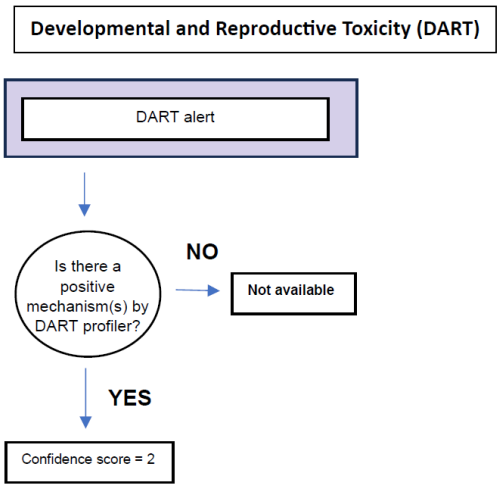

Mammalian *in vivo* data
In vivo mammalian
Receptor Mediated Toxicity
Is LOEL ≤ 10mg/kg bw?
NO
Is 10 < LOEL ≤ 500 mg/kg bw?
NO
Is LOEL > 500 mg/kg bw?
NO
Is NOEL ≤ 10mg/kg bw?
NO
Is 10 < NOEL ≤ 500 mg/kg bw?
NO
Is NOEL > 500 mg/kg bw?
NO
YES
YES
YES
YES
YES
YES
Hazard classification = 3
Hazard classification = 2
Hazard classification = 3
Hazard classification = 2
Single value: Conf.= 21
5th percentile Conf.= 26
Is DART Positive?
NO
Is DART Negative?
YES
YES
Hazard classification = 3
Hazard classification = 1
Data contain equivocal results: Conf.= 21
Data does not contain equivocal results Conf. = 26

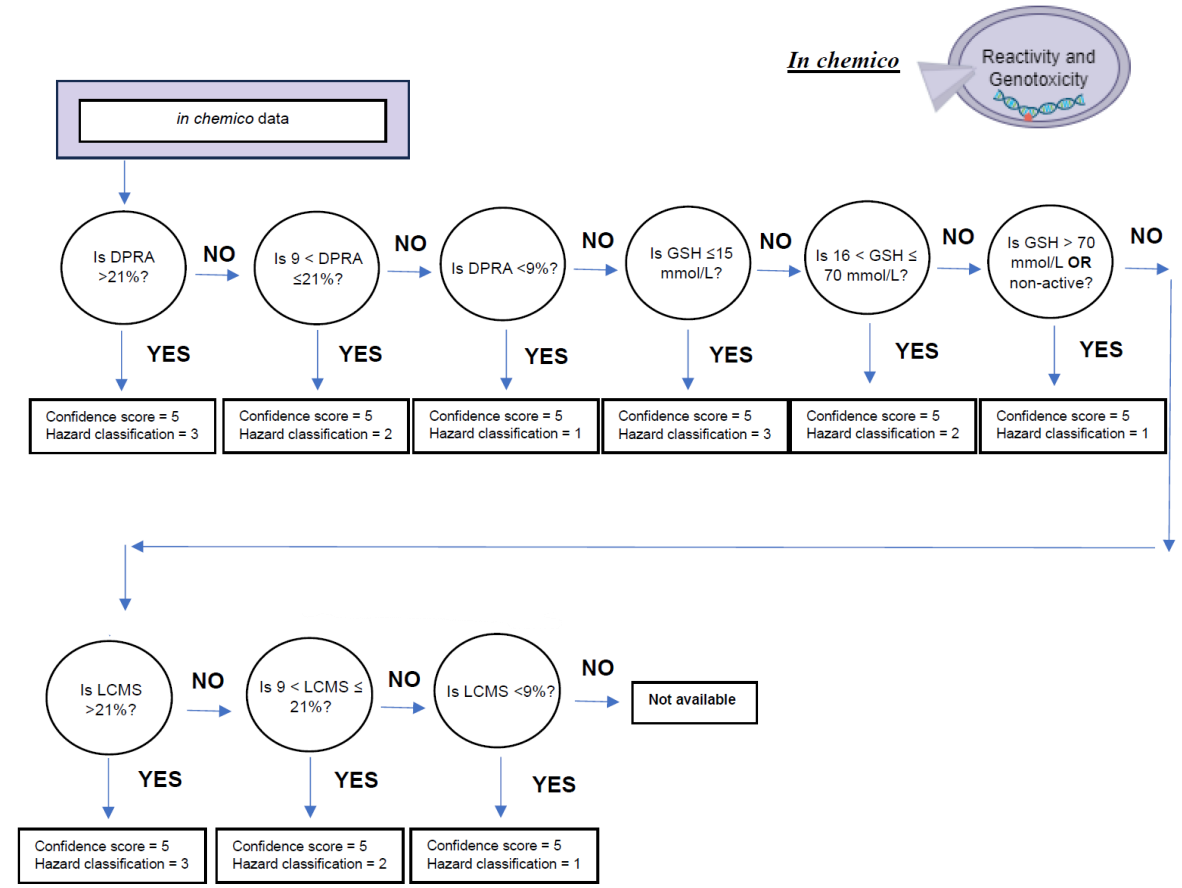

Chronic *in vivo* aquatic data
Chronic *in vivo* aquatic
Receptor Mediated Toxicity
Reactivity and Genotoxicity
Is LOEC/NOEC ≤0.1 mg/L?
NO
Is LOEC/NOEC >0.1 to ≤1.0 mg/L?
NO
Is LOEC/NOEC >1.0 mg/L?
NO
Not available
YES
YES
YES
Hazard classification = 3
Hazard classification = 2
Hazard classification = 1
Single value: Conf.= 21
5th percentile Conf.= 26

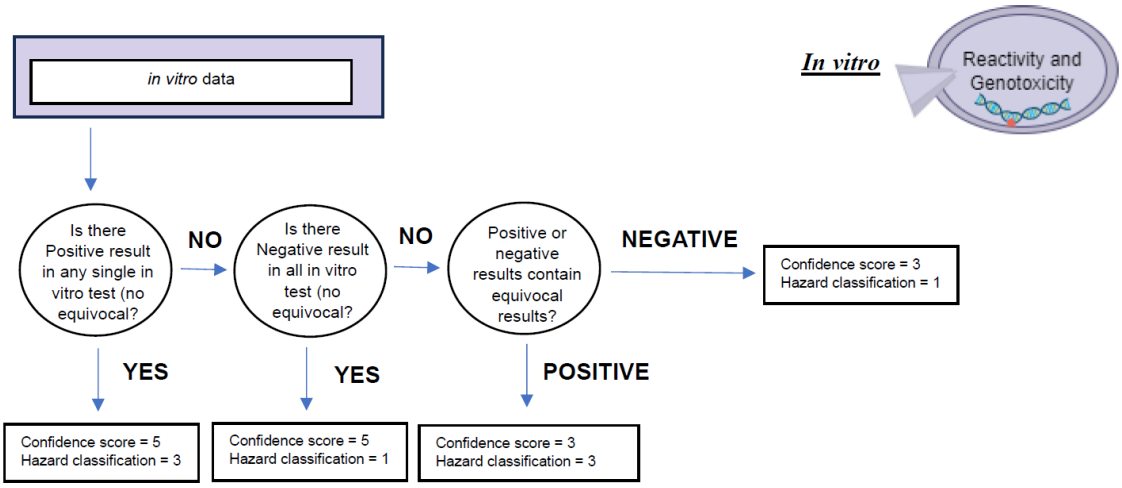

Acute *in vivo* aquatic data
Acute *in vivo* aquatic
Receptor Mediated Toxicity
Reactivity and Genotoxicity
Is EC/LC50 ≤0.1 mg/L?
NO
Is EC/LC50 >0.1 to ≤1.0 mg/L?
NO
Is EC/LC50 >1.0 mg/L?
NO
Not available
YES
YES
YES
Hazard classification = 3
Hazard classification = 2
Hazard classification = 1
Single value: Conf.= 21
5th percentile Conf.= 26

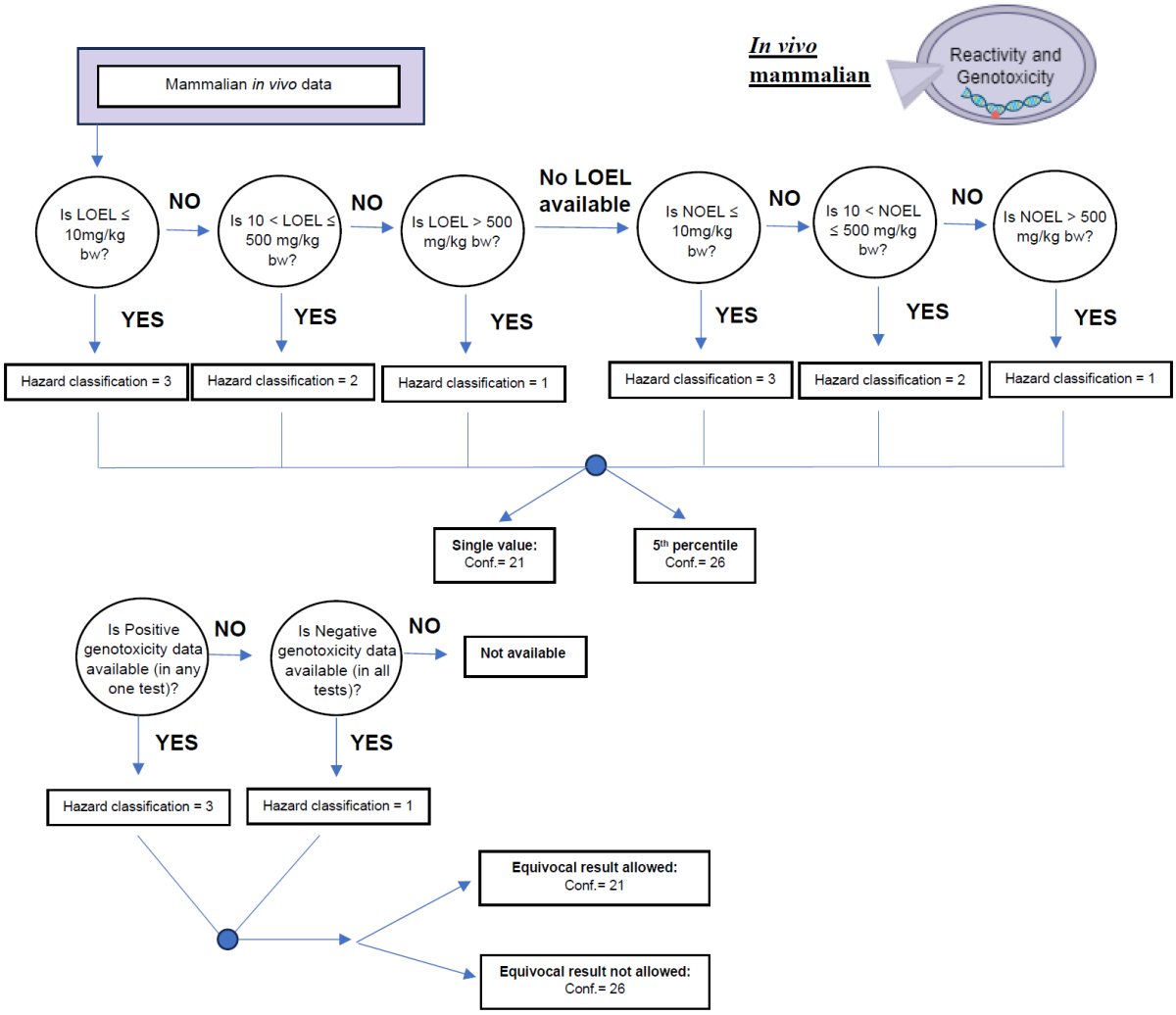

In silico
Reactivity and Genotoxicity
Protein binding potency
GSH (Protein binding potency by Toolbox)
h-CLAT (Protein binding potency by Toolbox)
DPRA Cys (Protein binding potency by Toolbox)
DPRA Lys (Protein binding potency by Toolbox)
4 Model positive consensus
NO
3 Model positive consensus
NO
2 Model positive consensus
NO
Single Model Positive consensus
NO
No Positive consensus in 4 models
NO
YES
Confidence score = 5
YES
Confidence score = 4
YES
Confidence score = 3
YES
Confidence score = 2
YES
Confidence score = 5
No positive consensus in 3 models OR NA in 2 models
NO
No possible predictions
Contd.
YES
Confidence score = 3
YES
Confidence score = NA
Protein Interactions
(1) OASIS Protein Binding
(2) OECD Protein Binding
(3a) OASIS Chrom. Abber./(3b) TIMES Chrom. Abber.
(4) OASIS Skin Sensitization
4 Model positive consensus
NO
3 Model positive consensus
NO
2 Model positive consensus
NO
Single Model Positive consensus
NO
No Positive consensus in 4 models
Contd.
YES
Confidence score = 5
YES
Confidence score = 4
YES
Confidence score = 3
YES
Confidence score = 2
YES
Confidence score = 5

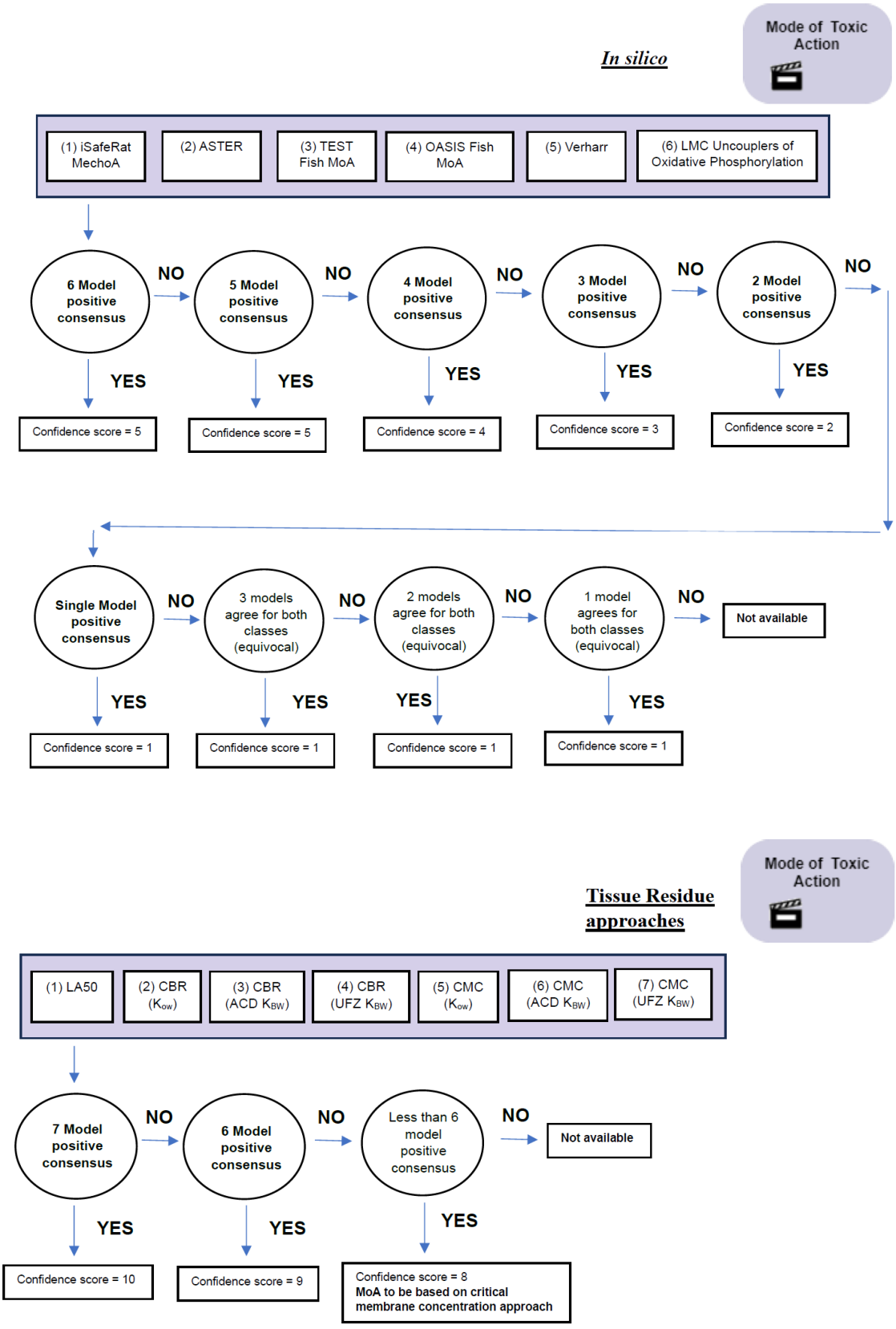

DNA Interactions
(1) TIMES COMET Assay
(2a) OASIS DNA Binding / (2b) TIMES TGR Assay
(3) ISS *in vivo* MNT
(4) ISS *in vivo* Ames Assay
4 Model positive consensus
NO
3 Model positive consensus
NO
2 Model positive consensus
NO
Single Model Positive consensus
NO
No positive consensus in 3 models OR NA in 2 models
NO
No Positive consensus in 4 models
YES
Confidence score = 5
YES
Confidence score = 5
YES
Confidence score = 4
YES
Confidence score = 3
YES
Confidence score = 2
YES
Confidence score = 3
Contd.

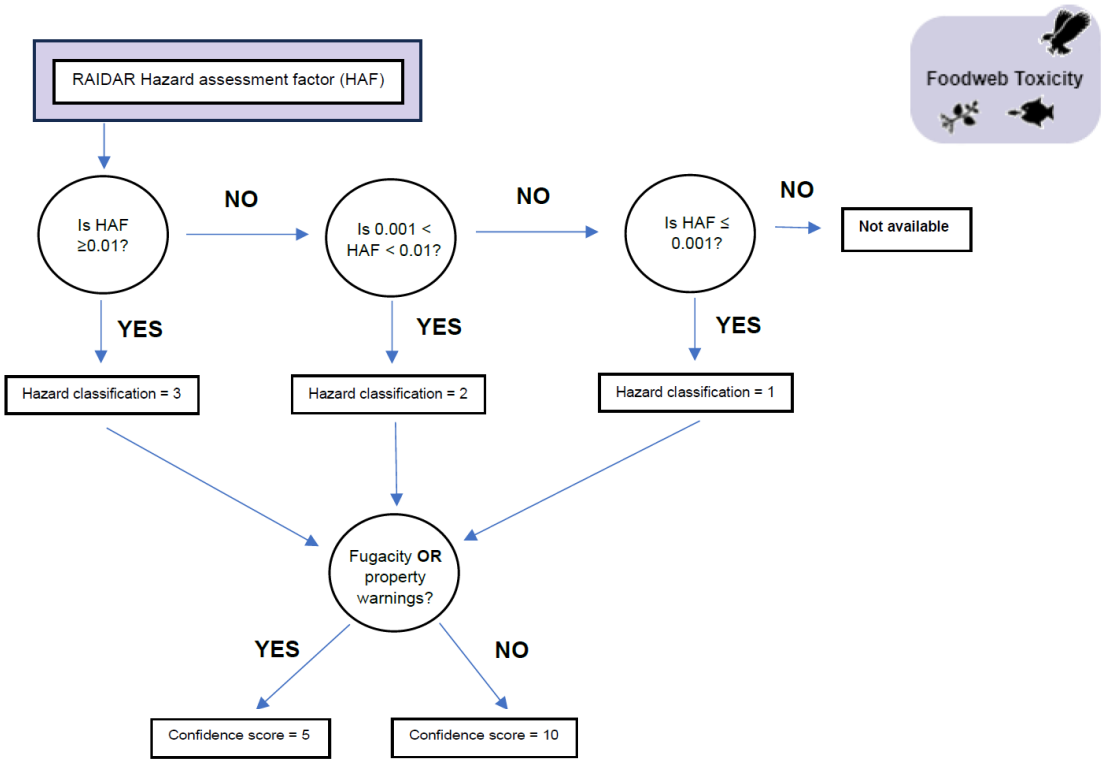

Developmental and Reproductive Toxicity (DART)
DART alert
Is there a positive mechanism(s) by DART profiler?
NO
Not available
YES
Confidence score = 2

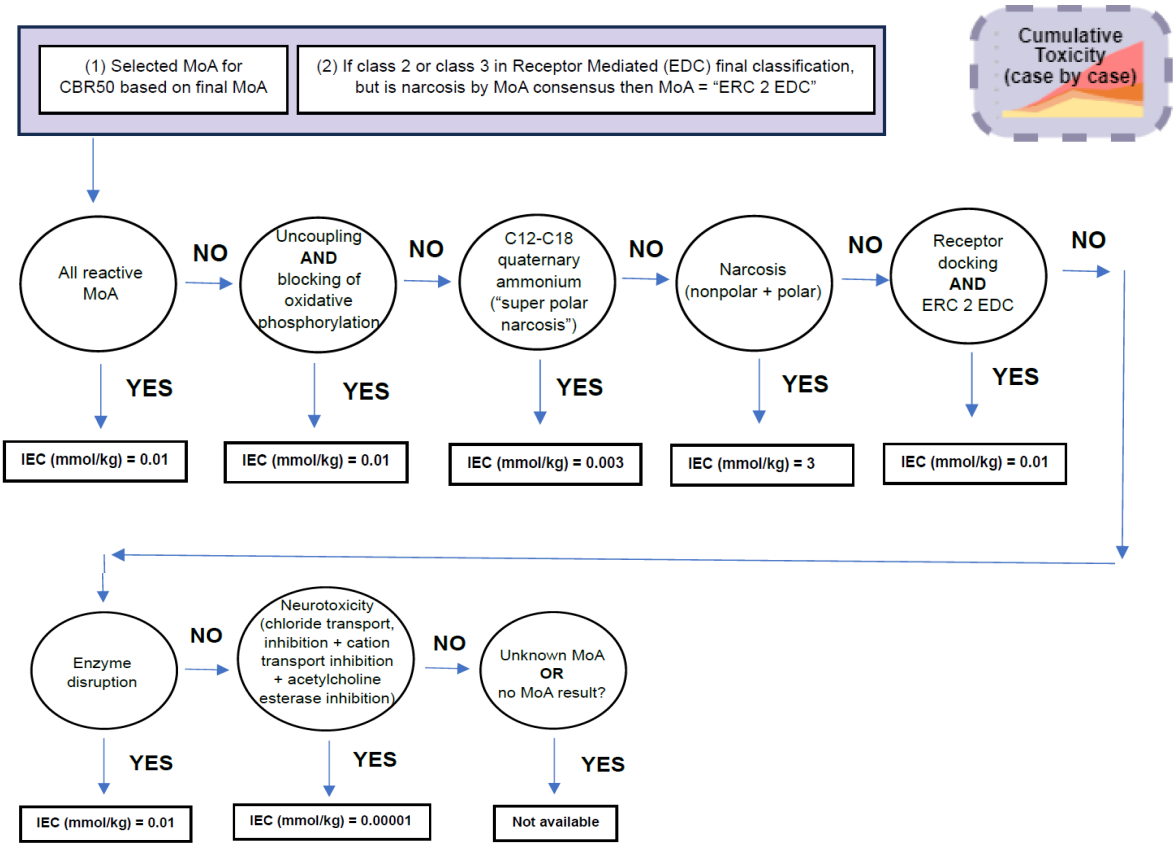

In chemico
Reactivity and Genotoxicity
in chemico data
Is DPRA >21%?
NO
Is 9 < DPRA ≤21%?
NO
Is DPRA <9%?
NO
Is GSH ≤15 mmol/L?
NO
Is 16 < GSH ≤ 70 mmol/L?
NO
Is GSH > 70 mmol/L OR non-active?
NO
YES
Confidence score = 5 Hazard classification = 3
Confidence score = 5 Hazard classification = 2
Confidence score = 5 Hazard classification = 1
Confidence score = 5 Hazard classification = 3
Confidence score = 5 Hazard classification = 2
Confidence score = 5 Hazard classification = 1
Is LCMS >21%?
NO
Is 9 < LCMS ≤ 21%?
NO
Is LCMS <9%?
NO
Not available
YES
Confidence score = 5 Hazard classification = 3
Confidence score = 5 Hazard classification = 2
Confidence score = 5 Hazard classification = 1


In vitro
Reactivity and Genotoxicity
in vitro data
Is there Positive result in any single in vitro test (no equivocal?
NO
Is there Negative result in all in vitro test (no equivocal?
NO
Positive or negative results contain equivocal results?
NEGATIVE
Confidence score = 3 Hazard classification = 1
YES
YES
POSITIVE
Confidence score = 5 Hazard classification = 3
Confidence score = 5 Hazard classification = 1
Confidence score = 3 Hazard classification = 3

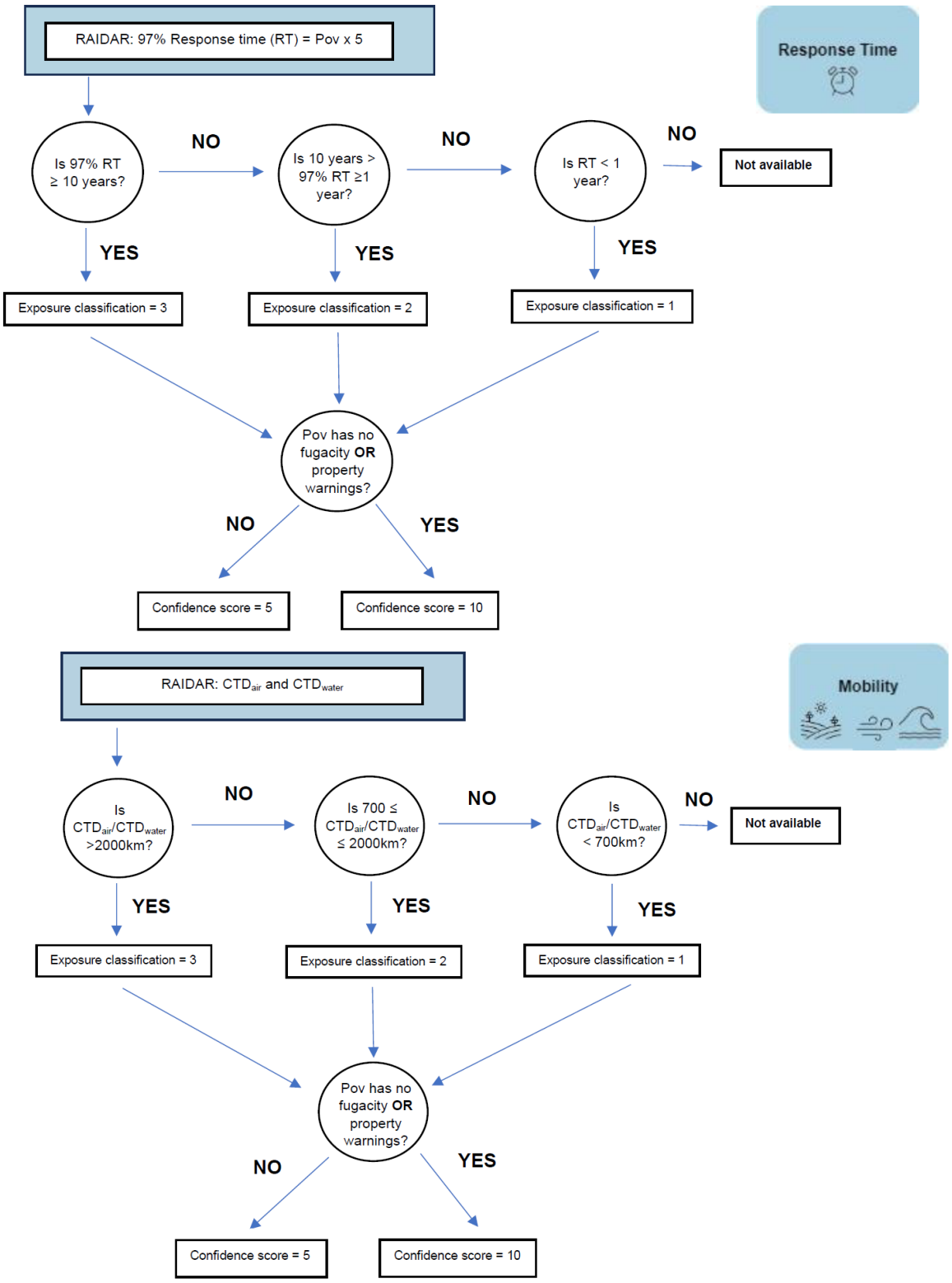

In vivo mammalian
Reactivity and Genotoxicity
Mammalian in vivo data
Is LOEL ≤ 10mg/kg bw?
NO
Is 10 < LOEL ≤ 500 mg/kg bw?
NO
Is LOEL > 500 mg/kg bw?
No LOEL available
Is NOEL ≤ 10mg/kg bw?
NO
Is 10 < NOEL ≤ 500 mg/kg bw?
NO
Is NOEL > 500 mg/kg bw?
YES
YES
YES
YES
YES
YES
Hazard classification = 3
Hazard classification = 2
Hazard classification = 1
Hazard classification = 3
Hazard classification = 2
Hazard classification = 1
Single value: Conf.= 21
5th percentile Conf.= 26
Is Positive genotoxicity data available (in any one test)?
NO
Is Negative genotoxicity data available (in all tests)?
NO
Not available
YES
YES
Hazard classification = 3
Hazard classification = 1
Equivocal result allowed: Conf.= 21
Equivocal result not allowed: Conf.= 26

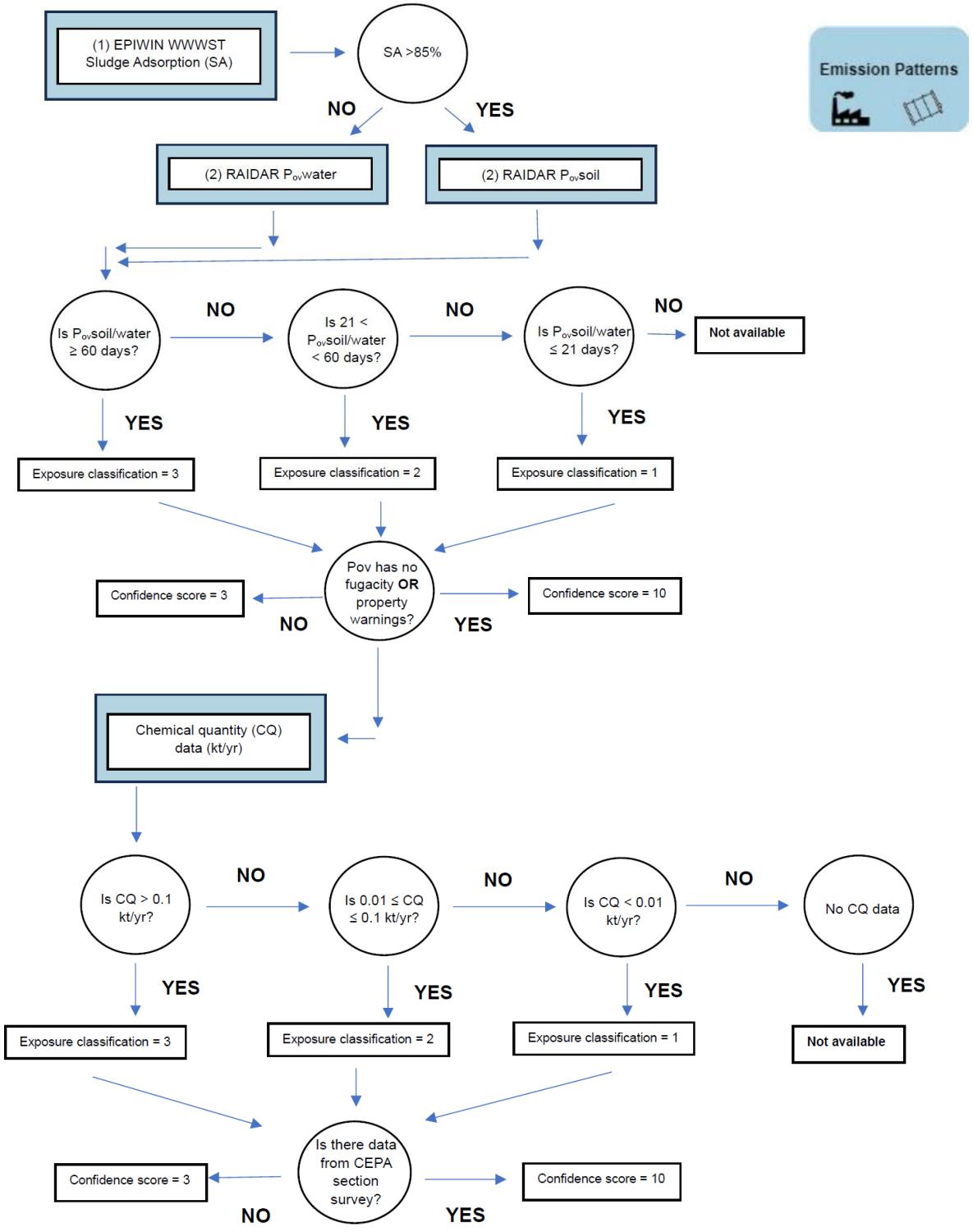

Mode of Toxic Action
In silico
(1) iSafeRat MechoA
(2) ASTER
(3) TEST Fish MoA
(4) OASIS Fish MoA
(5) Verharr
(6) LMC Uncouplers of Oxidative Phosphorylation
6 Model positive consensus
NO
5 Model positive consensus
NO
4 Model positive consensus
NO
3 Model positive consensus
NO
2 Model positive consensus
NO
YES
Confidence score = 5
Confidence score = 5
Confidence score = 4
Confidence score = 3
Confidence score = 2
Single Model positive consensus
NO
3 models agree for both classes (equivocal)
NO
2 models agree for both classes (equivocal)
NO
1 model agrees for both classes (equivocal)
NO
Not available
Confidence score = 1
Confidence score = 1
Confidence score = 1
Confidence score = 1
Mode of Toxic Action
Tissue Residue approaches
(1) LA50
(2) CBR (Kow)
(3) CBR (ACD KBW)
(4) CBR (UFZ KBW)
(5) CMC (Kow)
(6) CMC (ACD KBW)
(7) CMC (UFZ KBW)
7 Model positive consensus
NO
6 Model positive consensus
NO
Less than 6 model positive consensus
NO
Not available
YES
Confidence score = 10
Confidence score = 9
Confidence score = 8
MoA to be based on critical membrane concentration approach

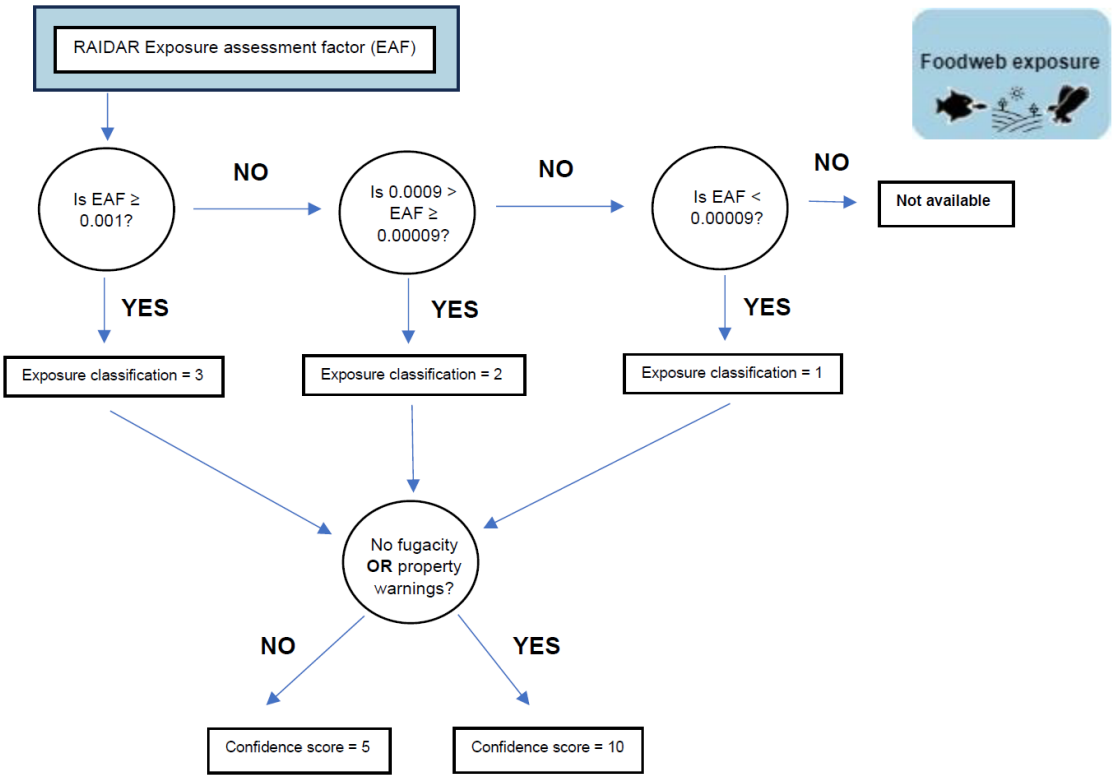

Foodweb Toxicity
RAIDAR Hazard assessment factor (HAF)
Is HAF ≥0.01?
NO
Is 0.001 < HAF < 0.01?
NO
Is HAF ≤ 0.001?
NO
Not available
YES
YES
YES
Hazard classification = 3
Hazard classification = 2
Hazard classification = 1
Fugacity OR property warnings?
YES
NO
Confidence score = 5
Confidence score = 10

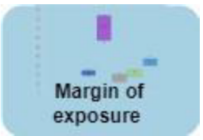

Cumulative Toxicity (case by case)
(1) Selected MoA for CBR50 based on final MoA
(2) If class 2 or class 3 in Receptor Mediated (EDC) final classification, but is narcosis by MoA consensus then MoA = "ERC 2 EDC"
All reactive MoA
NO
Uncoupling AND blocking of oxidative phosphorylation
NO
C12-C18 quaternary ammonium ("super polar narcosis")
NO
Narcosis (nonpolar + polar)
NO
Receptor docking AND ERC 2 EDC
NO
YES
YES
YES
YES
YES
IEC (mmol/kg) = 0.01
IEC (mmol/kg) = 0.01
IEC (mmol/kg) = 0.003
IEC (mmol/kg) = 3
IEC (mmol/kg) = 0.01
Enzyme disruption
NO
Neurotoxicity (chloride transport, inhibition + cation transport inhibition + acetylcholine esterase inhibition)
NO
Unknown MoA OR no MoA result?
YES
YES
YES
IEC (mmol/kg) = 0.01
IEC (mmol/kg) = 0.00001
Not available

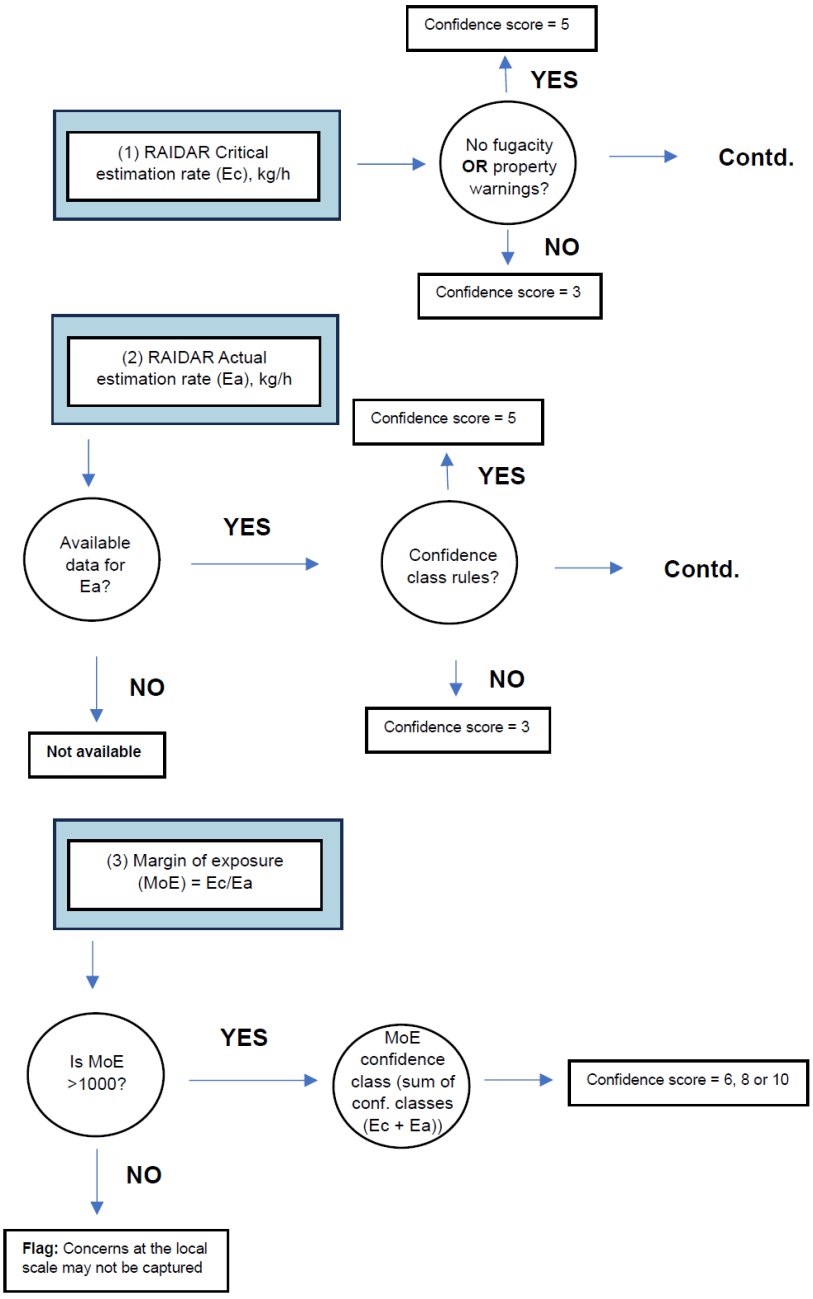
Appendix C: Exposure profiling




(1) EPIWIN WWWST Sludge Adsorption (SA)
SA >85%
NO
YES
Emission Patterns
(2) RAIDAR P_{ov}water
(2) RAIDAR P_{ov}soil
Is P_{ov}soil/water ≥ 60 days?
NO
Is 21 < P_{ov}soil/water < 60 days?
NO
Is P_{ov}soil/water ≤ 21 days?
NO
Not available
YES
YES
YES
Exposure classification = 3
Exposure classification = 2
Exposure classification = 1
Pov has no fugacity OR property warnings?
Confidence score = 3
NO
Confidence score = 10
YES
Chemical quantity (CQ) data (kt/yr)
Is CQ > 0.1 kt/yr?
NO
Is 0.01 ≤ CQ ≤ 0.1 kt/yr?
NO
Is CQ < 0.01 kt/yr?
NO
No CQ data
YES
YES
YES
YES
Exposure classification = 3
Exposure classification = 2
Exposure classification = 1
Not available
Is there data from CEPA section survey?
Confidence score = 3
NO
Confidence score = 10
YES


RAIDAR Exposure assessment factor (EAF)
Foodweb exposure
Is EAF ≥ 0.001?
NO
Is 0.0009 > EAF ≥ 0.00009?
NO
Is EAF < 0.00009?
NO
Not available
YES
YES
YES
Exposure classification = 3
Exposure classification = 2
Exposure classification = 1
No fugacity OR property warnings?
NO
YES
Confidence score = 5
Confidence score = 10

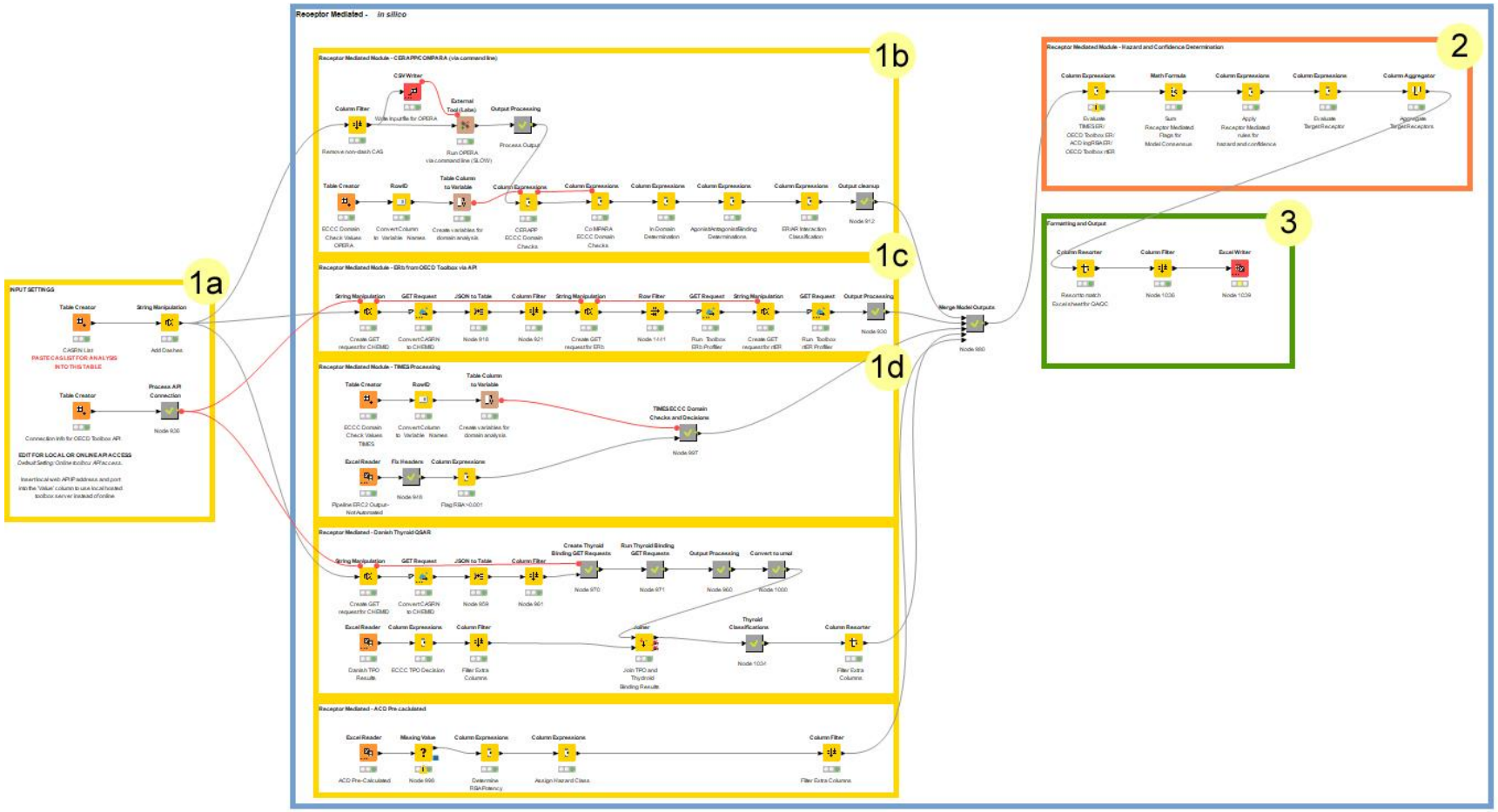

Margin of exposure


Confidence score = 5
YES
(1) RAIDAR Critical estimation rate (Ec), kg/h
No fugacity OR property warnings?
Contd.
NO
Confidence score = 3
(2) RAIDAR Actual estimation rate (Ea), kg/h
Confidence score = 5
YES
Available data for Ea?
YES
Confidence class rules?
Contd.
NO
NO
Confidence score = 3
Not available
(3) Margin of exposure (MoE) = Ec/Ea
Is MoE >1000?
YES
MoE confidence class (sum of conf. classes (Ec + Ea))
Confidence score = 6, 8 or 10
NO
Flag: Concerns at the local scale may not be captured

Appendix D: Supplementary Excel spreadsheet

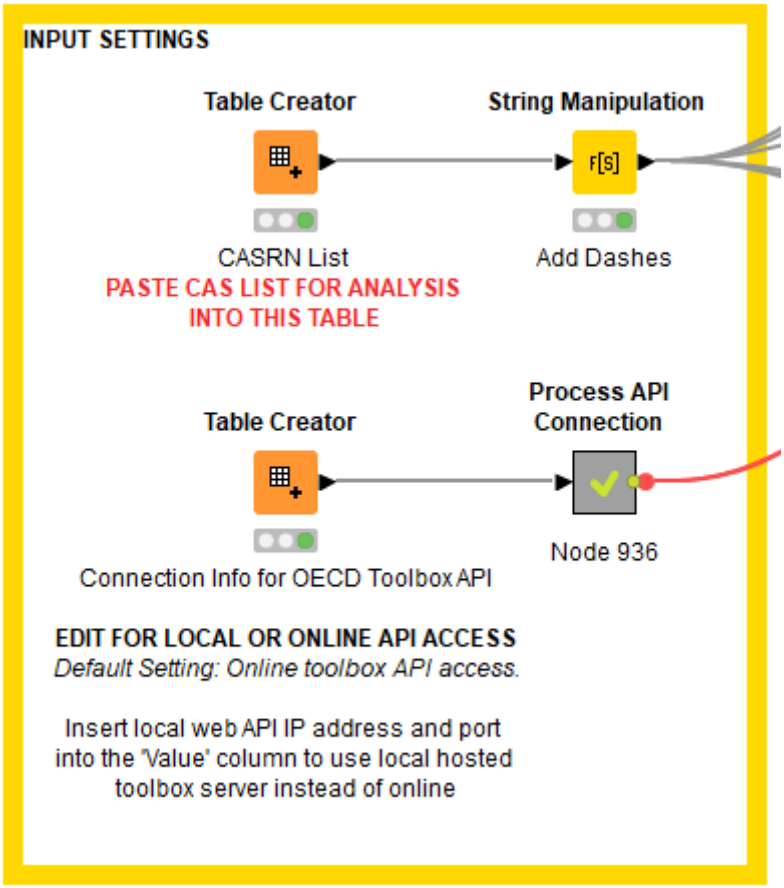
ERC 2 results for the 15 OFRs and 9 non-specific acting chemicals are provided in an Excel spreadsheet as supplement to this document. CAS RN 632-79-1, used as an example for ERC2 methodology, is highlighted.

Appendix E: Approaches to automation: *in silico* Receptor Mediated Toxicity descriptor



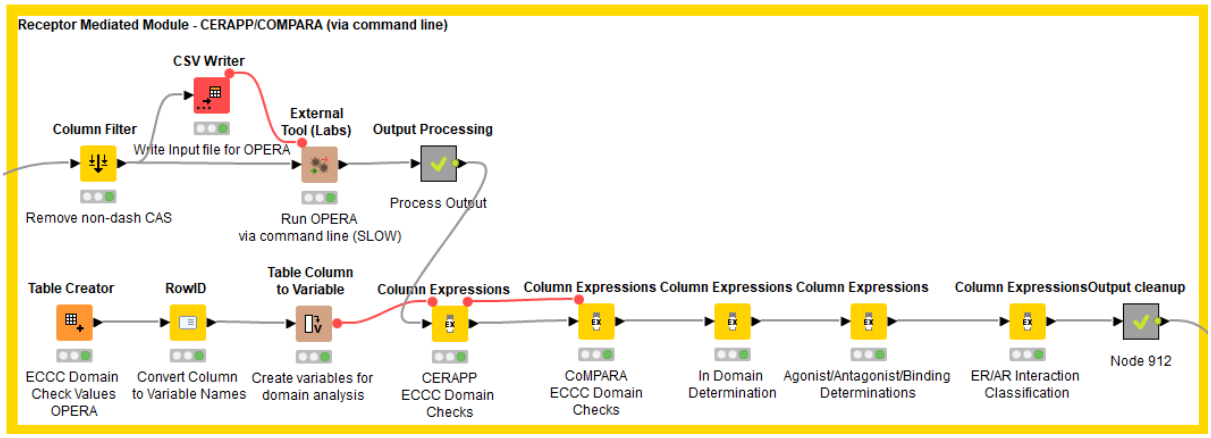
1a

The user pastes a list of CAS RNs into the CAS RN List Table Creator node. The node will process the CAS RNs and ensure that they have dashes added in the correct locations.
A second Table Creator node contains the connection information for the OECD Toolbox API.



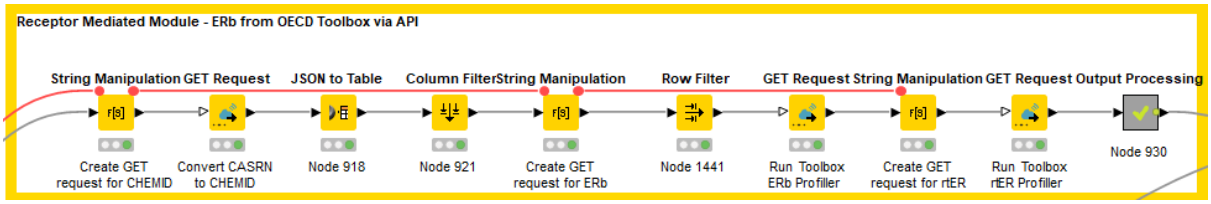
1b

CASRNs are run through OPERA (Mansouri et al. 2018) via an automated command line interface. The output will be parsed, and required information from CERAP/COMPARA will be evaluated (applying ECCC specific domain checks). ER/AR binding potentials are classified as per the ERC2 rules.



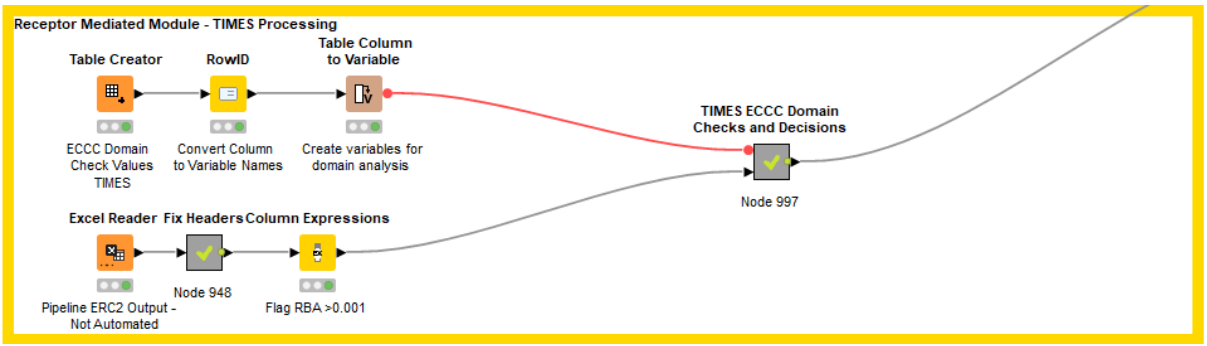
1c

The OECD Toolbox is accessed via API GET requests to collect information on ER binding for the chemicals specified in the input workflow. The information that is returned from the OECD Toolbox is parsed and classified as per the ERC2 rules.



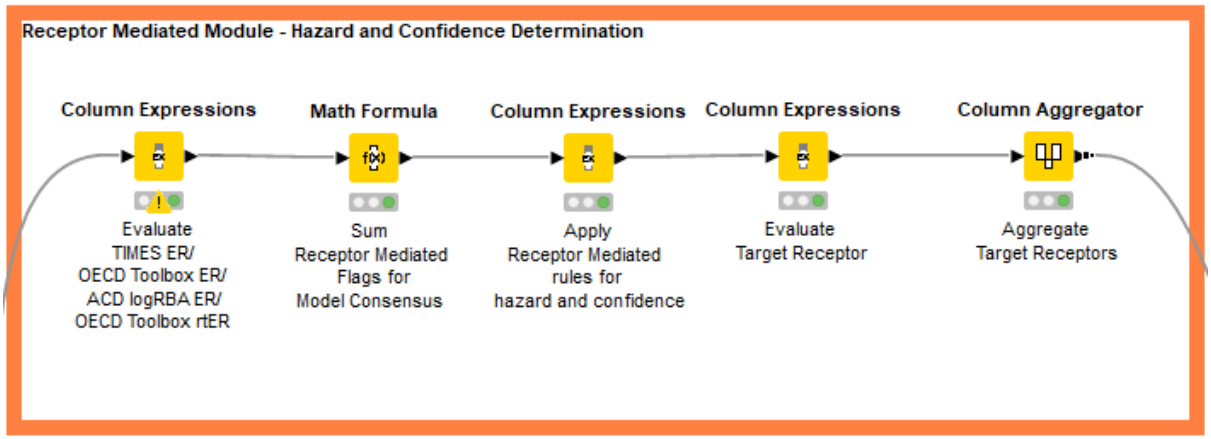
1d

Pre-populated outputs from the ECCC pipeline profiler are parsed and ER/AR binding potentials are evaluated. Hazard is classified as per the ERC2 rules.



2

Outputs from all of the *in silico* receptor mediated modules are joined and the overall hazard/confidence rules are applied.



3

The classified data results are formatted and can be either:

- Output to an Excel spreadsheet (shown below)
- Continue on in the workflow to be rolled up with results from other modules to create the ERC2 main classification sheet (not shown).