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Handling concentration data below the analytical limit in environmental mixture risk assessment: A case-study on pesticide river monitoring

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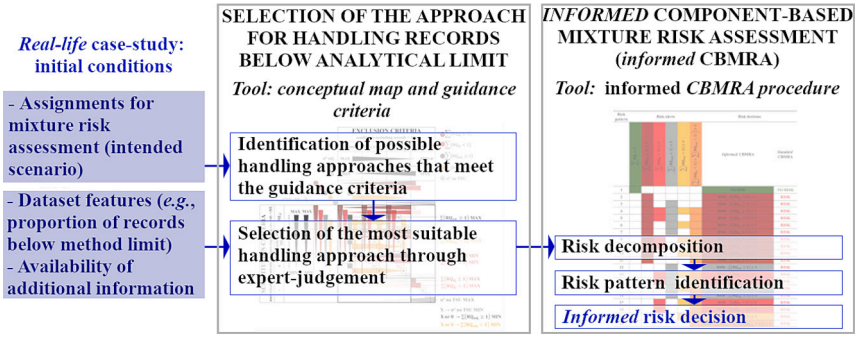
HIGHLIGHTS

- The *non-detects* can impact on Component-Based Mixture Risk Assessment (*CBMRA*).
- Guidance map and criteria are strategic for addressing *non-detects* in CBMRA.
- The *informed* CBMRA reduces the uncertainty about *non-detects* in risk decision.
- The approach used for handling *non-detects* in CBMRA must be clearly reported.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic organisms are exposed to ever-changing complex mixtures of chemicals throughout their lifetime. Component-Based Mixture Risk Assessment (CBMRA) is a well-established methodology for water contaminant-mixture management, the use of which is growing due to improved access to reference ecotoxicity data and extensive monitoring datasets. It enables the translation of measured exposure concentrations of chemicals into biological effect values, and thus to quantitatively estimate the risk of the whole water sample (*i.e.*, as a mixture). However, many factors can bias the final risk decision by impacting the risk metric components; thus, a careful design of the CBMRA is needed, taking into primary consideration the specific features of the dataset and mixture risk assessment assignments.

This study systematically addressed the effects of the most common approaches used for handling the concentrations of chemicals below the limit of detection/quantification (LOD/LOQ) in CBMRA. The main results included: i) an *informed* CBMRA procedure that enables the tracking of the risk decisions triggered by substances below LOD/LOQ, ii) a conceptual map and guidance criteria to support the selection of the most suitable approach for specific scenarios and related interpretation; iii) a guided implementation of the *informed* CBMRA on dataset of pesticide concentrations in Italian rivers in 2020 (702,097 records).

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1. Introduction

Throughout their lifetime, organisms are co-exposed to thousands of chemicals of anthropogenic origin (Scholz et al., 2022). Aquatic ecosystems are particularly threatened by pollution since they often serve as the final recipients of effluents from industry and municipal wastewater treatment plants, as well as of agriculture and surface runoff. This "cocktail of pollutants" (commonly including pesticides, biocides, pharmaceuticals, personal care products, surfactants, industrial chemicals, urban runoff, and non-intentionally produced substances) can pose a combined risk to aquatic life. Given the complexity of the chemical and toxicological profiles of environmental mixtures, assessing and managing them is a concern and a major objective for policy makers aiming to ensure good water quality (Bopp et al., 2018).

The impact of co-exposure on ecosystem health has not yet been addressed by regulation (Kienzler et al., 2017b). Worldwide, regulatory water monitoring programs typically focus on individual chemicals, assessing water concentrations and comparing them with individual effect thresholds. For instance, in the EU, the Water Framework Directive (WFD (Directive 2000/60/EC)) defines the *Good Chemical Status* in terms of the compliance with all the Environmental Quality Standards (EQS) established for 45 Priority Substances, together with various sets of river basin-specific pollutants defined at national level, without requiring any consideration of cumulative risk evaluations. However, given the growing pressure to progress towards a "non-toxic environment", the scientific community is actively involved in developing a multi-substance approach (Bopp et al., 2018; Brack et al., 2018; Posthuma et al., 2019a; Scholz et al., 2022) including: 1) advanced analytical chemical techniques enabling non-targeted screening of chemicals; 2) effect-based monitoring for the direct detection of cumulative effects (including unmonitored, unknown, and undetected substances) and possible identification of specific modes of action (Escher et al., 2020; Villeneuve et al., 2019); 3) effect-directed analysis of toxicity drivers (Brack et al., 2016); 4) concepts and computational methodologies to bridge the gaps in ecotoxicological data (Barron et al., 2021; Gutsell et al., 2015; Kienzler et al., 2017a); 5) chemical and toxicological quantitative footprints to summarize and communicate trends in chemical pollution and support management (Brack et al., 2018).

Within the latter pillar, Component-Based Mixture Risk Assessment (CBMRA) is a cost-effective tool for screening-level ecological risk assessment that can be applied across various levels of expertise (Posthuma et al., 2019a). CBMRA is a well-established and pragmatic methodology that assesses the cumulative toxicity of environmental mixtures using information on their chemical composition (*i.e.*, Measured Environmental Concentrations - MECs). CBMRA tools commonly involve the conversion of contaminant exposure concentrations into effect/risk-related values (*e.g.*, risk quotient (RQ); toxic unit (TU)) based on their effect on representative organisms or species assemblages (*e.g.*, regulatory or reference values, such as Predicted No Effect Concentration - PNEC, legally binding EQS, and/or metrics derived from Species Sensitivity Distributions - SSDs). Through the application of mixture effect models, these standardised "toxic equivalents" are used to estimate the overall risk, including the contribution of all the investigated substances resulting below the related effect thresholds or analytical limits (*i.e.*, summation of Toxic Units - $\sum TU$; the summation of RQ - $\sum RQ$; multi-substance Potentially Affected Fraction of species, – msPAF; pharmacologically based mixture models (Backhaus and Faust, 2012; Posthuma et al., 2019a)).

In practice, CBMRA can be applied to address a number of pragmatic objectives in mixture risk assessment, including: i) prioritizing water bodies and chemicals for action (Henning-de Jong et al., 2008; Posthuma et al., 2018), ii) controlling effluents from wastewater treatment plants (Finckh et al., 2022), and iii) screening the chemical toxic pressure on a large scale (Bradley et al., 2021; Chen et al., 2020; Corsi et al., 2019; Gustavsson et al., 2017; Lei et al., 2021; Markert et al., 2020; Rämö et al., 2018; Rodea-Palomares et al., 2023; Rorije et al., 2022). The application of CBMRA for these purposes has seen recent growth thanks to advancements in the accessibility of high-quality ecotoxicological data and MECs recorded during water quality monitoring programs worldwide (*e.g.*, open-access sources: US EPA ECOTOX DB, CompTox Chemistry Dashboard, QSAR TOOLBOX, NORMAN, European Chemical Agency (ECHA) information on chemicals, EnviroTox database, Pesticides Properties DataBase, IPCHEM). In fact, online platforms collecting and sharing consolidated experimental ecotoxicity data enable the identification of more sound threshold limit values for several pollutants, while *in silico* tools help bridge ecotoxicity data gaps for data-poor chemicals (*e.g.*, QSAR, read-across, grouping, EcoToxicological Threshold of Concern (Eco-TTC)); open-access repositories for chemical water pollution data support identification of pollution trends and typical mixtures on a larger scale (Barron et al., 2021; Beasley et al., 2015; Belanger et al., 2015; Connors et al., 2019; Olker et al., 2022; Williams et al., 2017).

Despite its increasing applicability, it is well acknowledged that CBMRA reliability depends on many factors associated with all components of the metrics (Jesenska et al., 2013; Markert et al., 2020). Therefore, the reasons behind any choice need to be evaluated in depth and reported. A factor that can significantly impact the CBMRA decision regards the approach used for handling data below the limits of detection (LOD) or quantification (LOQ) (from here on, they are generically referred to as records below the method limit: *records* < *ML*). *Records* < *ML* can represent a sizable part, or even the dominant part, of environmental monitoring datasets. Therefore, the issue can arise as to whether the approach to their handling can significantly drive the risk decision, resulting in insufficient protection or risk overestimation. Risk decisions of particularly difficult interpretation are those triggered by substances always assessed below their ML (from here on, non-detected substances, or *non-detects*). In fact, in routine applications of CBMRA, risk decisions can be triggered by: i) single *non-detects* monitored by the use of analytical methods not sensitive enough to assess low concentrations already toxic to the organisms (*i.e.*, method limits higher than the reference toxicity thresholds), or ii) a number of *non-detects* in a cumulative way (Gustavsson et al., 2017).

In CBMRA, there could be a significant difference in how *records* < *ML* are treated. Researchers often apply practical methods rather than strictly adhering to existing guidelines in various risk assessment (RA) domains (Helsel, 2010, 1990; USEPA, 1991, 2000). This is because systematically applying these guidelines can be challenging, especially when dealing with complex wide-scope pollution screening datasets aggregated from different sources and multiple surveys. Therefore, the majority of published CBMRA studies have adopted various approaches for managing *records* < *ML*, such as: i) the complete removal of *records* < *ML* from the dataset (Rorije et al., 2022), ii) treating them as true zero concentration (Finckh et al., 2022; Rodea-Palomares et al., 2023), or iii) replacing them with fractions of the ML values (Finckh et al., 2022) besides the use of ML values *tout court* (Rodea-Palomares et al., 2023). At times, authors have applied more than one approach to investigate the impact from different data treatments and deal with the related uncertainty (Finckh et al., 2022; Gustavsson et al., 2017; Kienzler et al., 2019; Rodea-Palomares et al., 2023). Nevertheless, it is often difficult to understand why a certain approach is selected over another; and how the results can be compared to the available alternative approaches; as well as how reliable the final CBMRA result is.

The present work highlights the importance of considering how the choice of approach for handling *records* < *ML* can impact the risk evaluation within the CBMRA context. To practically address this objective, the paper focused on:

1) defining an *informed* CBMRA procedure that traces the role of *records* < *ML* in the risk evaluation and discriminates between risk decisions triggered by *non-detects*;
2) developing a conceptual map and guidance criteria that facilitate: i) the selection of the most suitable approach for handling *records* <

ML under specific mixture risk assessment (MRA) assignments, and ii) the correct interpretation of the CBMRA outcome;

3) implementing the developed *informed* CBMRA procedure, the conceptual map, and the guidance criteria in a specific case-study, thereby validating these tools and demonstrating their practical effectiveness.

The paper's ambition was to develop tools that are sufficiently broad and flexible to accommodate a large variety of CBMRA end-goals and monitoring datasets and, possibly, find applications beyond the default approaches considered in the present study.

2. Materials and methods

2.1. Informed CBMRA procedure

The development of *informed CBMRA* procedure was based on: i) the use of RQ under the assumption of the concentration addition model; ii) the assumption of a unique ML value for each substance in a mixture; iii) the similar handling of records < LOD and records < LOQ; and iv) the aggregation of concentrations recorded within a selected time-span and monitoring area (hereafter, time-space unit, *TSU*). Briefly, the RQ is computed for each substance *i* (RQ_i) in the mixture (TSU) by dividing the exposure level (*i.e.*, measured environmental concentration, MEC_i) by the correspondent effect level (*i.e.*, reference toxicity threshold, TT_i; eq. 1). Then, the risk due to the simultaneous exposure to multiple

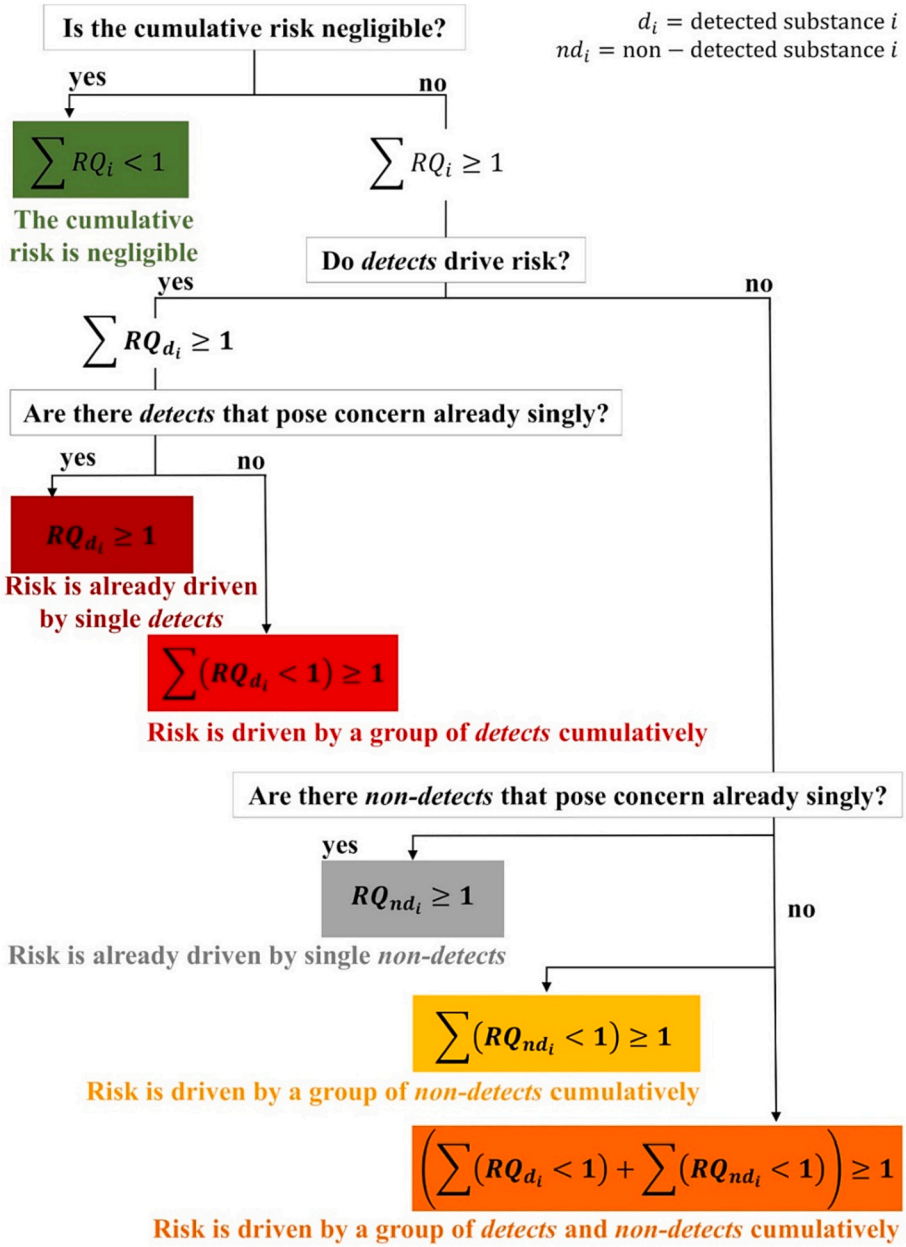

Fig. 1. Problem formulation outline underneath the *informed* CBMRA. Questions driving the transition of the *standard* CBMRA to the *informed* CBMRA. This advancement entails breaking down the risk complexity into separate subcomponents meaningful for the risk posed by i) single *detects*, ii) a group a *detects* cumulatively, iii) single *non-detects*, iv) a group of *non-detects* cumulatively. The diagram shows the intended five risk notifications (with the associated operational elements) in the priority order dictated by the selected problem formulation logic. Users can design alternative outlines, as needed. Abbreviations: RQ_i: risk quotient of the substance i; RQ_{d_i}: risk quotient of the detected substance i; RQ_{nd_i}: risk quotient of the non-detected substance i.

chemicals is calculated as a linear aggregation (sum) of the RQ_i computed for all substances in the mixture (eq. 2). When $\sum_{TSU} RQ_i$ is below 1, there is no appreciable risk to aquatic life.

$$RQ_i = \frac{MEC_i}{TT_i} \quad (1)$$

$$\sum_{TSU} RQ_i = RQ_x + RQ_j + RQ_y + \ldots \quad (2)$$

Compared to the *standard* RQ-based CBMRA, the *informed CBMRA* procedure was intended to provide the following additional information: 1) to notify whether the risk evaluation is decided by detected substances *vs* non-detected substances; 2) to notify when some chemicals can be of concern already individually or, contrarily, when risk is exclusively a matter of mixture exceedance. The diagram in Fig. 1 shows the problem formulation outline that underlies the *informed CBMRA* developed for the present study. It presents the questions addressed stepwise and the resulting risk notifications. This approach entails breaking down the risk complexity into four separate subcomponents assessing the harm specifically posed by i) single *detects*, ii) a group of *detects* cumulatively, iii) single *non-detects*, iv) a group of *non-detects* cumulatively. In this regard, users have the flexibility to design a different problem formulation outline for their case-study by applying different decision rules.

2.2. Conceptual map and criteria to guide the selection of the approach for handling records < ML

Being a solution-oriented tool for chemical prioritization and management, real-world CBMRA is commonly performed to meet specific MRA assignments (*e.g.*, setting priorities among impacted water bodies for further monitoring and action; identifying risk drivers; evaluating the impact of specific land uses; prioritizing chemicals for abatement or mitigation measures; anticipating the effects of future emission scenarios; monitoring pollution trends; exploring possible impacts of future developments in society (Posthuma et al., 2019a)). The MRA question at hand typically establishes the exposure scenario and the conditions under which it is necessary to make the risk-related decision (*e.g.*, conservative decision, over-protective decision, decision with the highest relative certainty, realistic decision, decision under worst/best scenario). The method for handling *records* < *ML* can affect how the planned decision setting is actually implemented.

The development of the conceptual map aimed to establish a coherent foundation for identifying the most suitable handling option to align with the MRA assignment, thereby securing the achievement of the final protection and management goal. It is intended to be used in conjunction with the *informed* CBMRA procedure. The map compares the roles of *detects* and *non-detects* in triggering single-substance and cumulative risk across common handling approaches, pinpointing the specific approaches that are the most and the least effective in accounting for these risks. This information is then used to set objective criteria for selecting the handling approach that best fits the exposure scenarios and the final MRA goal.

The map was generated from both exclusion and substitution criteria commonly used to deal with *records* < *ML*. More precisely, the selected exclusion criteria include: i) no *records* < *ML* eliminated, ii) *records* < *ML* with $ML_i \geq TT_i$ eliminated, iii) *records* < *ML* with $ML_i \geq$ a fraction of TT_i eliminated, and iv) all *records* < *ML* eliminated. The criterium iii) represents the possible circumstance where the minimal analytical performances required for the methods used under regulatory monitoring are retrospectively applied for selectively excluding *records* < *ML* (*e.g.*, for WFD monitoring, Directive 2009/90/EC requires that methods feature a LOQ value equal to or lower than 30 % of the associated EQS (Directive 2009/90/EC)). The selected substitution criteria involve replacing *records* < *ML* with: i) full ML values, ii) fixed fractions of ML values, and iii) zero value. The map is built on the simplest case in which each substance in a mixture (TSU) is assigned a single ML value.

2.3. Background considerations about the proposed case-study

The *informed* CBMRA procedure was applied to the 2020 Italian freshwater pesticide dataset (ISPRA, Rapporti 371/2022), handling the *records* < *ML* by ten different approaches closely following the conceptual map. The conceptual map and guidance criteria were then used to identify the most suitable approach to be used under representative MRA assignments.

In 2020, water sampling and analytical measurements were carried out by Italian Regional Environmental Agencies under the WFD monitoring program. The full dataset included 764,069 individual MECs (*i.e.*, a total of 406 chemicals resulting from 1836 sampling sites across Italian rivers); in particular, 740,656 records (*i.e.*, 96.9 % of the total dataset) referred to non-quantified concentrations (*records* < *LOQ*).

The average annual concentration of each monitored substance at each sampling station was considered to derive the co-exposure information (*i.e.*, TSU = sampling site – year combination). To calculate the RQ_i, the hazard concentration for 5 % of species (HC5) derived from chronic species sensitivity distribution (SSD_{NOEC}) was used as the reference toxicity threshold. This hazard metric characterizes the concentration below the No Observed Effect Concentration (NOEC) for 95 % of the species in an assemblage (Posthuma et al., 2001). Practically, HC5 values were extracted from the SSD_{NOEC} recently computed by Posthuma et al. (2019b) in an extensive effort to screen, aggregate, and analyse patterns in global ecotoxicity database for 12,386 chemicals.

The exclusion criteria applied to the proposed case-study included: i) no *records* < *LOQ* excluded, ii) *records* < *LOQ* with $LOQ_i \geq HC5_i$ excluded, iii) *records* < *LOQ* with $LOQ_i \geq 1/3\ HC5_i$ excluded, and iv) all *records* < *LOQ* excluded. Regarding the substitution criteria, the three numerical concentration values used to replace *records* < *LOQ* corresponded to: i) LOQ, ii) half LOQ, and iii) zero.

Before running the CBMRA, the dataset underwent a preliminary curation to meet the objectives of this exercise. We retained records reporting surficial MECs associated with substances with available SSD_{NOEC} in Posthuma et al. (2019b). Controls were conducted to identify possible duplicated data and typos. After the refining procedure, the consolidated dataset featured 702,097 records covering 353 chemicals and 1798 sampling locations; 680,094 records (*i.e.*, 96.9 % of the whole consolidated dataset) were *records* < *LOQ*. In order to validate the conceptual map, LOQ values were equalized when multiple values existed for the same substance at the same TSU (from here on, we refer to it as the *LOQ-equalized* dataset). The results of the *informed* CBMRA on the *LOQ-equalized* dataset are presented in this manuscript. However, to demonstrate the applicability of the developed tools to datasets with non-uniform LOQ values, the *informed* CBMRA was also carried out in the *original* dataset and results are reported in Supporting Information File 2.

Technically, the *informed* CBMRA procedure was implemented with the support of Microsoft Excel 2016.

3. Results

3.1. Informed CBMRA procedure: description

The classification and computation steps required by the *informed* CBMRA are presented in Tables 1–2. Initially, substances within each TSU are classified as *detects* or *non-detects* (Table 1, step 1). Briefly, substances measured at concentrations higher than their associated ML at least once within a TSU are classified as *detects*, whereas substances with their concentrations always below their ML are defined as *non-detects*. The risk quotient is then computed for each substance *i* (RQ_i) according to eq. 1 (step 2 and step 3), and classified based on its potential to harm (*i.e.*, TT_i exceedance; step 4). At the end of this two-step classification, all chemicals fall into one of the four RQ_i *classes*:

Table 1
Informed CBMRA workflow. a) Steps 1–3. The table refers to the model substances x, y, and z, each representing one of the three possible patterns of measured environmental concentrations (MECs; *i.e.*, x: all MEC_x above the method limit (ML_x); y: at least one MEC_y below ML_y; z: all MEC_z below ML_z). It provides the scheme to classify substances in a mixture (TSU) as *detects* and *non-detect* (step 1) and the equations for computing individual risk quotients (RQ_i; steps 2, 3). b) Steps 4–6. Starting from the output of step 3 (*i.e.*, RQ_i classified as detected RQ_i (RQ_{d_i}) and non-detected RQ_i (RQ_{nd_i})), the table provides the scheme to further classify individual RQ_i based on whether they exceed their associated toxicity thresholds (TT_i, step 4). Additionally, it provides equations for calculating each *risk component* (step 5) and the overall cumulative risk (step 6). Abbreviations: ML: analytical method limit; $\overline{d_i}$: average concentration of the detected substance *i*; nd_i: numerical concentration value substituting *record* < ML_i; TT_i: Toxicity Threshold of the substance *i*; RQ_{d_i}: risk quotient of the detected substance *i*; RQ_{nd_i}: risk quotient of the non-detected substance *i*.

a)

Time-space unit (TSU, i.e., mixture)		*Step 1: Substance* i *classification*	*Step 2: Per substance* i *concentration average*	*Step 3: Per substance* i *RQ computation*
Substance *i* (model substances)	**MEC (model patterns)**	$d_i; nd_i$	$\overline{d}_i;\ nd_i$	$RQ_{d_i};\ RQ_{nd_i}$
x	x_1; x_n	$detect_x$ (d_x)	$\overline{x} = \frac{x_1 + x_n}{n} \rightarrow \overline{d_x}$	$RQ_x = \frac{\overline{d_x}}{TT_x} \rightarrow RQ_{d_x}$
y	y_1; $<ML_{y,n}$	$detect_y$ (d_y)	$\overline{y} = \frac{y_1 + (<ML_{y,n})}{n} \rightarrow \overline{d_y}$	$RQ_y = \frac{\overline{d_y}}{TT_y} \rightarrow RQ_{d_y}$
z	$<ML_{z,1}$; $<ML_{z,n}$	$non\text{-}detect_z$ (nd_z)	$\overline{z} = \frac{(<ML_{z,1}) + (<ML_{z,n})}{n} \rightarrow nd_z$	$RQ_z = \frac{nd_z}{TT_z} \rightarrow RQ_{nd_z}$

b)

RQ_i	*Step 4: RQ_i classification*	*Step 5: Risk decomposition*	*Step 6: Cumulative risk computation*
	TT_i exceedance vs non-exceedance	Risk components	$\sum_{TSU} RQ_i$
RQ_{d_i}	$RQ_{d_i} \geq 1$	$\sum(RQ_{d_i} \geq 1)$	$\sum_{TSU} RQ_i = \sum(RQ_{d_i} \geq 1) + \sum(RQ_{d_i} < 1)$
	$RQ_{d_i} < 1$	$\sum(RQ_{d_i} < 1)$	
RQ_{nd_i}	$RQ_{nd_i} \geq 1$	$\sum(RQ_{nd_i} \geq 1)$	$+ \sum(RQ_{nd_i} \geq 1) + \sum(RQ_{nd_i} < 1)$
	$RQ_{nd_i} < 1$	$\sum(RQ_{nd_i} < 1)$	

– detected substance *i* that poses harm singly ($RQ_{d_i} \geq 1$)
– detected substance *i* that does not raise individual concern ($RQ_{d_i} < 1$)
– non-detected substance *i* that triggers risk at single substance level ($RQ_{nd_i} \geq 1$)
– non-detected substance *i* that does not trigger risk at single substance level ($RQ_{nd_i} < 1$)

Four *risk components* are then calculated to assess the cumulative risk posed by each RQ_i *class* (step 5). *Risk components* are operationally obtained by summing the RQ_i of substances belonging to the same RQ_i *class*, according to eq. 2 (*i.e.*, $\sum(RQ_{d_i} \geq 1)$, $\sum(RQ_{d_i} < 1)$, $\sum(RQ_{nd_i} \geq 1)$, $\sum(RQ_{nd_i} < 1)$). Thus, the overall risk of a mixture (TSU) corresponds to the sum of the four *risk components* (step 6).

By applying the definition of risk in CBMRA ($\sum_{TSU} RQ_i \geq 1$), ecological threat is not negligible when at least one of the four *risk components* is equal to or greater than 1 (*i.e.*, $\sum(RQ_{d_i} \geq 1) \geq 1$; $\sum(RQ_{d_i} < 1) \geq 1$; $\sum(RQ_{nd_i} \geq 1) \geq 1$; $\sum(RQ_{nd_i} < 1) \geq 1$), or when *components* that are not a concern by themselves have a cumulative risk of 1 or more (*i.e.*, $(\sum(RQ_{d_i} < 1) + \sum(RQ_{nd_i} < 1)) \geq 1$). These five conditions represent the *risk alerts* of the *informed CBMRA* procedure (Table 2). Finally, the last two steps entail identifying all "active" *risk alerts* in a TSU (step 7, Table 2) and the priority one (step 8), *i.e.*, the *risk alert* driving the final *informed* risk decision based on the priority order dictated by the CBMRA problem formulation (Fig. 1).

The *decision-key* within the CBMRA framework of Fig. 1 is reported in Table 2. It identifies twenty possible risk patterns and their corresponding *informed* risk decisions. This specific assessment primarily notifies when the risk is triggered by *detects* or *non-detects* and, secondly, when risk results from particular compounds exceeding their safe levels (*i.e.*, single substance risk) or, on the contrary, from the cumulative contribution of many substances (*i.e.*, cumulative risk). Emphasising that the system reacts to specific *alerts* leaving hidden some others that could potentially co-exist due to the chosen prioritization, users are advised to carefully tailor their decision logic at the problem formulation stage (*i.e.*, an *informed* CBMRA outline) in order to make risk conclusions informative of the risk type they deemed as the most critical.

As depicted in Table 2, the *informed CBMRA* decisions are consistent with the *standard* CBMRA decisions in terms of distinguishing between risk/no risk concern. However, in addition, they differentiate:

- TSUs where the risk is driven by *detects*. Within this group, the assessment further discriminates whether the risk is already driven by *detects* individually (*i.e.*, single substance risk) or, alternatively, when mixture components, although compliant with their own safety thresholds, sum up to unacceptable levels (*i.e.*, cumulative risk);

Table 2
Informed CBMRA workflow: steps 7–8. The table can be intended as the *decision-key* linked to the CBMRA outline of Fig. 1. It shows the five *risk alerts* of the *informed* CBMRA and lists all the *risk patterns* that can result from their possible co-occurrence. For each *risk pattern*, it indicates the *informed* CBMRA decision by identifying the priority *risk alert*. Operationally, for a given TSU, assessors identify the *risk pattern* based on the "active" *risk alerts* (step 7), and the associated *informed* CBMRA decision (step 8). Additionally, the *standard* CBMRA decision is provided here for the purpose of comparison. Abbreviation: RQ_{d_i}: risk quotient of the detected substance *i*; RQ_{nd_i}: risk quotient of the non-detected substance *i*.

Risk pattern n°	*Step 7:* *Risk pattern identification* — Risk alerts						*Step 8:* *Priority risk alert identification*	*Standard* risk decision
	$\sum RQ_i < 1$	$\sum(RQ_{d_i} \geq 1) \geq 1$	$\sum(RQ_{d_i} < 1) \geq 1$	$\sum(RQ_{nd_i} \geq 1) \geq 1$	$\sum(RQ_{nd_i} < 1) \geq 1$	$\left(\sum(RQ_{d_i} < 1) + \sum(RQ_{nd_i} < 1)\right) \geq 1$	*Informed* risk decision	
1							NEGLIGIBLE RISK	NEGLIGIBLE RISK
2							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
3							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
4							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
5							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
6							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
7							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
8							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
9							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
10							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
11							RISK - $\sum(RQ_{d_i} \geq 1) \geq 1$	RISK
12							RISK - $\sum(RQ_{nd_i} \geq 1) \geq 1$	RISK
13							RISK - $\sum(RQ_{d_i} < 1) \geq 1$	RISK
14							RISK - $\sum(RQ_{d_i} < 1) \geq 1$	RISK
15							RISK - $\sum(RQ_{nd_i} \geq 1) \geq 1$	RISK
16							RISK - $\sum(RQ_{nd_i} \geq 1) \geq 1$	RISK
17							RISK - $\sum(RQ_{d_i} < 1) \geq 1$	RISK
18							RISK - $\sum(RQ_{d_i} < 1) \geq 1$	RISK
19							RISK - $\sum(RQ_{nd_i} < 1) \geq 1$	RISK
20							RISK - $(\sum(RQ_{d_i} < 1) + \sum(RQ_{nd_i} < 1)) \geq 1$	RISK

- TSUs where the risk is triggered by a group of *non-detects* that cumulatively exceed the risk threshold of 1. These cases can raise the issue of dataset oversizing.
- TSUs where the risk is triggered by at least one single *non-detect* monitored *via* analytical methods not sufficiently sensitive to detect/quantify low concentrations already toxic for organisms (*i.e.*, the method limit is higher than the reference toxicity threshold). These cases call into question the sensitivity of the analytical methods employed to track target pollutants.

It is important to note that while the knowledge of the role of *detects vs non-detects* in the risk decision is informative for uncertainty, the knowledge of the risk type (single substance exceedance *vs* mixture exceedance) has a solution-oriented value as it helps to better focus the subsequent steps of the MRA path.

Technically, the *informed* procedure can be implemented through a spreadsheet which can operationalize the steps described above (*e.g.*, Microsoft Excel).

3.2. Conceptual map and guidance criteria

The developed map is shown in Fig. 2. It considers ten approaches for handling *records* < *ML* (10 squares in the central grid) obtained by combining the exclusion and substitution criteria selected in Section 2.2. Each substance monitored within a TSU is assumed to have a unique LOQ value.

The map develops from the systematic analysis of the variation pattern of three mixture elements sensitive to handling treatments of *records* < *ML*: 1) the number of *non-detects* (n° nd_i), 2) the concentration value assigned to *non-detects* (nd_i), and 3) the average concentration of *detects* ($\overline{d_i}$). The two external blocks graphically outline their variation when applying the exclusion and substitution criteria separately. These changes are closely linked to the variation of the four *risk components* (*i.e.*, $\sum(RQ_{d_i} \geq 1)$; $\sum(RQ_{d_i} < 1)$; $\sum(RQ_{nd_i} \geq 1)$; $\sum(RQ_{nd_i} < 1)$) and of the number of non-assessable TSUs (n° na TSUs), which are depicted in the lower part of the blocks.

The inner grid represents the core of the conceptual map, displaying

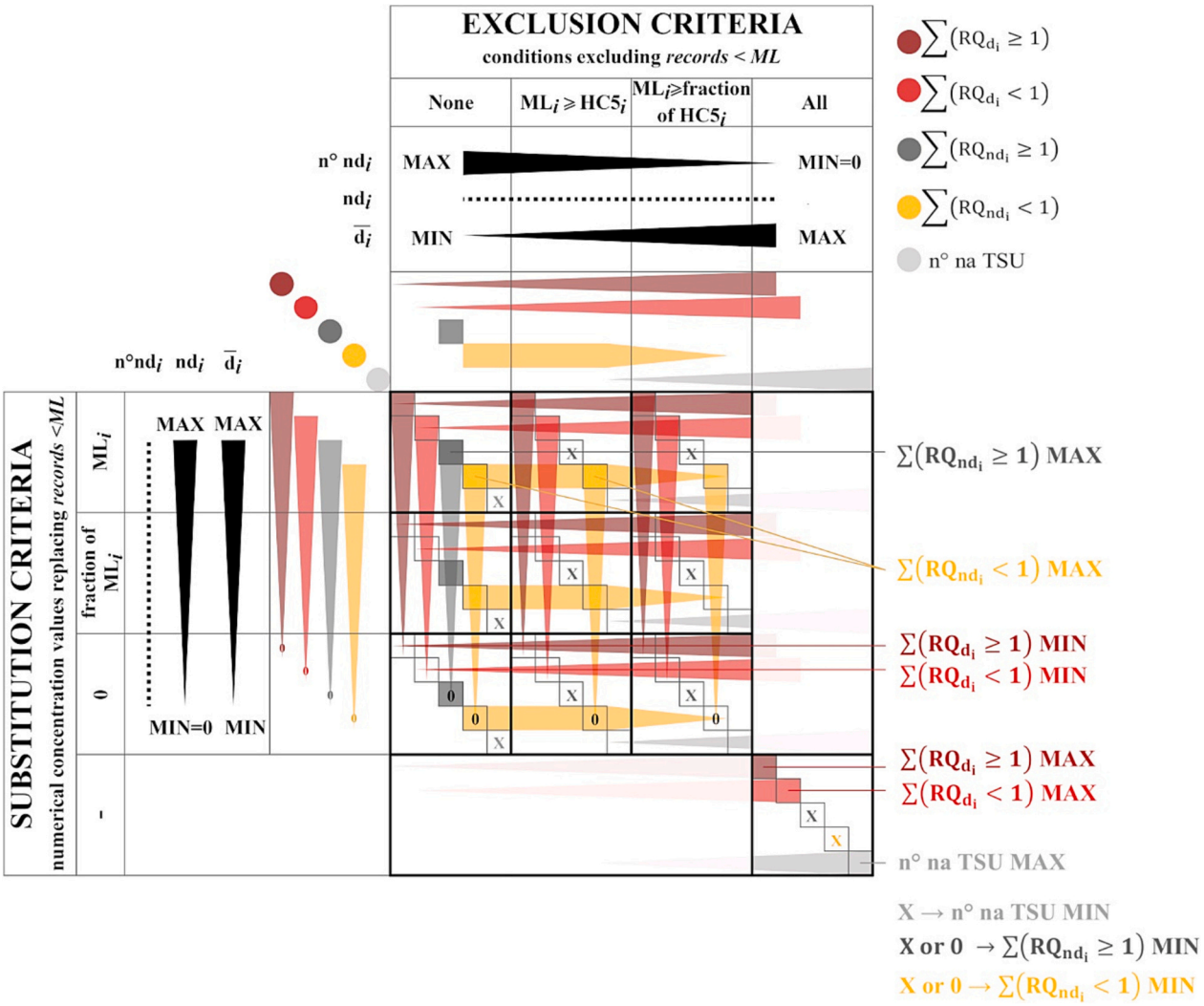

Fig. 2. Guidance Map. Map showing the relative magnitude of the *risk components* and number of non-assessable TSUs (n° na TSUs) in ten handling approaches applying increasingly selective criteria of exclusion and substitution of *records* < *ML*. The map assumes that each substance in a TSU has a unique ML value (*i.e.*, the mean concentration value assigned to *non-detects* (nd_i) corresponds to the concentration value chosen for replacing *records* < ML_i). In the two blocks dedicated to the exclusion and substitution criteria, the map shows: i) the pattern of variation of mixture elements which are sensitive to handling treatments of *records* < *ML* (*i.e.*, number of *non-detects* (n° nd_i), the concentration value assigned to *non-detects* (nd_i), and the average concentration of *detects* ($\overline{d_i}$)), ii) the resulting variation trends of the four *risk components* (*i.e.*, $\sum(RQ_{d_i} \geq 1)$; $\sum(RQ_{d_i} < 1)$; $\sum(RQ_{nd_i} \geq 1)$; $\sum(RQ_{nd_i} < 1)$) and n° of na TSUs. In the central grid, the map shows the relative magnitude of the *risk components* and n° of na TSUs for the ten approaches resulting from the combination of the overlying criteria (*i.e.*, graphically derived by the size of the overlapped area obtained by superimposing the variation trends of the *risk components* under the exclusion and substitution criteria separately). The map highlights the approaches where the highest and the lowest values for *risk components* and n° na TSUs are observed. Abbreviations: ML_i: analytical method limit of the substance *i*; $HC5_i$: hazard concentration safe for the 95 % of a species assemblage; n° nd_i: number of *non-detects*; $\overline{d_i}$: average concentration of the detected substance *i*; nd_i: numerical concentration value substituting *record* < ML_i; TT_i: toxicity threshold of the substance *i*; RQ_{d_i}: risk quotient of the detected substance *i*; RQ_{nd_i}: risk quotient of the non-detected substance *i*; n° na TSUs: number of non-assessable TSUs.

the pattern of variation of the four *risk components* and n° na TSUs across the 10 handling approaches. This variation pattern is visually depicted by the size of the overlapped areas obtained by superimposing the variation trends of the *risk components* and n° na TSUs under the exclusion and substitution criteria separately (intense colour).

The notes on the right side of the map identify in which handling approach the magnitude of each *risk component* and the number of na TSUs are biased at the maximum and at the minimum. As explained below, this information represents the foundation for identifying guidance criteria supporting the selection of the most suitable handling approach on a case-by-case basis.

The guidance for decisions of risk/no risk concern, as depicted in Fig. 3, addresses 4 default CBMRA assignments: a) decision of risk with the relative highest certainty, b) decision of risk under the worst-case scenario, c) decision of risk under the conservative scenario, and d) decision of no risk concern under the conservative scenario. For each decision setting, it specifies: i) the relevant *informed* risk notifications to consider, ii) the conditions underpinning the intended decision of risk/no risk concern (*i.e.*, *guidance criteria*), and iii) the handling approaches that implement these criteria (framed approaches in the map). The suite of reference CBMRA assignments is not exhaustive, and more specific scenarios can be addressed by users by extending its rationale.

Overall, Fig. 3 points out that the criteria underlying a planned decision may lead to either distant handling approaches (Fig. 3c, d) or approaches that could be considered questionable under common circumstances (Fig. 3a, b). On the latter aspect, for example, replacing *records* < *ML* with a true zero concentration usually demands strong weight-of-evidence arguments by assessors, as it can result in the underestimation of exposure to potentially sensitive organisms. Similarly, deleting all *records* < *ML* can lead to the loss of valuable information. In

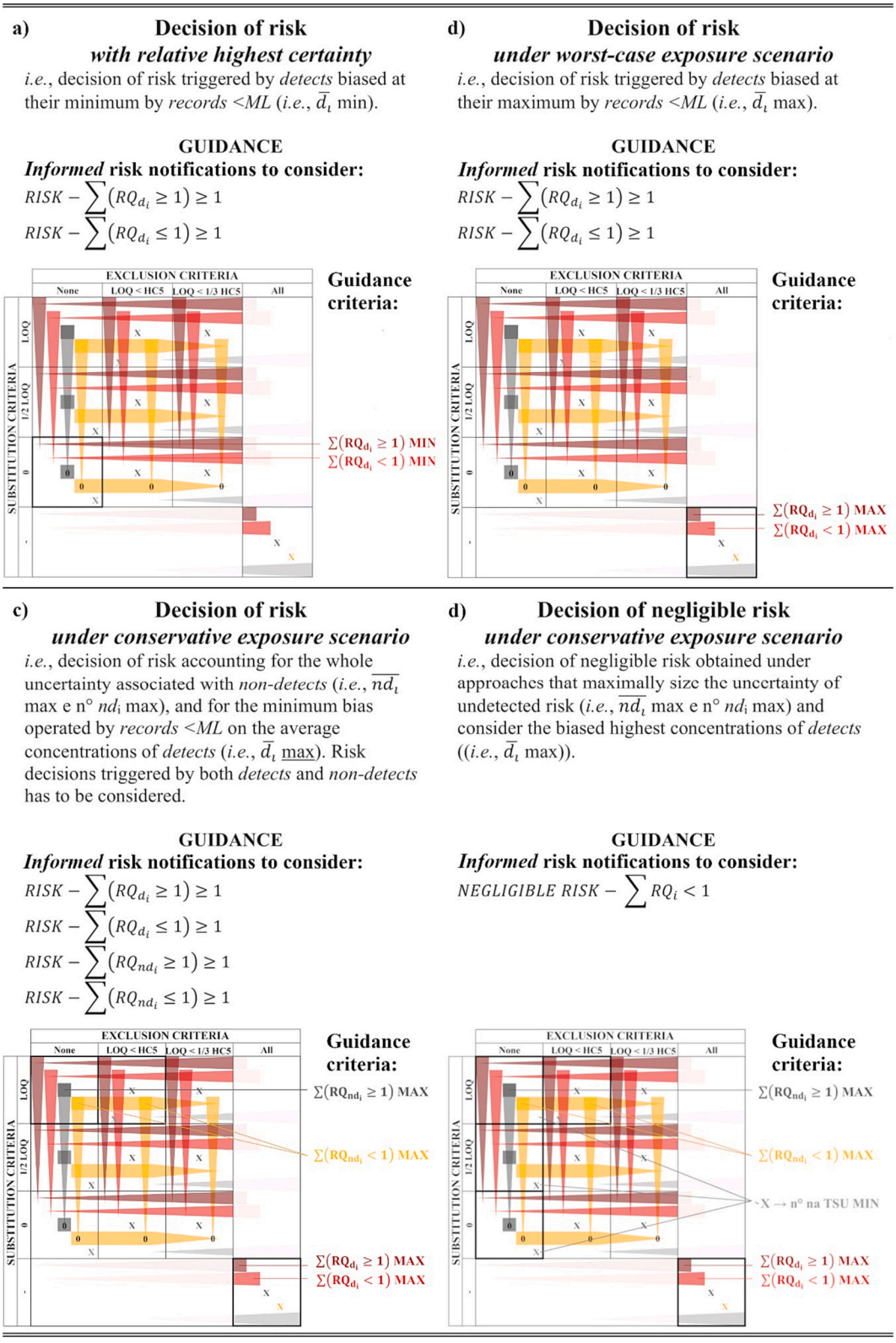

Fig. 3. Guidance criteria to select the approach for handling *records* < *ML* under default MRA assignments. The guidance provides instructions for i) identifying the *informed* decisions that require attention, ii) understanding the conditions that support these decisions (*i.e.*, guidance criteria), and iii) identifying the handling approaches that implement the identified criteria (framed approaches in the map). The guidance is provided with reference to the following MRA assignments: a) decision of risk with the relative highest certainty, b) decision of risk under the worst-case scenario, c) decision of risk under the conservative scenario, and d) decision of negligible risk under the conservative scenario). Abbreviations: LOQ: limit of quantification; $HC5_i$: hazard concentration safe for 95 % of a species assemblage; n° nd_i: number of *non-detects*; $\overline{d}_i$: average concentration of the detected substance *i*; nd_i: numerical concentration value substituting *record* < ML_i; TT_i: toxicity threshold of the substance *i*; RQ_{d_i}: risk quotient of the detected substance *i*; RQ_{nd_i}: risk quotient of the non-detected substance *i*; n° na TSUs: number of non-assessable TSUs.

fact, this procedure can turn mixtures that did not reveal hazardous contamination (*i.e.*, mixtures featuring only *non-detects* with $\sum RQ_{nd_i} < 1$) into non-assessable TSUs. Furthermore, this approach can lead to an unrealistic overestimation of the average concentrations of *detects* ($\overline{d_i}$), justifiable only under the requirement of worst-case scenarios.

Due to these limitations, the final decision should rely on "expert-evaluation", where assessors evaluate the obtained guidance on a case-by-case basis, considering additional factors (*e.g.*, the relative proportion of detected and non-detected risk in the dataset, the availability of additional information, level of realism, *etc.*). The disentanglement of the risk complexity into four distinct *risk components*, meaningful for

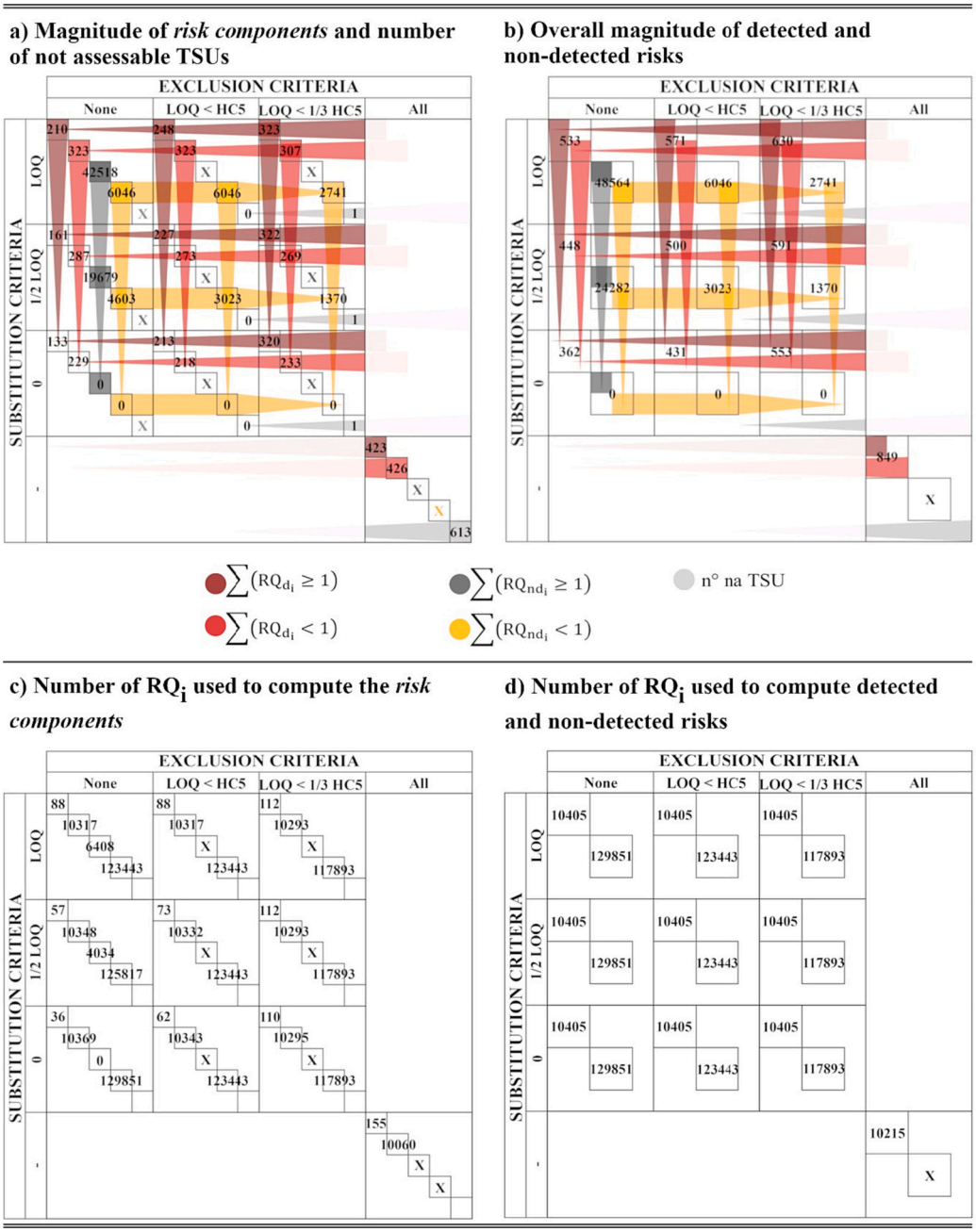

Fig. 4. Map validation. Magnitude of the *risk components* and number of non-assessable TSUs output by the *informed* CBMRA performed on the case-study dataset (LOQ-equalized) treated according to the ten target handling approaches. Information is provided at the dataset scale. Within each approach, the numbers along the diagonal correspond to: a) the magnitude of the four *risk components* (*i.e.*, $\sum(RQ_{d_i} \geq 1)$; $\sum(RQ_{d_i} < 1)$; $\sum(RQ_{nd_i} \geq 1)$; $\sum(RQ_{nd_i} < 1)$) and the number of na TSUs; b) the overall magnitude of detected risk (*i.e.*, $\sum RQ_{d_i} = \sum(RQ_{d_i} \geq 1) + \sum(RQ_{d_i} < 1)$) and non-detected risk (*i.e.*, $\sum RQ_{nd_i} = \sum(RQ_{nd_i} \geq 1) + \sum(RQ_{nd_i} < 1)$); c) the number of RQ_i used to compute each *risk component* (*i.e.*, $n^\circ(RQ_{d_i} \geq 1)$; $n^\circ(RQ_{d_i} < 1)$; $n^\circ(RQ_{nd_i} \geq 1)$; $n^\circ(RQ_{nd_i} < 1)$); d) the total count of detected and non-detected RQ_i (*i.e.*, $n^\circ RQ_{d_i}$; $n^\circ RQ_{nd_i}$). Validation is proved by the consistency between the trends outlined by the conceptual map and those indicated by the computed risk magnitude (a, b). Fig. 4d highlights that the approaches using the same exclusion criteria are based on the same sub-datasets (*i.e.*, $n^\circ RQ_{d_i}$ and $n^\circ RQ_{nd_i}$). Furthermore, it demonstrates that applying increasingly stricter exclusion criteria progressively reduces the $n^\circ RQ_i$, as visible for the non-detected *risk component* ($n^\circ RQ_{nd_i}$). The unchanged number of $n^\circ RQ_{d_i}$ across the approaches (with the exception of the approach eliminating all *records* < *LOQ*) has to be considered a haphazardness associated with the specific features of the case-study dataset. Abbreviations: LOQ: limit of quantification; HC5: hazard concentration safe for 95 % of a species assemblage.

uncertainty and risk type (*i.e.*, single substance *vs.* mixture), helps assessors identify the key elements that should influence the final risk decision, and thus the most appropriate handling option. Risk managers would take a similar approach, but in the opposite direction, to correctly interpret CBMRA outcomes. In fact, given the data handling pipeline, they would correctly evaluate the risk decision considering the uncertainty brought by the treatment applied for handling *records < ML*.

3.3. A perspective of the informed CBMRA application from the case-study

The *informed* CBMRA procedure was applied to the 2020 Italian freshwater pesticide dataset (LOQ-equalized) to achieve the following goals: 1) to provide a real-world interpretative context for the conceptual map and guidance criteria, 2) to compare the *informed* CBMRA outcomes obtained under the application of ten handling approaches, and 3) to critically evaluate the use of the map and criteria to guide the selection of the most suitable approach under specific MRA assignments. The following three subsections describe the evidence in response to these scopes. An example of the spreadsheet used for computation is provided in Supporting Information File 1. The environmental significance of CMBRA results is not commented on here due to being out of the scope of the present work, and further aspects, beyond the handling of *record < ML*, would have been considered for this purpose (*e.g.*, $HC5_i$ quality score, minimum number of samples to ensure meaningful annual concentration average, minimum number of chemicals to ensure meaningful mixture metrics) (Price et al., 2012).

3.3.1. Conceptual map validation

The magnitude of the *risk components* and the number of non-assessable TSUs (n° na TSUs) computed upon treating *records < LOQ* is reported in Fig. 4 according to the ten target handling approaches. More precisely, Fig. 4a reports the sum of RQ_i featuring each single *risk component* (*i.e.*, $\sum(RQ_{d_i} \geq 1)$; $\sum(RQ_{d_i} < 1)$; $\sum(RQ_{nd_i} \geq 1)$; $\sum(RQ_{nd_i} < 1)$), whereas Fig. 4b presents the same information in relation to the overall detected risk (*i.e.*, $\sum RQ_{d_i} = \sum(RQ_{d_i} \geq 1) + \sum(RQ_{d_i} < 1)$) and non-detected risk (*i.e.*, $\sum RQ_{nd_i} = \sum(RQ_{nd_i} \geq 1) + \sum(RQ_{nd_i} < 1)$). In support of this information, the numbers of RQ_i underpinning summations are reported symmetrically in the two maps below (Fig. 4c and d, respectively; see comments in the caption).

The conceptual map's proof-of-concept is demonstrated by the consistency between its relative trends and the risk magnitude values computed from the study, as detailed below.

Focusing on the detected *risk components*, Fig. 4b proves that their overall magnitude ($\sum RQ_{d_i}$) aligns with the map's trend. Correctly, this pattern is not visible when looking at the two detected *risk components* individually (Fig. 4a, *i.e.*, $\sum(RQ_{d_i} \geq 1)$ and $\sum(RQ_{d_i} < 1)$). This phenomenon can occur because, as more stringent exclusion criteria are applied, the "dilution effect" of *records < LOQ* on the average concentration of *detects* ($\overline{d_i}$) progressively reduces, affecting the relative proportion of the two detected *risk components* (*i.e.*, some $RQ_{d_i} < 1$ can turn into $RQ_{d_i} \geq 1$).

With respect to the non-detected *risk components* (*i.e.*, $\sum(RQ_{nd_i} \geq 1)$ and $\sum(RQ_{nd_i} < 1)$), Fig. 4a confirms that they are present when no *record < LOQ* is excluded, and they are absent when all such records are eliminated. Gradually stricter exclusion criteria in-between result in the elimination of $\sum(RQ_{nd_i} \geq 1)$ and in the reduction of $\sum(RQ_{nd_i} < 1)$. Fig. 4a also highlights a distinctive feature of the *informed* CBMRA in LOQ-equalized datasets: the two approaches substituting with full LOQ values i) all *records < LOQ* and ii) the ones that remain after removing those with LOQ ≥ HC5, yield the same $\sum(RQ_{nd_i} < 1)$.

Fig. 4b demonstrates that the choice of substitution has a significant impact on the magnitude of the non-detected risk. It highlights that the overall $\sum RQ_{nd_i}$ halves when halving the replacing concentration value (*i.e.*, from LOQ to ½ LOQ), and it zeros when substituting with zero. This phenomenon does not apply to detected *risks*.

Lastly, Fig. 4a indicates that the approach eliminating all *records < LOQ* results in the highest proportion of non-assessable TSUs, as predicted by the conceptual map.

3.3.2. Informed risk decision

A summary of the *informed* risk decisions obtained under the ten handling approaches is represented in Fig. 5. Each pie chart shows the proportion of the TSUs that were evaluated to be: i) unconcerned about the cumulative risk, ii) at risk according to the five risk notifications, and iii) not assessable (na) since they had less than two substances present. More detailed results are reported in Supporting Information File 1 (*i.e.*, risk evaluations assigned to each TSU under the 10 handling approaches).

Overall, the general outcome is aligned with the pattern anticipated by the map. Eliminating all *records < LOQ* resulted in a high proportion of na TSUs. As mentioned in Section 3.2, many of these na TSUs were assessed unconcerned about risk under other approaches (Supporting Information File 1), confirming the potential loss of valuable information when using this option.

When evaluating TSUs with no appreciable risk, the best scenario was obtained with the true zero substitution, whereas the worst scenario was obtained with the replacement of all *records < LOQ* with the full LOQ value.

Risk decisions triggered by *non-detects* were absent in the approaches that eliminated all *records < LOQ* or replaced them with zero. Single *non-detects* triggering risk decisions were present only when no *records < LOQ* were eliminated. The full deletion of these records in the approaches applying different elimination criteria reveals, conversely, situations of no concern or risk triggered by *non-detects* cumulatively (Supporting Information File 1). This outcome is a consequence of prioritizing *risk alerts* based on single substance exceedance over mixture exceedance such as in the proposed case-study (Fig. 1 and Table 2).

Using increasingly stricter exclusion criteria progressively reduced the proportion of TSUs deemed at risk in favour of negligible risk decisions.

3.3.3. Guidance for expert-evaluation

The case-study results according to the guidance provided in Fig. 3 for four representative CBMRA assignments are reported in Fig. 6. They highlight the handling approaches that match the criteria (framed approaches) and display the number of TSUs that respond to each target decision (numbers on the top-right corners). This guidance represents the foundation for assessors to make their expert-evaluation and select the handling approach that better fits the case-study's features, as exemplified below.

In the pesticide case-study, the high prevalence of *records < LOQ* in the dataset (97 %) is especially important when assessing risk and no risk concern in conservative exposure scenarios (Fig. 6c, d), where non-detected *risk components* influence the decision (*i.e.*, criteria: $\sum(RQ_{nd_i} \geq 1)MAX$; $\sum(RQ_{nd_i} < 1)MAX$). In such circumstances, substituting all *records < LOQ* with their full LOQ values is preferred to maximize consideration of undetected risk. In contrast, for risk assessments with higher relative certainty (Fig. 6a) or under worst exposure scenarios (Fig. 6b), where decisions rely solely on detected *risk components* (*i.e.*, criteria: $\sum RQ_{d_i}MIN$ and $\sum RQ_{d_i}MAX$, respectively), the prevalence of *non-detects* becomes irrelevant.

In the evaluation of risk with higher relative certainty, experts should ensure that there is sufficient evidence to support substituting values below LOQ with zeros to avoid underestimating risks, and consider the best alternative option, if needed (*e.g.*, substituting with half the LOQ value).

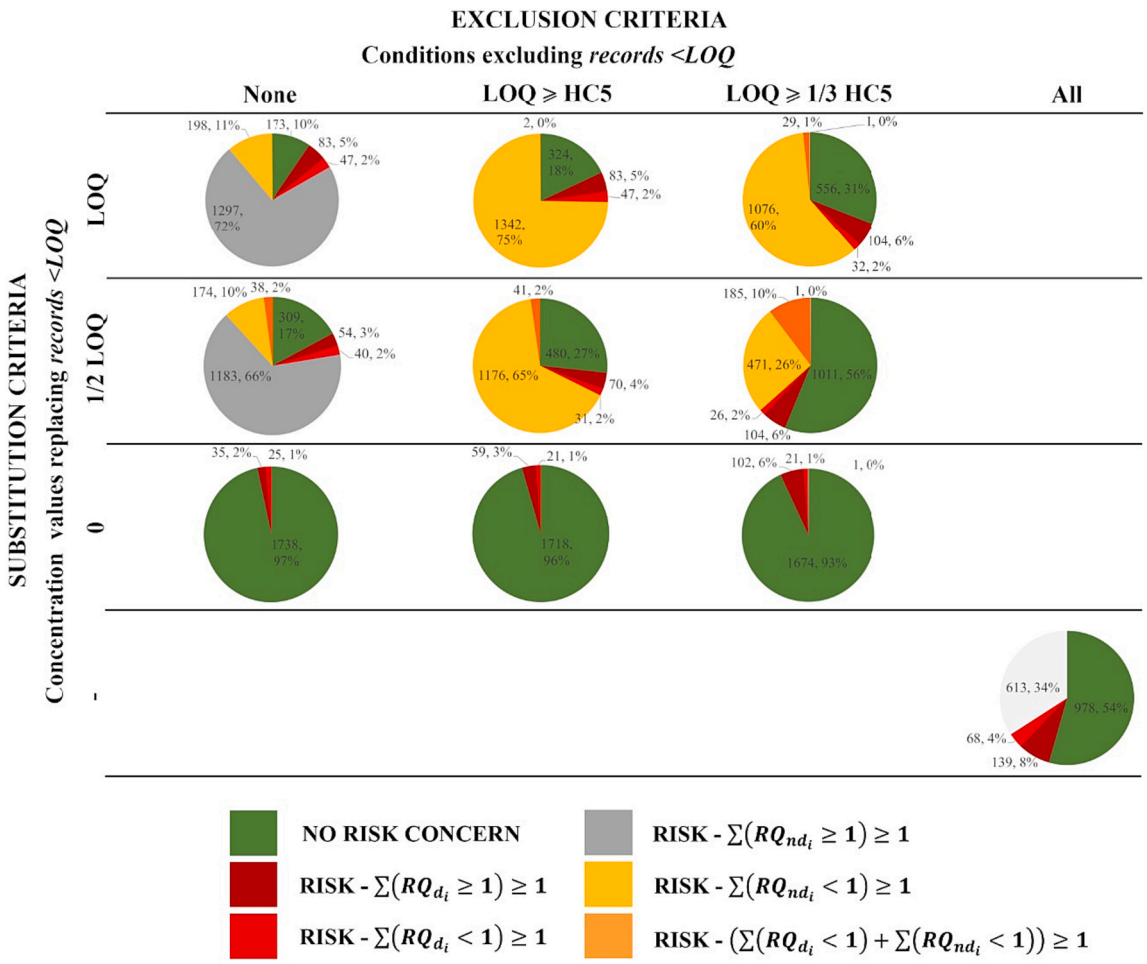

Fig. 5. Results of the *informed* CBMRA performed on the case-study dataset treated according to 10 approaches for handling *records* < *LOQ*. For each handling approach, the pie chart reports the number of TSUs, along with their relative percentages, evaluated as either posing negligible concern for cumulative risk, causing risk under the five uncertainty notifications, or being non-assessable. Abbreviations: LOQ: limit of quantification; HC5: hazard concentration safe for 95 % of a species assemblage.

4. Discussion

All in all, the case-study well-exemplified the significant impact that *records* < *ML* may have in real-world CBMRA and, in particular, the major uncertainty that can arise from data referring to substances always assessed below their LOD/LOQ values (*i.e.*, *non-detects*). In the daily practice of CBMRA, the number of *non-detects* can result in a high proportion because MEC dataset are often gathered from several chemical monitoring programs having distant scopes (*e.g.*, extensive investigative monitoring, surveillance, targeted diagnostic programs). Therefore, especially in the so-called "big data" case-studies, it is possible that the suite of monitored substances is excessive, and that many substances are monitored through routine analytical methods that are not powerful enough to determine whether the retrospectively selected toxicity benchmarks are exceeded. In the long term, it is likely that analytical methods with lower detection limits will reduce this source of uncertainty.

Through the joint application of the developed tools, the uncertainty associated with *records* < *ML* can be traced and kept to a minimum when interpreted at its best, but it cannot be eliminated. In fact, assessors, advised by the conceptual map and guidance criteria, can select the approach that minimizes the magnitude of this uncertainty based on the MRA question at hand and the available data. Furthermore, through the application of the *informed* CBMRA procedure, they can identify the cases (*i.e.*, TSUs) in which risk decisions are driven by *non-detects*, and therefore undeniably affected by higher uncertainty compared to *detects*-triggered risk decisions. This information can facilitate both risk assessors and managers in better focusing their efforts during the subsequent steps of the risk process. In fact, when additional information is available, enabling the acquisition of exposure data from modelling or the realistic evaluation of the likelihood of *non-detects*' presence (*e.g.*, chemical fingerprints for major anthropogenic sources in the area, background patterns of water bodies), assessors may attempt to refine the assessment. Similarly, risk managers who are aware of these aspects can avoid drawing misleading risk conclusions, and take better decisions.

To conclude, it is important to emphasise that the bias introduced by the approach for handling *records* < *ML* is just one of the causes of uncertainty in CBMRA. Other notable biases include missing data on toxicity and exposure, methods used for bridging data gaps, data curation and validation approaches, CBMRA assumptions, reference values, *etc.* (Hahn et al., 2014; Scharmüller et al., 2020). Therefore, to coherently tackle the overall uncertainty, CBMRA is usually performed within more structured frameworks, adopting the principle of a tiered analysis (Kortenkamp et al., 2018). According to these systematic tiering processes, the assessment commonly begins with simplified worst-case assumptions concerning chemical exposures and hazards, and progresses to the subsequent levels with step-wise refinements and more sophisticated concepts only if concerns are pointed out. Within these overarching frameworks, the tools developed in the present work can provide additional support for dealing with CBMRA complexity and interpreting results.

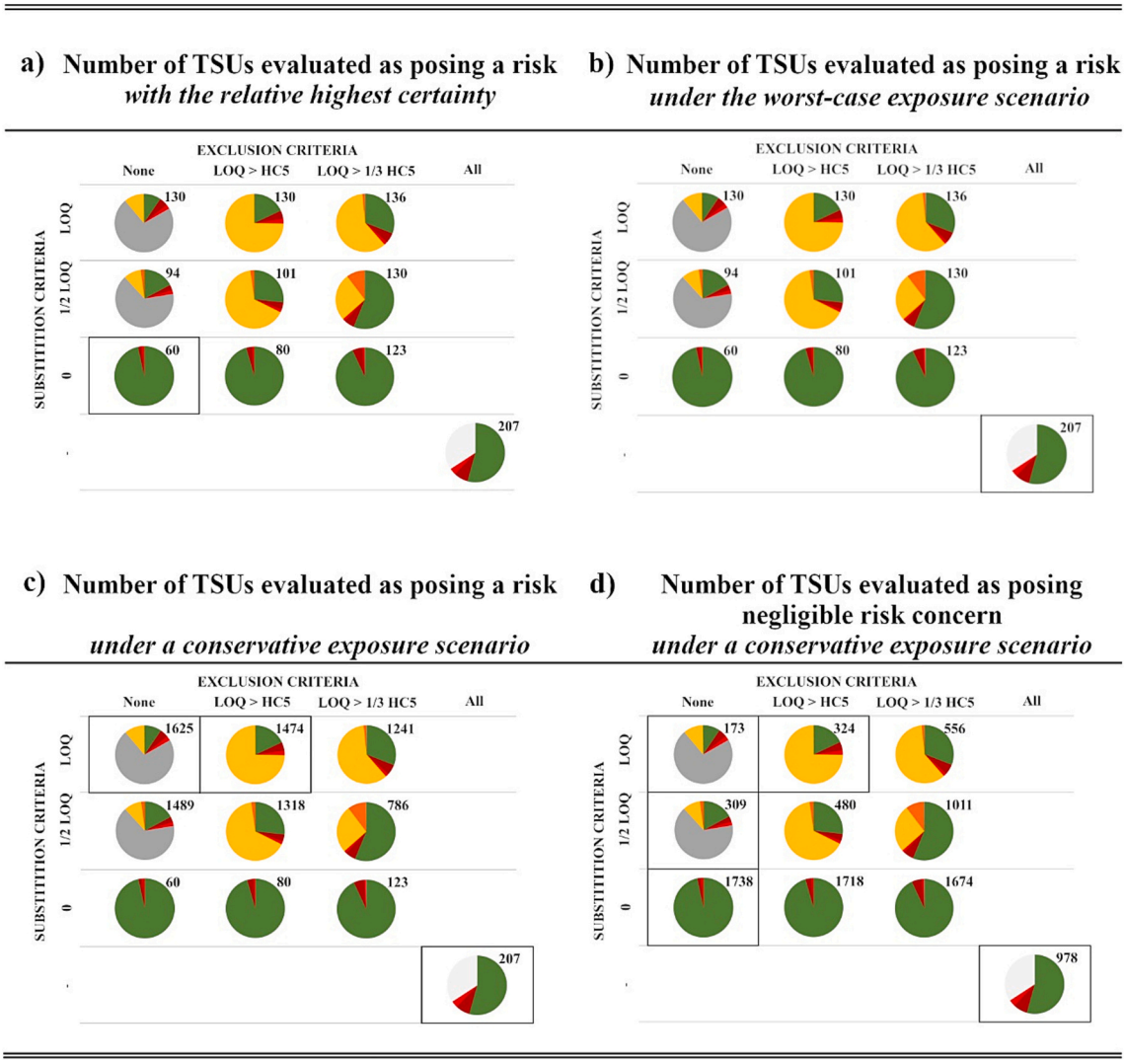

Fig. 6. Map-guided expert-evaluation of the results of the *informed* CBMRA performed on the case-study dataset treated according to ten approaches for handling *records* < *ML*. The case-study results presented in Fig. 5 are assessed here based on the four MRA assignments addressed in Fig. 3 a-d. The numbers in the top-right corners of each pie-chart indicate the TSUs meeting the intended decision. The frames highlight the handling approaches that meet one or more of the specific MRA assignment's guidance criteria. Abbreviations: LOQ: limit of quantification; HC5: hazard concentration safe for 95 % of a species assemblage.

5. Conclusions

This study focused on the impact of commonly used approaches for handling *records* < *ML* in the context of CBMRA and stressed the importance of working out and transparently reporting the choice of the handling method.

The developed *informed* CBMRA procedure is a useful addition to the *standard* CBMRA as it traces uncertainty in the final risk decision due to substances always assessed below their LOD/LOQ. The conceptualised map, in combination with the guidance criteria, can effectively support assessors in coherently accommodating the *informed* CBMRA to a variety of assessment situations. The obtained *informed* outcomes facilitate risk professionals to focus on single chemicals or co-exposures that are more likely to matter most and take effective measures. The present work made these tools ready for use in CBMRA, as demonstrated by their application to a real-world case-study. The tools are easy to implement and flexible for applications beyond the default approaches considered in the present work.

The utility of CBMRA approaches for obtaining a good approximation of the combined toxicity of mixtures is well recognised. Its use is in line with the holistic principles of contemporary water protection policies.

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CRediT authorship contribution statement

Seta Noventa: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Visualization, Writing-original draft. **Emanuela Pace**: Conceptualization, Resources, Data curation, Writing - review & editing. **Dania Esposito**: Conceptualization, Resources, Data curation, Writing - review & editing. **Giovanni Libralato**: Supervision, Writing - review & editing. **Loredana Manfra**: Conceptualization, Supervision, Project administration, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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