BTO 2024.025 | March 2024

Three new methods for refining risk characterization of chemicals in water

Assessment of less-than-lifetime risk, probabilistic risk, and human exposure

Joint Research Programme

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Three new methods for refining risk characterization of chemicals in water
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Management samenvatting

Drie nieuwe methoden voor de verfijning van toxicologische risicobeoordeling van waterrelevante stoffen

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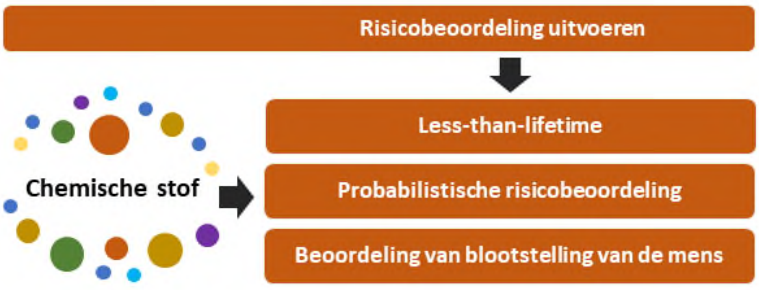
Er zijn drie nieuwe methoden ontwikkeld voor verschillende aspecten van de toxicologische risicobeoordeling in de context van waterkwaliteit. De 'less-than-lifetime'- of LTL-risicobeoordeling is geschikt voor het beoordelen van stoffen, vooral wanneer de concentraties gedurende een bepaalde periode veranderen. De PRA (probabilistic risk assessment) -methode helpt om een meer realistisch beeld te schetsen van de gezondheidsrisico's van chemische stoffen door de onzekerheden bij elke stap van de risicobeoordeling te beoordelen. Voor HEA (human exposure assessment) is een methode opgezet om de blootstelling van de mens aan chemische stoffen te beoordelen, inclusief blootstellingen die in het verleden hebben plaatsgevonden, die nu plaatsvinden of naar verwachting in de toekomst zullen plaatsvinden. Deze methode houdt ook rekening met verschillende blootstellingsroutes, zoals via drinkwater en huidcontact, en met verschillende leeftijdsgroepen. Het toenemende gebruik van antropogene stoffen in de samenleving vormt een belangrijke uitdaging voor de waterkwaliteit. Ondanks de vooruitgang in risicobeoordelingsmethoden ontbreken er elementen in de aanpak voor het evalueren van gezondheidsrisico's in verband met antropogene stoffen die aanwezig kunnen zijn in het watersysteem. De drie methoden die nu ontwikkeld zijn op basis van bestaande kennis en de nieuwste ontwikkelingen op het gebied van toxicologische risicobeoordeling kunnen daaraan bijdragen. De beschreven methoden maken gefundeerde besluitvorming mogelijk en ondersteunen geïnformeerde acties in de watersector. Hoewel de methoden in eerste instantie worden gepresenteerd voor de risicobeoordeling van individuele stoffen, kan toekomstig onderzoek op dit gebied worden uitgebreid om methoden te ontwikkelen voor de beoordeling van mengsels van chemische stoffen, waarmee een kritieke kennislacune in de huidige risicobeoordelingspraktijken wordt aangepakt. Aangezien er de komende jaren nieuwe ontwikkelingen worden verwacht op het gebied van toxicologische risicobeoordeling, is het aan te raden om deze voortdurend te volgen om op de hoogte te blijven van nieuwe wetenschappelijke methoden en kaders.



Belang: veranderende situatie en inzichten steeds meenemen in beoordeling chemische risico's

De chemische waterkwaliteit verandert voortdurend onder invloed van veranderende milieu-omstandigheden, menselijke activiteiten en uiteenlopende blootstellingsroutes. Er komen steeds nieuwe verontreinigende stoffen in het milieu en de wetenschappelijke inzichten in de daardoor veroorzaakte gevaren en de risicobeoordelings-methoden ontwikkelen zich steeds verder. Dit vormt een aanzienlijke uitdaging voor de toxicologische risicobeoordeling: er is voortdurende verfijning nodig

van risicobeoordelingsstrategieën in een drinkwatercontext. Traditionele risico-beoordelingsmethoden zijn vaak niet in alle situaties inzetbaar, waardoor hiaten ontstaan in de beoordeling van mogelijke gezondheidseffecten. Drinkwaterbedrijven en -laboratoria en overheden hebben behoefte aan een eenduidige aanpak en betere risicobeoordelingsmethoden die hen helpen de gezondheidsrisico's door blootstelling aan chemische stoffen via drinkwater nauwkeurig te evalueren. Dit vraagt om methoden die rekening houden met verschillende blootstellingsscenario's en onzekerheden in de risicobeoordelingen.

Aanpak: literatuuronderzoek, bestaande kennis en recente ontwikkelingen verwerkt in methoden

Voor dit onderzoek is informatie over bestaande kennis en recente ontwikkelingen in de toxicologische risicobeoordeling verzameld. Er is literatuuronderzoek uitgevoerd om relevante risicobeoordelingsmethoden te identificeren, gebaseerd op collegiaal getoetste data uit (inter)nationale (meta)databases en websites en rapporten van gerenommeerde instituten en autoriteiten voor de bescherming van de menselijke gezondheid, waaronder de Wereldgezondheidsorganisatie (WHO), het Amerikaanse Environmental Protection Agency (US EPA), de Europese Autoriteit voor Voedsel en Veiligheid (EFSA), het Europees Agentschap voor chemische stoffen (ECHA), Public Health England (PHE), het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) en andere relevante databases. De kennis is verwerkt in nieuwe beoordelingsmethoden die zijn getest in casestudies.

Resultaten: LTL-, PRA- en HEA-methoden om risico's rond waterkwaliteit te beoordelen

Er zijn drie nieuwe methoden ontwikkeld voor verschillende aspecten van de toxicologische risicobeoordeling in de context van waterkwaliteit. De 'less-than-lifetime'- of LTL-risicobeoordeling is geschikt voor het beoordelen van stoffen, vooral wanneer de concentraties gedurende een bepaalde periode veranderen. De PRA (probabilistic risk assessment) -methode helpt om een meer realistisch beeld te schetsen van de gezondheidsrisico's van chemische stoffen door de onzekerheden bij elke stap van de risicobeoordeling te beoordelen. Voor HEA (human exposure assessment) is een methode opgezet om de blootstelling van de mens aan chemische stoffen te beoordelen, inclusief blootstellingen die in het verleden hebben plaatsgevonden, die nu plaatsvinden of naar verwachting in de toekomst zullen plaatsvinden. Deze methode houdt ook rekening met verschillende blootstellingsroutes, zoals via drinkwater en huidcontact, en met verschillende leeftijdsgroepen. In de toekomst kan deze methode op basis van verder onderzoek worden uitgebreid om ook de blootstelling aan mengsels van chemische stoffen te beoordelen en daarmee een kritieke kennislacune in de huidige risicobeoordelingspraktijk aan te pakken.

Toepassing: complexe vragen over risico's rond waterkwaliteit beter beantwoorden

De nieuwe methoden maken een gerichte aanpak voor risicobeoordeling mogelijk en bieden een beter inzicht in de potentiële bedreigingen van chemische stoffen in water. Door rekening te houden met verschillende blootstellingsscenario's en kwetsbare bevolkingsgroepen, stellen deze methoden drinkwaterbedrijven in staat om hun besluitvorming te funderen en op maat te maken. Gebruik van de nieuwe methoden zorgt voor een realistische interpretatie van potentiële risico's van chemische stoffen in water, waardoor drinkwaterbedrijven prioriteiten kunnen stellen en daarop kunnen handelen (bijvoorbeeld binnen risicogebaseerde monitoringprogramma's). Samen kunnen deze methoden drinkwaterbedrijven helpen de veranderende uitdagingen op het gebied van waterkwaliteit voor te blijven, naleving van de regelgeving te garanderen en het vertrouwen in de chemische waterkwaliteit te bevorderen. Omdat ook de komende jaren nieuwe ontwikkelingen worden verwacht rond toxicologische risicobeoordeling, is het aan te raden deze te blijven volgen om op de hoogte te blijven van nieuwe wetenschappelijke methoden en kaders.

Rapport

Dit onderzoek is beschreven in het rapport: Drie nieuwe methoden voor de verfijning van toxicologische risicobeoordeling (BTO 2024.025).

Meer informatie
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General Introduction

The increasing production and use of chemicals is resulting in an increased number and concentration of contaminants in (drinking) water sources, which pose considerable challenges to (drinking) water quality. An important task for water companies and regulators is to ensure that (drinking) water quality does not compromise public health and confidence in water safety. A key component of this effort is toxicological risk assessment which evaluates the safety of chemicals that may be present in water systems. Although there has been considerable progress in risk assessment methods, a clear approach to the risk assessment of (drinking) water-relevant chemicals is currently lacking. Building on our previous BTO report describing a workflow for assessing potential hazards to human health from drinking water contaminants (Baken et al., 2018), this follow-up project presents the methods for health risk assessment under different exposure scenarios and focuses specifically on addressing uncertainties in risk assessment. To develop these methods, we used the existing knowledge and incorporated new developments in toxicological risk assessment. An extensive literature search was conducted to identify relevant risk assessment methods, drawing from peer-reviewed and (inter)national (meta)databases and websites or reports published by renowned institutes and authorities for human health protection including the World Health Organisation (WHO), US Environmental Protection Agency (USEPA), European Food and Safety Authority (EFSA), European Chemical Agency (ECHA), Public Health England (PHE), and the Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

The report is structured into different sections. The first section (chapter 1) presents the method for assessing the less-than-lifetime (LTL) exposures, particularly applicable for substances with varying concentrations in (drinking) water over time. The framework presented is a decision tree that assists experts in determining whether a measured or predicted LTL exposure level of a chemical may lead to adverse health effects. The second section (chapter 2) includes a chapter on the probabilistic risk assessment (PRA), providing a realistic understanding of the potential risks associated with water contamination. PRA assesses the variability and uncertainty at each step of the health risk assessment, enhancing credibility in interpretating threats to human health from potential (drinking) water contaminants. The third section (chapter 3) presents the method for human exposure assessment (HEA) in a retrospective and prospective scenario. The method considers different ways through which humans may be exposed to chemicals in water, including drinking and skin contact. The aim is to provide a more accurate evaluation of health risks, considering different vulnerable population groups such as children and adults. A subsequent section (chapter 4) provides a general discussion of methods introduced in the report. The report concludes with a final section (chapter 5), presenting three case studies (Appendices I – III), corresponding to preceding chapters (chapter 1-3). These case studies illustrate the application of the proposed approaches in realistic scenarios, thereby enriching the understanding and applicability of the approach outlined in the report. In addition, this chapter includes information on external developments (Appendix IV) that may be relevant to the broader context of water quality and human health. Finally, reflection and look-ahead has been included (Appendix V), which provides a review and foresight of the methods presented in the main report. This section assists in preparing for emerging topics and the relevance of the proposed methodologies in light of evolving scientific developments.

The methods presented in this report can be used in specific cases depending on the nature of the (drinking) water-related questions. These methods can be used by risk assessors as a tool to improve the understanding of human exposure to contaminants in drinking water, and inform on risk-based monitoring. In addition, these methods allow for customised investigations and responses leading to a more nuanced view of water quality and its implications on public health. An important area for future investigation is development of a risk assessment methodologies for aggregated exposure and exposures to complex chemical mixtures that may be of concern for water companies. In addition, development of methodology for measuring internal exposure doses of water relevant contaminants is considered a priority for future research, as the present study focused exclusively on external doses. This will ensure a more accurate characterisation of health risk and development of targeted risk reduction strategies for specific exposure scenarios.

1 Less-than-lifetime risk assessment for drinking-water quality

1.1 Introduction

People are exposed to chemicals in many ways, which can lead to adverse health effects. Chemicals can be released into the environment from a variety of sources, including agriculture, domestic use, and industries (Golovko et al., 2021; Villanueva, 2014). Once released, these chemicals may disperse into the air, water, soil, and food and may come in contact with people through inhalation, ingestion, or dermal contact. The number and concentrations of chemicals detected in (drinking) water sources are increasing due to increased production, use, longer periods of reduced river flows due to climate change and improved detection techniques (Béen et al., 2021; Sjerps et al., 2017). This poses greater challenges to water quality. Populations at extra risk, including populations that are more sensitive or having a relatively high exposure (e.g., unborn embryo or foetus (exposure via mother), infants, children, elderly, patients) are more susceptible to harmful effects of some contaminants. In the European Union (EU), risks of chemical substances are assessed by sector (e.g., pesticides, pharmaceuticals, and industrial chemicals), and the assessment systems for these sectors differ, resulting in different risk assessment methods. A key factor for this is that the information required for different frameworks varies. To harmonise the risk assessment process, the EU has proposed the "one substance - one assessment" approach as part of the Green Deal and Zero - Pollution targets (Escher et al., 2022; van Dijk et al., 2021). There are many factors that influence whether a contaminant can have health effects via drinking water exposure, such as, the type of contaminant, its concentration in the water, individual susceptibility of exposed persons, the amount of water consumed, frequency and the duration of exposure. The duration of exposure is an important and critical element in the assessment and estimation of risks to human health (de Oliveira et al., 2021; Schwela, 2014). Continuous steady-state life-time exposure to a constant exposure level of contaminant(s) is generally not realistic (Amachree et al., 2013; 2014). In our previous report (BTO 2018.030), we presented a workflow for assessing potential human health hazards and/or health risks from drinking water chemical contaminants (Baken et al., 2018). The health effects due to less-than-chronic (or less-than-lifetime) exposure may differ from the effects of chronic (or lifetime) exposure, therefore, evaluation of these short-duration exposure risks may require different approaches. The aim of this research is therefore to develop a general framework for dealing with chemical exposures via (drinking) water that correspond to exposure scenarios, referred to here as less-than-lifetime (LTL) exposures. In line with the implementation of other existing quantitative methods related to drinking water quality (human exposure assessment, probabilistic assessments and the application of new toxicological models and test systems), this project provides a risk assessment framework to better evaluate any potential health risks resulting from LTL exposures. A case study (refer to Appendix I) is incorporated to illustrate the practical application of the proposed framework.

1.2 Less-than-lifetime risk assessment

A health risk assessment is an important process aimed to estimate the nature and probability of adverse health effects in individuals potentially exposed to chemicals in contaminated environmental mediums, either presently or in the future. In this study, the terms “LTL risk assessment” refers to evaluating the risk posed by chemicals to human health may be exposed (via drinking water) for a period shorter than a lifetime which is generally estimated to be 70 years. Such exposures, termed LTL exposure, include intermittent (not continuous) or fluctuating (irregular) exposures, occurring for acute/very short (1-14 days), short (more than 14 days to 1 year) or intermediate durations (more than 1 year to 7 years) (see Figure 1). The suggested choice of exposure durations is based on the framework for the LTL risk assessment of carcinogens developed by Felter et al., (2011) and are intended only as examples. Intermittent exposure could be short-term, intermediate, or chronic, with the main characteristic being that the exposure is discontinuous (Felter et al., 2011). More precisely, an intermittent exposure can be defined as an exposure separated by a sufficiently long duration so that the steady state of exposure is not maintained (equilibrium

of concentrations between the external environment and the body compartments is not achieved). Such irregular exposure patterns may potentially alter the body's response mechanism. Determination of potential risk(s) to human health from chemical exposures generally relies on *in vivo* (animal experimental data), *in vitro* and/or in some cases relevant human data (experimental and/or epidemiological) over different time periods (Goeden et al., 2018; PHE, 2019; EFSA 2021). Classical risk assessment methodologies prioritise chronic duration studies as the primary source of toxicity information for prolonged, including lifetime exposures, such as drinking water consumption. These long duration (continuous) studies provide sufficient time to manifest the adverse effect of exposure (see Table 1 for types of toxicological studies). Values based on such chronic exposure are considered protective for shorter, therefore less-than-lifetime (LTL) exposures (Felter et al., 2011; Geraets et al., 2016; Goeden et al., 2018).

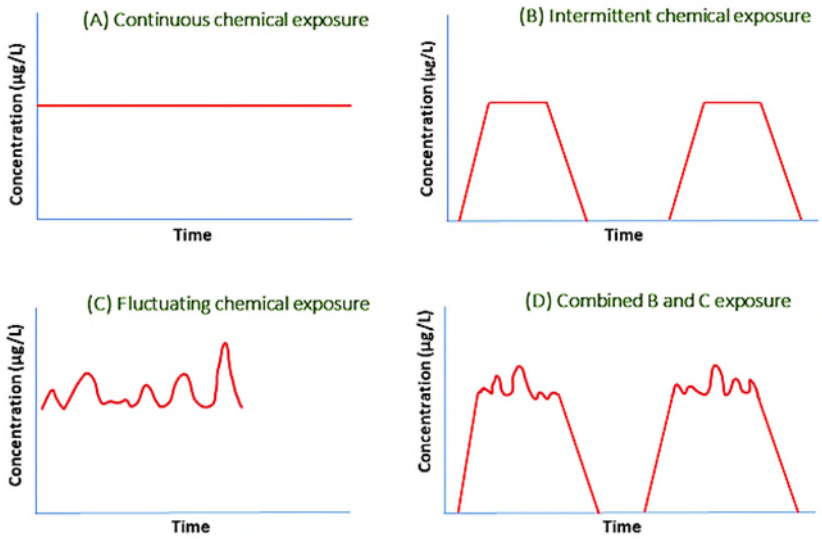

Figure 1. Different chemical exposure categories: *(A) continuous, (B) intermittent, (C) fluctuating exposure and (D) a combination of intermittent and fluctuating exposure (adapted from: Geraets et al., 2016).*

When assessing the risks associated with chemical exposures, consideration should be given to the relevance of chronic health-based guidance values (HBGVs) for LTL exposure patterns. HBGV is a science-based recommendation for the maximum (oral) exposure to chemical substance (exhibiting threshold of toxicity) that is not expected to result in an appreciable health risk (considering the existing safety data, uncertainties in data and the likely exposure duration) (EFSA, 2021). Important among HBGVs is the Acceptable Daily Intake (ADI) (expressed as mg/kg/day), which is the estimate of "safe" levels of intake of a chemical substance (food additives, pesticide residues and veterinary drugs) via food or drinking water (EFSA, 2021; Gray, 2023; Herrman & Younes, 1999). In addition, reference dose (RfD) and tolerable intakes (TI), such as, Tolerable Daily Intake (TDI) and Tolerable Weekly Intake (TWI) are the other commonly used HBGVs. These guidance values typically rely on experimental animal data and are adjusted using safety factors (uncertainity factors) to account for inter-species, inter-individual variability, and the lack of strong evidence. The RfD, utilised by the US Environmental Protection Agency (USEPA) is an alternative to ADI which sometimes results in lower values for acceptable intakes. The procedure for establishing RfDs is somewhat more detailed than for ADI and includes the use of additional modifying factors ranging from 1-10 (based on professional judgement). (Barnes & Dourson, 1988; Watts & Teel, 2014; retrieved from USEPA 2023). RfDs incorporate additional safety factors to account for hypersensitivity reactions and extrapolation from experimental animal data to humans (Barnes & Dourson, 1988; Watts & Teel, 2014). These have become a widely used indicator of chronic toxicity and have been established for oral and inhalation routes. TDIs are used for substances that are not expected to be found in food (as opposed to ADI for substances such as additives, pesticide residues or veterinary drugs which are expected to be found in food).

Table 1. *Different types of toxicological studies used for testing chemicals, including pesticides or active substances for crop protection and industrial chemicals.*

Type of Study	Duration	Animal species	OECD Test Guideline
Long-term	Major portion of lifespan, including: 24 months exposure durations (18 months for specific mice strains).	Rodents (rats and mice)	OECD TG 451, OECD TG 453
Chronic	Long duration (12 months) or major portion of lifespan, (18 or 24 months) or shorter (6 or 9 months)	(mainly) Rodents (rats and mice)	OECD TG 452, OECD TG 453
	9 months	Non-rodents (dog, beagle; other species: swine, mini-pigs)	OECD TG 452 combined with OECD TG 409, with appropriate modifications outlined in OECD Guidance Document No. 116
Sub-Chronic	Part of lifespan Intermediate duration (90 days)	Rodents (preferably rat) Non-rodents (dog, beagle; other species: swine, mini-pigs)	OECD TG 408 OECD TG 409
	Short-term (Sub-acute) (28 days)	Rodents (preferably rat)	OECD TG 407
Acute	Part of lifespan	Rodents (preferably rat)	OECD TG 420, OECD TG 423, OECD TG 425

In addition to chronic exposure studies, acute exposure studies are used to determine acute reference dose (ARfD) (USEPA, 2006), which may be used as a basis to develop short term exposure values (STEV) (Leusch et al., 2020). Such short term or acute exposure limits are particularly relevant for LTL exposure scenarios involving relatively high acute or short-term exposures of chemicals e.g., in situations to deal with emergencies involving accidental or intentional chemical release or other catastrophic events that are single and non-repetitive. For guidance on setting an ARfD we recommend the readers to refer to the publications of Solecki et al. (2005). STEVs are not suitable for all chemicals, especially those that pose serious health risks. Therefore, STEV should only be developed for chemicals for which relevant drinking water guideline is based on acute toxicity (Leusch et al., 2020). The World Health Organisation (WHO) has developed an approach to derive short-term health-based guidelines for chemical in case of emergency situations using existing toxicity data (such as ARfD) and allocating relative source contribution 100% to drinking water in the short term (WHO, 2017a). Similar methods have also been used by the USEPA and UK Water Industry Research (UKWIR) to derive short-term guideline values. These limits can be used to assess the severity of the event, determine potential consequences, and decide what protective measures should be taken. In advance of an uncontrolled release, these limits can also be used to assess the consequences and plan a response (DOE, 2016). The decision on whether it is necessary to establish an acute reference limit for a chemical of concern is based on the hazard profile of a substance and on specific endpoints that may be particularly relevant to the effects of acute exposure (Bos et al., 2010; WHO/IPCS, 2020). The decision as to whether the application of an ARfD is necessary should be based on the hazard profile of a chemical, as well on specific endpoints which may be particularly relevant to acute effects, such as, irritation of skin, eye, mucous membrane/gastro-intestinal tract, or mucous membrane/respiratory tract) (ECHA, 2013; Solecki et al., 2005).

HBGVs are used to establish allowable levels of contaminants in water (and food) for the protection of public health. There are also some chemicals (such as residues of pesticides, veterinary medicines, or biocidal products) for which the

Maximum Residue Limit[1] (MRL) can be used. MRL is the maximum amount of residue that is legally tolerated (regulatory limit) in or on food and animal feed when applied correctly (Good Agriculture Practice) and is expected to be without any health concerns. MRLs are derived for individual products. Summation of the total dose from all the products is compared with an HBGV (e.g., an ADI) to verify whether MRLs may lead to health risks or not. If no substance specific MRL is available, a default MRL for pesticides of 0.01 mg/kg is used (EU Commission, 2008).

In case of drinking water health-based statutory drinking water standards, called Guideline Values (GLVs) are in place for a limited number of chemicals known to appear in drinking water. GLVs are established to ensure the safety of drinking water supplies. Main information sources on drinking water standards and guidelines are provided in Table 2. Often, for chemicals that emerge in surface and groundwater, drinking water guideline levels have not yet been derived (e.g., due to uncertainty in database, calculated value is below the level that can be achieved through treatment methods, or the value is below the detection level). For such chemicals a provisional or indicative guideline value may be applied for interim assessment (WHO, 2022). If any LTL exposure to a chemical is below a chronic health-based guideline value, no acute or chronic effects are expected. However, if LTL exposure exceeds the chronic health-based guideline value but is below the acute reference value, acute effects can be ruled out but not any (sub)chronic effects. Therefore, in a (drinking) water context, LTL risk assessment of chemicals is especially relevant in the circumstances if the chronic health-based guideline values are exceeded for a certain period of time. For example, lead is rarely found in source waters, but can enter drinking water if lead containing plumbing materials corrode (especially if the water is high in acidity or low in mineral content). As such, there is no safe level of Pb exposure (Flora et al., 2012; Vorvolakos et al., 2016; WHO, 2022), but in such situations where lead leaches from the material in contact with water, people may be (temporarily) exposed to lead for what is considered LTL (see case-study in Appendix I). Studies have shown that the concentration of chemical substances in (drinking) water sources is subject to variations (Chen et al., 2010; Sjerps et al., 2017; Yang et al., 2017), which may result in periods of higher concentrations alternating with periods of lower concentrations. For example, the (short) periods of extremely low river discharge may temporarily result in higher chemical concentrations in drinking water sources (due to reduced dilution of point source) than under normal conditions (Sjerps et al., 2017; ter Laak, 2018), and thus potentially higher exposures during periods as short as 3-months, making LTL risk assessment also relevant for such situations and possible corresponding exposure scenarios. An approach to LTL risk allows assessing potential health risks from LTL exposures which are currently not often considered (BTO2018.030). This can also help the (drinking) water utilities as well as government and authorities in deciding what and if any control measures need to be taken to ensure protection of human health This is because the occurrence of such chemicals is only at concentrations far below those that would be of health concern or there are inadequate data to establish a formal guidance value.

In 2011, Felter et al., proposed a framework that provides a guidance on factors that should be considered in cancer risk assessment decisions for LTL exposures based on available toxicity and exposure data (Felter et al., 2011). In addition, several chemical safety guidance documents for biocides, veterinary medicines, cosmetics, and industrial chemicals, address the issue of fluctuating or intermittent exposures, but a clear-cut approach on dealing with such exposures, particularly in the (drinking) water context is currently lacking (ECHA, 2012a; ECHA, 2012b; ECHA, 2012c; ECHA, 2015; EMA, 2010; Geraets et al., 2016; SCCS, 2012).

[1] The accepted MRL and/or maximum permitted concentration for most individual pesticides in drinking water is 0.1 µg/L. This is not a health-based standard; it is based on the limit set by the European Commission in 1980 to reflect the limitations of analytical techniques at that time and as an environmental policy measure to limit pesticides in general. The Directive also set a standard of 0.5 µg/l total pesticides (the sum of all the substances detected in a sample). Stricter separate health-based standard of 0.030 µg/L exist for four organochlorine pesticides (aldrin, dieldrin, heptachlor and heptachlor epoxide) (Eu Commission, 2020).

1.3 Approach

To identify relevant risk assessment methods and other relevant information for the LTL risk assessment, a literature search was performed to complement the KWR knowledge base on risk assessment[2]. A search was conducted to identify both peer-reviewed and (inter)national (meta)databases and websites or reports published by renowned institutes and authorities for human health protection including the World Health Organisation (WHO), US Environmental Protection Agency (USEPA), European Food and Safety Authority (EFSA), European Chemical Agency (ECHA), Public Health England (PHE), Rijksinstituut voor Volksgezondheid en Milieu (RIVM). The terms "short term exposure + chemicals + oral", "risk assessment + less-than-lifetime", "sub-chronic toxicity + health risk", "low-level chemicals + drinking water" + short term exposure + intermediate exposures" were used during the search. The relevant literature was summarized, placed in water relevant context, and discussed with LTL risk assessment experts. Finally, the framework was developed and applied to LTL risk assessment using exposure to lead (Pb) as a case study in a (drinking) water context.

1.4 Implementation of LTL risk assessment in drinking water quality

Here we propose a framework for assessing the health risk of chemicals in LTL exposure scenarios for drinking water quality using existing quantitative methods. The framework presented is a decision tree that helps water quality and risk experts determine whether a measured, or predicted LTL exposure level may lead to adverse health effects. The framework consists of four steps (see Figure 2). The first step is to identify chemicals in drinking water. Additional information could be sought for similar and/or the co-occurring chemicals. The second step describes the assessment of exposure. The assessment of potential hazard of contaminants identified in drinking water is described in the third step. In this step, a method for assessing both carcinogenic and non-carcinogenic effects is presented based on the framework proposed by Felter et al (2011), in combination with principles developed by Public Health England (PHE, 2019) and Geraets et al. (2016) and Baken et al (2018). These studies are chosen as they are the most relevant sources we found in literature and as they can be applied to the chemicals in the (drinking) water context. The final step of the framework is risk characterisation and uncertainty analysis. A case study is included to illustrate the practical application of the recommended framework in a drinking water context.

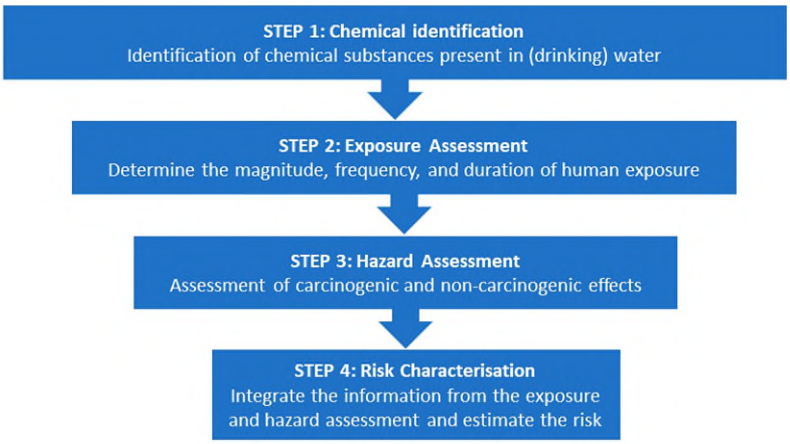

Figure 2. *Workflow of the framework for LTL risk assessment of chemicals in (drinking) water.*

[2] Tools for human health risk assessment of emerging chemicals, BTO 2018.030; Human health impact of chemical contaminants in drinking water – usefulness of the DALY concept, BTO 2011.044; Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water; Visie op mengseltoxiciteit in Drinkwater, BTO 2008.009; Toxicological relevance of emerging contaminants for drinking water quality, BTO 2009.022.

Step 1: Identification of drinking-water contaminants

The LTL risk assessment method for drinking-water contaminants begins with identification of chemicals detected in drinking water and associated health risks (see Step 2). Without identification, the toxicological/health risk assessment is not possible. In our previous study, we developed a workflow for the evaluation of potential human health hazards and/or health risks of drinking water contaminants (Baken et al., 2018). In this study, we additionally present the methods for the identification of chemicals in (drinking) water. The most straightforward method for identifying potentially hazardous chemicals is through sampling and chemical analysis. Two types of analysis can be applied to identify chemicals in (drinking) water; targeted and non-targeted analysis (Hollander et al., 2019; Wang et al., 2022). In targeted analysis, known chemicals are analysed based on the reference standards for identification and quantification. However, targeted chemical analysis alone is no longer sufficient because of the multitude of micropollutants present in water, and advanced approaches such as non-target analysis can be applied. Non-targeted analysis allows for the simultaneous detection and characterization of a wide range of (unknown) chemicals, including chemicals of emerging concern (CECs) (Hinnenkamp et al., 2021; Pourchet, 2020). However, non-target screening (NTS) is most often only qualitative in nature, meaning that concentrations of the compounds are not assessed. NTS is therefore often used as screening technique. From the detected compounds a selection of those judged most relevant (based on hazard) can be made, that need to be determined quantitatively. Since many different types of contaminants may be present in the water distribution system, a case-by-case approach may be required for accurate qualitative and quantitative analysis of the selected chemicals (Zulkifli et al., 2018). Parent chemical compounds can be degraded in the environment and during drinking water treatment, producing degradation products that may be of smaller, similar or higher toxicity as that of the parent compounds (Li & Mitch, 2018) and the formation of degradation products should therefore be considered explicitly in exposure assessment.

Step 2: Exposure assessment for LTL scenario

Exposure assessment is a critical step in risk assessment process and includes estimation (modelling) or measurement of chemical concentration in the relevant medium (drinking water) and estimation of the duration of exposure (Locey, 2005) (Figure. 4). If the exposure turns out to be LTL and the estimated or measured concentration of chemical of concern lies within the health-based drinking water standards/limits (GLVs) no LTL risk assessment is required. Whereas, if the exposure levels exceed the GLVs for the given LTL exposure duration, LTL risk assessment can be conducted. When calculating exposure based on measured/modelled data, three types of calculations can be obtained (USEPA, 2003): the average daily dose (ADD) (when the contaminant is known to cause non-carcinogenic or non-chronic effects), the lifetime average daily dose (LADD) (when the contaminant is known to cause carcinogenic or chronic effects), and the acute dose rate (ADR). The difference between these three exposure measures is the averaging time (AT). In case of ADD, AT is set to the exposure duration over which exposure has occurred. In case of LADD, the average duration is set to a lifetime even though the exposure does not occur over the entire lifetime (average 70 years). ADR is also calculated using the same equation as ADD and LADD, but AT is equal to one day. Toxicity may primarily depend on peak exposure concentrations (Figure. 3), which may be concentration or dose driven [i.e., independent of duration (but must be maintained for a minimal time) or frequency of occurrence) (Haber et al.,2016). Therefore, to evaluate toxicity, it is important to determine whether the toxicity is due to concentration of the chemical during peak exposure periods or the total dose (i.e., concentration multiplied by time). Depending on whether the concentration or the total dose is responsible for driving the adverse effect, dose averaging may be chosen over the exposure period of interest in combination with an evaluation of the peaks. The peaks may be evaluated by comparison with an ARfD, if available. If no ARfD is available, some pragmatic approach may for instance be used depending on the factor by which an HBGV for longer exposure duration has been exceeded. Dose averaging requires averaging exposure periods with non-exposure periods, hence potentially underestimate the actual health risks associated with the chemical. If peak exposure is relevant or reoccurring, we suggest using dose averaging in an LTL exposure scenario by considering only the peak exposure and assuming that it remains the same over the entire exposure period. This can be used as a starting point for risk assessment to ensure that actual health risks are not

underestimated. In the present example (see case study), we describe the LTL exposure assessment in a fluctuating exposure scenario. The method for estimating the exposure rate is described in TEXT BOX 1.

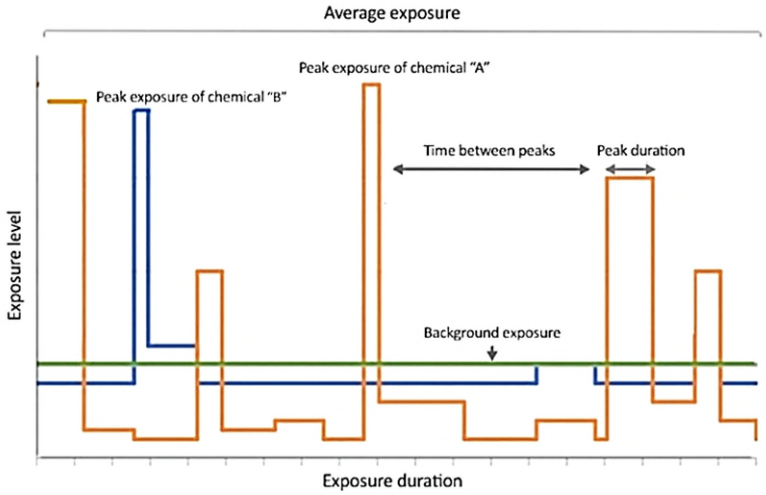

Figure 3. *Fictional peak exposure of chemicals (orange and blue peaks) of chemicals relative to background exposure (green line).*

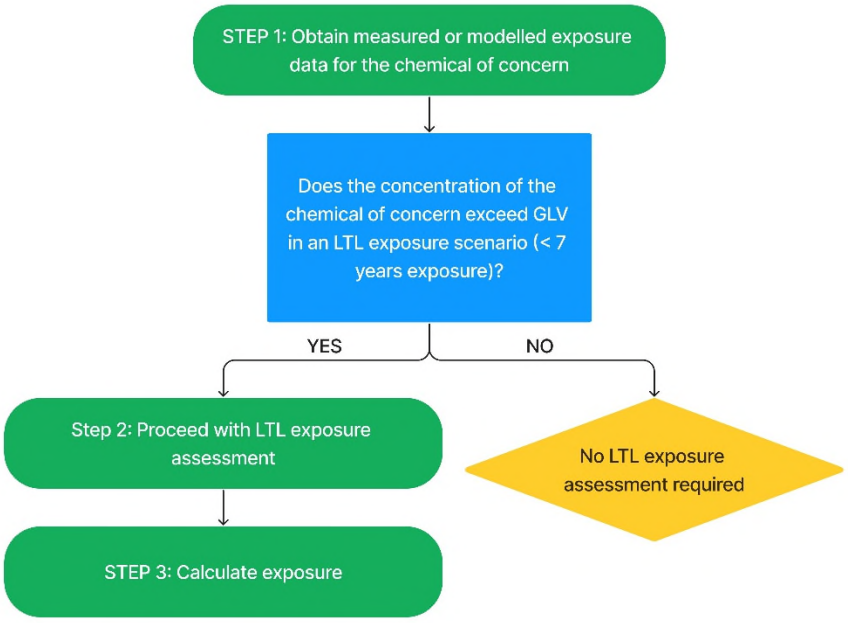

Figure 4*. Workflow for LTL exposure assessment.* ***Green*** *= Start and end of steps;* ***Blue*** *= Process;* ***Yellow*** *= Decision.*

During exposure assessment, whole populations can be considered, including all life stages in one calculation. However, consideration should be given to specific life stages of the exposed individuals (infants, toddlers, children, elderly). Some age groups may have higher susceptibility (e.g. the unborn and the elderly) and should be considered separately in the risk assessment. Attention should be paid to whether the exposure is retrospective or prospective (see chapter 3). A retrospective risk assessment involves determining the individuals who have been exposed to the chemicals of concern already released in the environment (e.g., during an accidental release), and a prospective risk assessment involves determining the population that are undergoing exposure or are anticipated to be exposed in the future (Borghi et al., 2020; OECD, Test No. 296; PHE, 2019). The prospective approach is typically used for

regulatory purposes and can be aimed at risk mitigation and choice of risk management options (Borghi et al., 2020). In addition, several other considerations must be taken into account to estimate exposure, such as considering the likeliness of cumulative exposure (i.e., combined exposure to multiple chemicals through different exposure pathways over a period of time). This is particularly important in assessing drinking water quality, as simultaneous exposure to multiple low-level chemicals from drinking water is common (WHO, 2017a). The method for the assessment of chemicals in a combined or aggregate exposure scenario is out of the scope of present study.

TEXT BOX 1

The Averaging Time (AT), used in calculating ADD is usually different for non-carcinogenic and carcinogenic risk estimates. For non-carcinogenic chemicals, the average exposure during the contact with a chemical is generally the relevant exposure duration for risk assessment [e.g., AT = Exposure Duration (ED) * Exposure Frequency (EF)]. When actual dose is being calculated, for example in case of acute exposure AT = ED (e.g., one day) (USEPA, 2003). For cancer risk assessment, the average duration is set to a lifetime, which is usually assumed to be 70 years (70 x 365 = 25,550 days) in risk assessments (WHO/IPCS, 2021). The difference is based on the different mechanisms for cancer and non-cancer effects. For cancer, it is based on the idea that all exposures add up and manifest any time in lifetime. For non-cancer effects recovery may occur in between exposure periods in case of intermittent exposures hence the doses of multiple exposure periods cannot be added up.

$$\mathbf{ADD}_{\mathbf{oral}} = \frac{\mathbf{C_W \times IR \times EF \times ED}}{\mathbf{Bw \times AT}}$$

Where:

ADD	*= Average daily dose (intake) [mg/kg bw-day]*
Ingestion	*= amount of substance consumed via oral intake*
C_w	*= Concentration of target chemical in water [mg/L]*
IR	***=** Ingestion rate of water [default intake = 2L/day adult (70 kg bw), 1L/day children (12 kg bw)*
EF	*= Exposure frequency i.e. number of exposure events over the length of time [days/week or year]*
ED	*= Exposure duration is the length of time over which exposure occurs [days or weeks or years]*
BW	*= Body weight [kg] [default BW = adult (70 kg), children (12 kg), bottle-fed infants (5 kg)]*
AT	*= Averaging time (days) is the period of time over which the exposure is relevant for health risk characterization*

Step 3: Hazard assessment of chemicals identified in drinking water in LTL scenario.

Exposure assessment should be followed by the toxicological assessment to identify potential risks to human health and to set priorities for monitoring and abatement (Baken et al., 2018). In an LTL exposure scenario, the toxicological assessment of chemicals can be conducted from the available scientific data (e.g. epidemiological, animal and in – vitro studies) on chemicals of concern. If effect data from epidemiological studies, or experimental animal studies (or in some cases in-vitro studies) are incomplete or unavailable, non–testing (in silico) approaches may be used to assess hazards. Such tools do not provide safe exposure levels but are useful for rapidly identifying potential hazards, prioritising compounds for further testing, and providing mechanistic information (Baken, 2018). Nevertheless, expertise is needed to select non-testing tools (on case-by-case basis) and to perform and evaluate hazard prediction, where often multiple non-testing approaches should be combined to obtain the best prediction of toxicity (BTO 2014.009; BTO 2018.030). A tiered approach to LTL risk assessment of carcinogenic and non-carcinogenic chemicals is presented in figure 5 and 6. The proposed methodology attempts to address specific issues related to quantitative aspects of risk assessment for LTL exposure to chemicals in drinking water and provides guidance on factors that should be considered in risk assessment decisions.

Table 2. *Information sources for drinking water standards and guidelines values (source Baken, 2018).*

Legal standards	Drinking water decree (Netherlands)	*https://wetten.overheid.nl/BWBR0030111/2015-11-28*
	Regulation of materials and chemicals for drinking and hot tap water supply (Netherlands)	*https://wetten.overheid.nl/BWBR0030279/2017-07-01*
	EU Council Directives	*https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:01998L0083-20151027*
	USEPA Drinking Water Regulations	https://www.epa.gov/dwreginfo/drinking-water-regulations
	OEHHA California Public Health Goals	*https://oehha.ca.gov/water/public-health-goals-phgs*
Guideline values	Australia Drinking Water Guidelines	https://www.nhmrc.gov.au/about-us/publications/australian-drinking-water-guidelines
	WHO Drinking-water quality guidelines	https://www.who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/water-safety-and-quality/drinking-water-quality-guidelines
	USEPA Drinking Water Regulations	https://www.epa.gov/dwreginfo/drinking-water-regulations
	USGS Health-Based Screening Levels	https://www.usgs.gov/programs/environmental-health-program/science/usgs-health-based-screening-levels-available-online
	Health Canada	https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality/guidelines-canadian-drinking-water-quality-summary-table.html
	National Institute for Public Health and the Environment (Netherlands)	https://rvszoeksysteem.rivm.nl/

1.5 Hazard assessment of carcinogenic effects from LTL exposure

Carcinogens are substances or agents capable of causing cancer or increasing the incidence of cancer in humans or experimental animals (Schrenk, 2018). Carcinogens can be classified as non-genotoxic or genotoxic based on their MoA. Non-genotoxic carcinogens are known to induce tumours by disrupting cellular structures and by altering the rate of either cell proliferation or processes that increase the risk of genetic error (Hartwig et al., 2020; Nohmi et al., 2018; Lee et al., 2013). Genotoxic carcinogens cause tumours by directly affecting the genetic material (e.g. DNA, chromosomes). Such substances can induce gene mutations, structural chromosome mutations and genome mutations (Hartwig et al., 2020; Nohmi et al., 2018). If the carcinogenic MoA is not identified, then from the precautionary perspective the carcinogenic substance is usually assumed to be a genotoxic carcinogen (EFSA, 2005). The threshold for a carcinogen is the exposure level below which there is no increased risk of cancer. There is a general consensus among scientists and regulators about acceptance of on increased cancer risk of one in a million (10^{-6}) (USEPA, 2005). An increased lifetime cancer risk of one in one million ($\leq 1 \times 10^{-6}$ or less) is acceptable and is generally not considered a significant public health concern. The cancer risk levels are policy decisions of health authorities and are 1-in-100,000 ($\leq 1 \times 10^{-5}$) according to WHO and 1-in-1,000,000 ($\leq 1 \times 10^{-6}$) in the Netherlands. A risk level of 1 in a million implies a likelihood that up to one person, out of one million equally exposed people would develop cancer if exposed to the specific chemical concentration continuously (24 hours per day) over 70 years (an assumed average lifetime). For non-genotoxic carcinogens, it is generally assumed that a threshold exists, because the mechanism leading to carcinogenesis has a lowest “effect level”. In contrast, for genotoxic carcinogens, it is generally assumed that there is no threshold and that even a very low dose can contribute to adverse effects, therefore, a “no effect level” (i.e. no observed adverse effect level or NOAEL) cannot be derived (Hartwig et al., 2020; SciCom, 2018).

Step 1: Refer to step 1 of method section of this document; “Identification of drinking-water contaminants”.

Step 2: Refer to step 2 of method section of this document; “LTL exposure assessment”.

Step 3: For assessing the carcinogenic effects, the first step should be to determine if carcinogenicity data are available for the chemical of interest. If chemical-specific data on the carcinogenicity of compound are available, a cancer risk assessment can be performed for that compound. Mechanistic and toxicokinetic data should be used. The use of mechanistic data (e.g., the studies on the interactions of the chemical of interest with cellular macromolecules) can lead to a better selection of critical effects, identification of a susceptible population, increased confidence in hazard identification, elucidate the relevance of animal data to humans, susceptibility, mode of action, and/or support biological plausibility linking a chemical exposure to an adverse outcome such as carcinogenicity. Toxicokinetic data which include a description of the rates of distribution of chemicals in tissues, allow for better interpretation and prediction of the fate of chemicals in the body (Vandenberg et al., 2010). However, toxicity and kinetic data are often lacking (Knepper, et al., 2020; Kolkman et al., 2021). In such cases, data gaps can be filled (whenever possible) by using read-across and structure-activity relationships (Q)SAR models (Felter et al., 2011). After evaluating the available data and confirming that carcinogenesis is the most relevant endpoint for risk assessment, consideration should be given to relevant MoA by which the chemical (and degradation or transformation products if any) contributes to tumour formation. Determining whether the data supports a non-linear (non-genotoxic /threshold carcinogens) or linear dose (genotoxic / non-threshold carcinogens) - response relationship dependent on the criteria set in the risk assessment requires an understanding of MoA. For illustration, the criteria to decide on linearity are provided in Table 3. When a specific MoA is established, workflow described under Step 3A should be followed. The risk assessment of non-genotoxic carcinogens should be performed by using or deriving a health-based guidance value (HBGV), and by applying appropriate UFs wherever possible (if a lifetime HBGV has been established for the carcinogenic effect, then that should be used, and if no lifetime HBGV is available, then that should be derived that fits the LTL exposure period and used). The preferred point-of-departure (POD) for the HBGV is the benchmark dose (BMDL10). However, if BMDL10 is not available, the NOAEL [or Lowest-observed-adverse-effect level (LOAEL)] with appropriate (UF) may be used. Appropriate UFs should be chosen to reflect differences in toxicokinetics and toxicodynamics between animals and humans and between humans, and default UFs applied may vary because of interindividual variability of population sub-groups (pregnant women and their foetuses, new-borns, young children, or elderly). If data are insufficient to establish a HBGV, a margin of exposure (MOE) approach may be used based on the most appropriate POD and taking uncertainty into account. The MOE is the ratio of POD (preferably the BMDL10) obtained from animal toxicology studies to the predicted, and/or estimated human exposure level. It should be noted that the use of HGBV or MOE based on long-term toxicity studies may be considered precautionary when applied to short-term LTL scenarios. If the LTL exposure scenario being evaluated indicates a higher exposure than the HBGV, refinements of risk assessment may be considered appropriate, e.g., visualising uncertainty in the exposure and toxicity data (e.g. by using Risk21 software; this is not covered in this study). In addition, the use of a shorter-term study to establish a short-term HBGV may be appropriate. There are often mixed MoAs, as well as dose-dependent transitions in MoA, which contribute to the complexity of understanding the potential for a linear or nonlinear dose-response in the low-dose range (Slikker et al, 2004, Felter et al., 2011). Although some regulatory agencies assume of a “linear, no threshold” model for mutagenic carcinogens, this assumption is increasingly being challenged, as studies indicate nonlinearity of mutagenic compounds (Lutz, 2009). We follow the developments in science and formal guidances by competent authorities in our assessments.

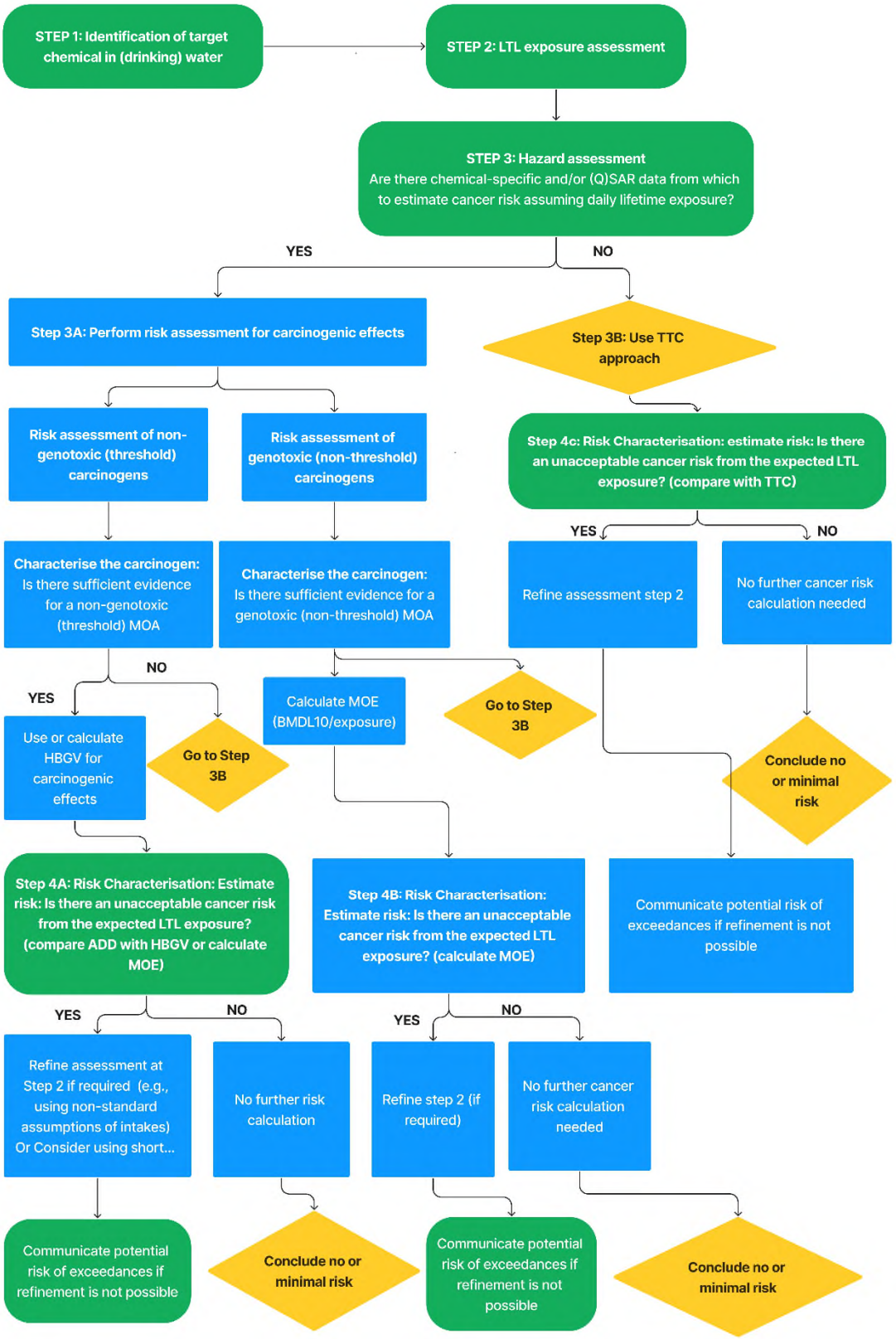

Figure 5: Flow chart for assessing risk from LTL exposure to carcinogenic chemicals in drinking water. *The scheme is based on the framework proposed by Felter et al (2011) for assessing risks from LTL exposures to carcinogens, combined with the principles developed by Public Health England (PHE, 2019).* ***Green*** *= Start and end of steps;* ***Blue*** *= Process;* ***Yellow*** *= Decision.*

If a cancer risk cannot be estimated for the chemical, then a default approach such as the threshold of toxicological concern (TTC) may be considered (Baken & Sjerps, 2016). The TTC is a pragmatic, science-based method for assessing low-level exposure to chemicals with limited chemical-specific toxicity data and unknown toxicity (EFSA, 2019; Ellison et al., 2021). It was developed to screen and prioritise the risk assessment of substances with known chemical structures (with or without structural alerts for genotoxicity and for cancer and non-cancer endpoints) for which human oral exposure can be estimated to be relatively low. If exposure exceeds the relevant TTC value, case-by-case considerations should be applied. Some authors suggest that higher TTC values should be set for short-term and less-than-lifetime than for chronic exposures in pharmaceutical contaminants (EMA, 2006; Muller et al., 2006), cosmetics (Kroes et al., 2007), and trace chemicals with structural alerts for genotoxicity (Felter et al., 2009, 2011). In an expert workshop, it was recommended that LTL TTC values require the development of a specific database for acute or other LTL toxicity (EFSA & WHO, 2016a). Such LTL TTC values could be very useful for LTL risk assessment and are a starting point for additional research (out of scope of current study). If exposure to a chemical is below the TTC value, the chemical can be considered as having a low probability of risk with reasonable confidence (Ellison et al., 2021; Munro et al., 1996). TTC levels and TTC based Drinking water guideline values are given in Table 5 (Baken et al., 2018).

Table 3: *Criteria for linear and non-linear MoA (adapted from: Villanueva et al., 2014; Ceritti et al., 2016; Kirkland et al., 2016).*

Mode of Action	Non- Threshold or Linear (genotoxic)		Threshold or Non-Linear (non – genotoxic)
Description	Cause tumors by:		Cause tumors by:
	▪ directly acting on DNA (these are reactive and do not require metabolic conversion)	▪ indirectly acting on DNA (require metabolic activation and conversion in the body to reactive chemicals)	▪ indirectly affecting structures of DNA or gene expression to alter chromosomal number/integrity. (e.g., peroxisome proliferators, hormones, and local irritants)
Mechanism	Interaction at a specific location or base sequence of the DNA structure causing lesions, breakage, fusion, deletion, mis-segregation or non- disjunction leading to damage and mutation		Cytotoxicity, receptor-mediated endocrine modulation, non-receptor mediated endocrine modulation, tissue-specific toxicity and inflammatory responses, immunosuppressants, or gap junction intercellular communication inhibition, altered methylation, oxidative stress.
In – silico test result	Structural alert: positive		Structural alert: negative
in - vitro test result	Ames test: positive		Ames test: negative
In – vivo test result	Carcinogenic in both rats and mice and in more than one organ		Carcinogenic in single species and single organ in rodents
Some examples of chemicals in (drinking) water	Acetaldehyde, Acrylonitrile, Aromatic amines, Azo dyes, AflatoxinB1, Bromate, Benzo(a)pyrene, Nitrosamines, Dimethylbenzanthracene, Insecticides, Fungicides, Trichloroethylene, Formaldehyde, Trichloroethylene		Bisphenol A (BPA), Cadmium, Diethanolamine, Dieldrin

Table 4: *Existing UFs used for deriving HBGV (Adapted from: Bercu et al., 2021; ECHA 2012c).*

Classification	TTC (µg/day)	Reference	TTC-based drinking water target value (µg/L)	Reference
Cramer class I (low toxicity)	1800	*Munro et al. 1996*	37.7	*Baken & Sjerps, 2016*
Cramer class II (medium toxicity)	540	*Munro et al. 1996*	-	
Cramer class III (high toxicity)	90	*Munro et al. 1996*	4.0	*Baken & Sjerps, 2016*
Organophosphates and carbamates	18	*Kroes et al. 2004*	-	
Carcinogens	1.5	*TOR rule ('80)*	0.1	*Mons et al. 2013*
Genotoxic substances *(except aflatoxins, azoxy- or N-nitroso compounds*	0.15	*Kroes et al. 2004*	0.01 0.02	*Mons et al., 2013* *Baken & Sjerps, 2016*
(-) No values were calculated. The threshold values were only derived for compounds that were relevant to drinking water. These substances mostly shared structural similarity with Cramer classes I and III and genotoxic compounds.				

Table 5. *TTC levels and TTC based Drinking water guideline values (Source: Baken et al., 2018).*

Source of uncertainty	Default safety factor
In the absence of chemical-specific data on kinetics and/or dynamics	Default UF = 100 (10 for inter-species variability x 10 for intra-human variability)
If available and relevant, remaining components, for which data are not available	inter-species variability in toxicokinetics = species dependent (e.g., 4.0 for rats and 7 for mice) inter-species variability in toxicodynamics = 2.5 intra-human variability in toxicokinetics = 3.16 intra-human variability in toxicodynamics = 3.16
Deficiencies in the data available for the assessment	Getting additional data to improve the quality of the dataset is recommended. If additional data cannot be obtained or requested, use additional justified UF on a case-by-case basis
Accounting for the absence of a No-Observed-Adverse-Effect-Level (NOAEL)	case-by-case basis
Severity and nature of the observed effect	case-by-case basis
Conversion factors for administration of test substances in drinking water	no recommendations for conversion factors for toxicity studies where the substance is administered in the drinking water

1.6 Hazard assessment of non - carcinogenic chemicals from LTL exposure

Step 1: Refer to step 1 of method section of this document; "Identification of drinking-water contaminants".

Step 2: Refer to step 2 of method section of this document; "LTL exposure assessment".

Step 3: For assessing the non-carcinogenic effects of LTL exposure, the first step should be to determine whether there is chemical-specific toxicity (acute, short-term, sub-chronic) and/or QSAR data from which to estimate human

health risk. If the data are available, step 3A should be followed. Here, the first step is to determine whether a reference value (RfV) [health-based guidance value (HBGV)] is available for the given exposure scenario, or for a scenario that is similar to that on which the reference values are based. If an RfV for a sufficiently similar exposure is not identified, the next step should be to determine whether the study that forms the basis for the RfV is similar in duration to the scenario. If the study that forms the basis for the RfV is based on a duration similar to that in the scenario of interest, the RfV can be modified to remove adjustments that were made to extrapolate from the original toxicity study. For example, if the RfV included an UF for extrapolation from a subchronic to chronic study. In that case, a RfV relevant to a subchronic exposure can be used by removing the UF for subchronic to chronic extrapolation. If no such study is available, the next step is to compare it to the toxicity study of relevant exposure duration and apply uncertainty factors (UF). If the reference value is modified by eliminating the UF, it is important to consider whether another endpoint could become the critical effect for the duration of interest. When no chemical-specific toxicity and/or QSAR data are available, step 3B should be followed. In order to determine whether a comparison of exposure should be made only to a short-term reference value or also to a long-term value, both the toxicokinetics and toxicodynamics of the chemical should be considered. This is to determine whether the chemical (or its degradation product) of interest is likely to persist due to low elimination relative to the interval between exposure periods (or episodes), and, secondly, whether the compound is sufficiently persistent to metabolism to accumulate between exposure episodes (Haber et al., 2016). If either determination results in a positive result, the exposure must be compared to a reference value that applies to the entire exposure period. In order to estimate whether the chemical would accumulate in the body, the elimination half-life of the chemical or its active metabolite from the body can be used (Haber et al., 2016). According to Haber et al., (2016), a chemical is considered non- accumulating if the interval between successive exposure episodes is at least five elimination half-lives (if elimination mechanisms are not saturated). If the elimination of the chemical (or its metabolite) is known to be biphasic or multiphasic – meaning that there is an early more rapid phase/s of elimination with shorter half-life(ves) and a terminal elimination phase with a longer half-life - the longer terminal elimination half-life should be used to be protective (unless the use of shorter half-life is justified). For example, if a small amount of the chemical is stored in a depot–such as bone or fat, and mobilized only under specific conditions, it may be appropriate to use the shorter half-life in specific cases.

An important aspect of the intermittent exposure scenario is whether effects persist between successive exposure episodes (i.e., whether effects accumulate) or whether the effects are reversible or irreversible. For this step, the key question is whether biological changes that are not adverse will progress with repeated exposure to concentrations below those that cause adverse effects (Haber et al., 2016). It may be important to consider not only whether the exposure is above or below than the RfV, but by how much.

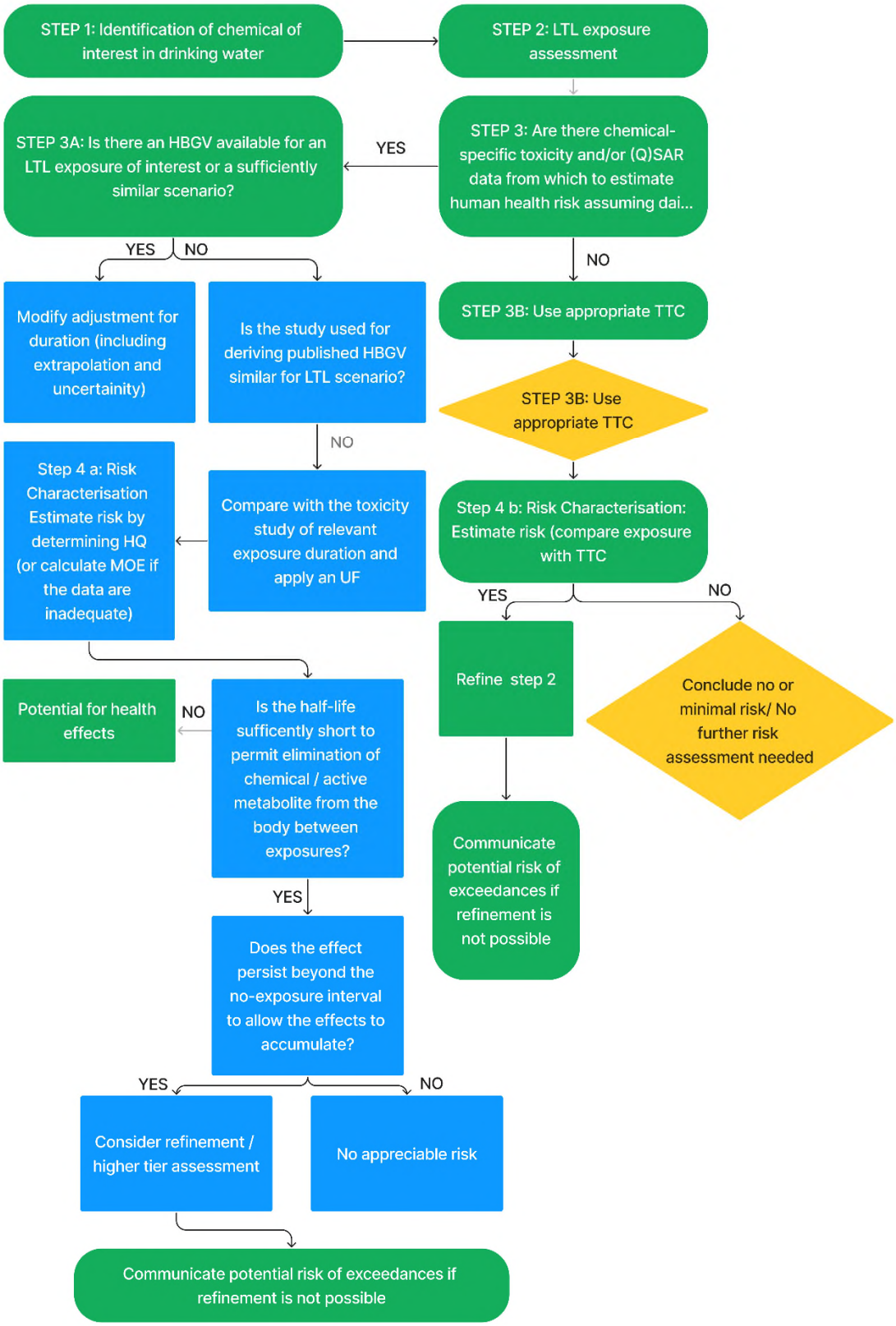

Figure 6: *Flow chart for assessing risk from LTL exposure to non-carcinogenic chemicals in drinking water. The scheme is modified from Haber et al. (2016).* ***Green*** *= Start and end of steps;* ***Blue*** *= Process;* ***Yellow*** *= Decision.*

1.7 Risk characterisation

Risk characterisation is the final stage of the risk assessment process and integrates information from the exposure and hazard assessment process and includes a synopsis and synthesis of all the data that should contribute to a conclusion about the nature and extent of the risk to chemicals during an LTL exposure scenario, i.e., whether adverse effects can be expected from the given LTL exposure (carcinogenic or non-carcinogenic effects). For non-carcinogenic chemicals a risk can be characterised by comparing estimated (or measured) exposure (ADD) with the available non-cancer health guidelines such as RfDs and this is called Hazard Quotient (HQ) (see TEXT BOX 2). In case there are inadequate data, MOE should be calculated. For carcinogenic effects, risk characterisation depends on the mechanisms of carcinogenicity and the relationship between dose and carcinogenic response. Different approaches have been used to characterise the risk for toxic effects that are considered to have a threshold or non-threshold (see TEXT BOX 3). For genotoxic effects, it is generally assumed that there may be no threshold and that there is some risk at any level of exposure. Therefore, substances that are genotoxic and carcinogenic the exposure levels should be controlled, so that exposure is as low as reasonably practicable (ALARP) (PHE, 2019). It should be noted, however, that some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms, and HBGV would be appropriate for such chemicals.

TEXT BOX 2: Risk characterisation for non-carcinogens

(a) HQ = ADD / RfD or MRL

Where:
HQ = Hazard quotient
RfD = Reference Dose [mg/kg/day]
MRL = Minimal Risk Level [mg/kg/day]

HQ>1, implies significant non-carcinogenic health risk,
HQ≤ 1 implies that exposure may not lead to non-carcinogenic health risk

Or

(b) Calculate MOE if the data are inadequate

MOE = BMD10 (or NOAEL)/Estimated exposure dose (ADD or DAD)
MOE ≥ 100 is generally considered to be protective

Or

(c) Use TTC in absence of chemical specific data

ADD > TTC: risk unacceptable
ADD ≤ TTC: risk acceptable

$HI = HQ_1/RfD_1 + HQ_2/RfD_2 + \ldots + HQ_n/RDV_n$

Where:
HI = Hazard Index
HQ_n/RfD_n = Hazard Quotient of "n" number of chemicals in a mixture
HI>1, implies significant non-carcinogenic health risk,
HI≤ 1 implies that exposure may not lead to non-carcinogenic health risk

TEXT BOX 3: Risk characterisation for carcinogens

(a) Calculating lifetime cancer risk associated with less than lifetime (LTL) exposures

$$ILCR = \frac{D \times CSF \times ADAF \times ED}{LT}$$

D *Exposure dose [mg/kg bw-day]*
CSF *Cancer Slope Factor* [mg/kg-day]*
ED *Exposure duration [years]***
LT *Lifetime [years] (considering 70 years average expectancy)*
ADAF *Age dependent adjustment factors (10 for children 0 < 2 years; 3 for children to < 16 years; = 1 for children ≥ 16 and adults.*

Note: To obtain the overall risk for a 70-year period (initiated at birth), the risk is calculated for each age group and exposure periods and then added together to obtain the total. It is also possible to compute cancer risk can also be calculated for any exposure duration combined with the doses for the target age group (USEPA, 2011). In the present study, only one age group i.e. < 16 years is selected for demonstration.

Note: Cancer risks will be considered negligible where the estimated cancer risk is 1-in-100,000 (≤ 1 x 10^{-5}) (WHO, 2022) or 1-in-1,000,000 (≤ 1 x 10^{-6}) (van der Aa et al., 2017). The risk exceeding 1-in-10,000 (≥ 1 x 10^{-4}) is considered unacceptable (USEPA, 1992; Nag & Cummin, 2022).

≥ 1 × 10^{-3} *: High risk*
≥ 1 × 10^{-4} but <1 × 10^{-3} *: Moderate risk*
≥ 1x10^{-6} but <1 × 10^{-4} *:Low risk*
<1 × 10^{-6} *: Very low risk*

(b) Calculate MOE if data are inadequate

MOE = BMD10/ Estimated exposure dose (ADD)

MOE banding
MOE > 1,000,000 *: highly unlikely to be a concern*
MOE 10,000 - 1,000,000 *: unlikely to be a concern*
MOE < 10,000 *: may be a concern*

(c) *Use TTC in absence of chemical specific data*

ADD > TTC: risk unacceptable
ADD ≤ TTC: risk acceptable

TEXT BOX 4

For carcinogenic chemicals, the US Environmental Protection Agency (USEPA) has an oral slope factor (or an inhalation unit risk). Oral slope factor can be used for oral exposure of chemicals, for example via drinking water (or food) to calculate an increased Lifetime Cancer Risk (ILCR) that may occur from the LADD. This number can tell the cancer risk above the existing likelihood of developing cancer. The method for determining ILCR is given below and is based on the publication of Aendo et al., (2019) and Mohammadi et al. (2019). The cancer slope factor (CSF) converts estimated daily intake averaged over a lifetime of exposure directly to incremental risk of an individual developing cancer (USEPA, 1989).

2 Probabilistic risk assessment for drinking-water quality

2.1 Introduction to Probabilistic Risk Assessment

Toxicological risk assessment is challenging due to high uncertainties and variability in toxicodynamics and toxicokinetics between and within species (including humans) and different types of studies (Chou & Lim, 2020; Testai et al., 2021). Probabilistic risk assessment (PRA) aims to characterize these uncertainties and support better risk assessment thereby improving risk management decisions. A detailed description of PRA is provided below. Uncertainty can result from limitations in knowledge (e.g. limited availability of empirical information) and by biases or imperfections in the instruments, models and/or techniques used. The uncertainty can be reduced by improving our knowledge base (e.g., by improving the data quality by further research), models and/or techniques (EFSA, 2012a; Jager et al., 2001). A list of the most important sources of uncertainties (in general) in risk assessment is provided in Table 6 and is also applicable in a (drinking) water context. Variability refers to an inherent heterogeneity or diversity of data in an assessment that is beyond a researcher's control and cannot be reduced (e.g., through further studies or measurement) (ECHA, 2012; IPCS, 2017). Examples include variability in responses between species, within species (e.g., due to age, sensitivity, physiology, behaviour), variability in environmental characteristics (e.g., temperature, wind, homogeneity) and variability in time and space (ECHA 2012). However, some sources of variability associated with exposure, such as variability in the concentration of a substance in water, can be reduced by developing appropriate sampling protocols and/or through improving treatment techniques (Triantafyllidou et al., 2021; Uwamungu et al., 2022). There can also be uncertainty about the degree of variability, which can be reduced by quantifying and accounting for uncertainty of known variability (ECHA 2012; WHO/IPCS, 2017), or by increasing the number of measurements to make a more precise estimate of the variability. After quantifying individual uncertainties in effect and/or exposure assessment (e.g., by accounting for parameter uncertainty and/or scenario uncertainty), the next step is to combine the individual uncertainties into the overall uncertainty of the final target human dose (the dose that complies with the specified health protection goals) by probabilistic approaches and finally sensitivity analysis of the uncertainties (WHO/IPCS, 2017).

Classical and current approaches to risk assessment tend to rely on single-point estimates, often called deterministic risk assessment (DRA), which include brief qualitative descriptions of uncertainties as a basis of assessment factors (AFs). To cover the uncertainties in the various aspects of a (deterministic) risk assessment conservative/worst case values are generally used in such risk assessments. DRA may indicate "no appreciable risk" even in a worst-case scenario. However, if the worst-case assessment shows that risks cannot be ruled out, a more realistic assessment may be required to provide a better and more quantified information about the severity of effect (Bokkers et al., 2009). This can be achieved by integrating a description of uncertainties and variabilities in a PRA. PRA is a general term for risk assessments that use probability models to represent the likelihood of different levels of risk in a population. This provides risk managers with information about the uncertainties in the data, models, assumptions, and outcomes. A fundamental feature of PRA is that it does not provide a single point estimate as conventional approaches do, but a distribution over which a particular exposure, dose or effect will occur. For example, in a point estimate approach, a single numerical value (i.e., point estimate) is chosen for each variable such as a drinking water intake of 2 L/day and a body weight of 60 kg for an adult. A single risk estimate is calculated based on the choices that are made for each variable (EFSA, 2020). In a PRA approach, all inputs to the risk equation (PoD, AFs, exposure) are distributions describing the variability in a population and /or the uncertainties of each input (parameter) that can assume different values for different receptors in the population and can be defined mathematically by a probability distribution (USEPA, 2014).

Table 6: *Sources of uncertainty in toxicological risk assessment (ECHA, 2012).*

Sources of Uncertainity related to effect assessment	
Model uncertainty	▪ Adequacy of the model, e.g. QSAR, toxicokinetic and mechanistic models of effects: - oversimplification - dependency errors - use out of the validity / applicability domain
Parameter uncertainty (physico-chemical and hazard)	▪ *Measurement errors:* e.g. influence of the methodology used, technical inadvertence (e.g., while conducting an *in vitro* or *in vivo* study). ▪ *Sample uncertainty:* representativeness of the data set, e.g. a small sample may not give the entire range of values found in reality; the sample may be biased towards lower or higher values as a result of the selection criteria used to take the sample; averaging methodologies. ▪ *Selection of the data used for assessing the risk*: i.e. use of default data (e.g. TGD (technical guidance document) default data are frequently used for exposure assessment) or choice of the dose descriptor (i.e. uncertainty in choosing one data among others for risk assessment purpose). ▪ *Extrapolation uncertainty*: i.e. use of alternative methods (e.g. QSAR, *in-vitro* test, read-across for similar substances) or use of assessment factors (e.g. inter-species, intra-species, acute to chronic, route to route, lab to field extrapolation).
Sources of Uncertainity related to exposure assessment	
Scenario uncertainty	▪ Adequacy of exposure scenario assumptions, e.g., emission sources ▪ Exposed population (e.g. children) ▪ Spatial and temporal setting (e.g. local, regional, short- or long- term) ▪ Environment of exposure (e.g. conceptual model of working place or natural environment) ▪ Exposure pathway(s) / route (s) (e.g. disregarding an important exposure pathway / route) ▪ Exposure event(s) (e.g. magnitude and frequency of the event) ▪ Assumed efficacy of risk management measures (e.g. usage)
Model uncertainty	▪ Extrapolation (i.e. use of a model outside the domain for which it was developed) ▪ Modelling errors (i.e. non-consideration of parameters in the model structure itself, etc.) ▪ Dependency errors (i.e. lack of consideration of correlations between parameters)
Parameter and data uncertainty	▪ *Measurement uncertainties:* e.g., low sample size, measurement error ▪ *Selection of data:* e.g., conservativeness in estimation of emissions, choice of the exposure concentration used for the exposure assessment, adequacy of default values, assumed effectiveness of risk management measures. ▪ *Extrapolation:* e.g., read-across for similar substances/scenario's. ▪ *Variability:* e.g., Environmental variability (temperature, wind, homogeneity etc.), variation in behaviour (related to exposure potential), variation in time and space, relating to any of the above. *Selection of data*: e.g., conservativeness in estimation of emissions, choice of the exposure concentration used for the exposure assessment, adequacy of default values, assumed effectiveness of risk management measures.

PRA can be applied to one or more phases of the risk assessment paradigm, including hazard characterization (i.e. deriving a PoD and AFs) as well as exposure assessment (e.g. uncertainties in concentrations) and risk characterisation (Bokkers et al., 2017; Slob et al. 2014; USEPA, 2014; van der Voet 2007; WHO/IPCS, 2017). PRA approaches are complementary to deterministic approaches (and not a substitute), as they introduce more realism by using distributions to represent the range of variation and uncertainty (EFSA, 2012a). PRA can be considered as an option for more advanced assessment in cases where deterministic approaches are insufficient to reach a risk management decision, e.g., where the deterministic assessment indicates cause for concern and more refined estimates are considered to investigate the probability of exposures above the reference dose (RfD), which is an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects during a lifetime (Baynes, 2012; EFSA, 2012a).

2.2 Implementation of PRA for (drinking) water quality assessment

The challenge for (drinking) water providers and regulators are to ensure that the quality of the (drinking) water does not affect public health and the confidence in the safety of water (Drinkwaterbesluit, 2021; EU Commission, 2020). Using PRA to assess the health risk of chemicals provides an indication of the likelihood of health effects that might actually occur by assessing the variability and uncertainty at each step of the health risk assessment. This can add credibility to interpretations of potential threats to human health from potential (drinking) water contaminants. According to Bokkers et al (2017), protection goals (i.e., the magnitude of the effect in individuals and the proportion of the population that should be protected from that magnitude of effect) as well as the level of conservatism (i.e., the probability of not underestimating the risk) are made explicit in PRA and can be tailored to the decision context. In this way, a water quality expert may ask for assessments based on various defined protection goals or levels of conservatism. It can also indicate whether risk-mitigation measures are needed, or additional information is needed to assess this. If the probabilistic results are still inconclusive with respect to health risk, the results of the probabilistic analysis can still help to identify the aspects in the overall risk assessment as a starting point for the collection of additional data.

Developing a general approach to PRA for drinking water quality is a challenging task. While some guidance has been developed on the use of probabilistic methods for modelling dietary exposure to chemicals (EFSA, 2012a), a framework for implementing the PRA to (drinking) water quality is lacking. A complete PRA approach for drinking water quality to assess the uncertainties in health risks associated with the chemicals in drinking water is relevant to ensure the protection of human health. Here we propose a framework for PRA of chemicals in the context of drinking water quality. This chapter describes the development of a general approach for PRA, that allows the analysis of variability and uncertainty in the exposure and/or risk assessments of chemical exposures that occur via drinking water. A case study is included to illustrate the application of the recommended approaches.

2.3 Method

PRA includes several major steps, that are consistent with the accepted environmental health risk assessment process (USEPA, 2014) and can be applied in the context of drinking water quality. These steps are illustrated in figure 7 and are elucidated in more detail below.

Step 1. Problem formulation: For a PRA of chemicals relevant to drinking water quality, the first step should be the problem formulation, which is a systematic approach developed here that identifies all factors critical to risk assessment, such as, the occurrence of the chemical(s) of concern in (drinking) water, the regulatory objective and the intended outcome relevant to the decision-making process and stakeholders, the analytical approaches and the limitations of the analysis, (Beronius et al., 2020; USEPA, 2014). Identification of chemicals of public health concern in drinking-water should be based on the health hazard of chemicals (of interest) and the likelihood of exposure via (drinking) water and/or other routes. This is because the presence of a particular chemical in drinking-water may not necessarily result in human exposure to concentrations of concern (e.g., the concentration of the chemical may be far below the health-based guideline value. It must be noticed that a complete toxicological dossier is often lacking for chemicals that appear in the water cycle because they have only recently come into use or the chemical analytical methods could not detect them earlier (Baken et al., 2018).

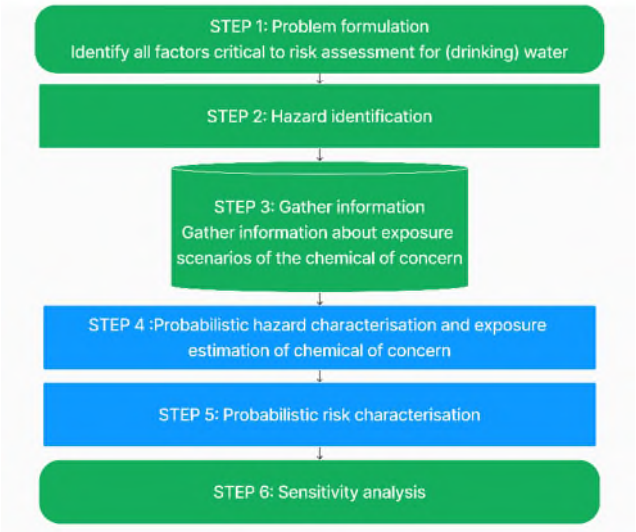

Figure 7. *Scheme for PRA of chemicals for (drinking) water quality. The steps shown in blue are specific to PRA, whereas the steps in green are in general used in toxicological/health risk assessment.*

Step 2. Hazard identification: The purpose of this step is to be determine whether exposure to the chemical via (drinking) water can lead to an adverse human health effect. Potential risks to human health from (drinking) water chemical contaminants can be determined by defining safe intake levels of chemicals and comparing them to measured or estimated (drinking) water concentrations (Baken et al., 2018). However, health-based regulatory drinking water standards exist only for a limited number of chemicals known to be present in drinking water. Therefore, to obtain information on the potential toxicological properties of a chemical of concern a tiered approach can be applied, starting with obtaining the measured data to derive guideline values based on established acceptable daily intake levels or toxicity data based on animal experiments (Baken et al., 2018). As such, epidemiological studies may show associations between effects and substances. However, such studies lack causal evidence and are often unavailable due to significant ethical concerns, therefore, data from animal studies (rats, mice, etc) and animal-free new approach methodologies (to some extent) can be relied on to draw conclusions about the potential hazard to humans (WHO/IPCS, 2021). In the absence of complete toxicity data, non-testing tools for example QSARs and read-across approaches can be applied to predict the toxicity of the chemical of concern. If QSAR and read-across results are inconclusive, further assessment can be done by considering exposure of a chemical of concern to the corresponding 'Threshold of Toxicological Concern' (TTC) Cramer Class (EFSA, 2012b). TTC is a concept that refers to the establishment of an exposure level for all chemicals, whether there are chemical-specific toxicity data, below which there is no appreciable risk to human health. The concept assumes that for many chemicals, including those with unknown toxicity, a low exposure threshold with negligible risk can be determined based on knowledge of their chemical structures (EFSA 2012b; Kroes & Renwick., 2005).

Step 3. Gathering information on exposure scenarios: Information is needed from the stakeholders and the experts (e.g., at government agencies, water utilities, research institutions, who understand the risk factors affecting both (drinking) water quality and availability across exposure scenarios to be evaluated, starting with defining the exposed population. Consideration should be given to susceptible population, because some age groups may have higher susceptibility to chemical exposures (e.g. the unborn, infant, toddler, children, pregnant women, and the elderly) and should be considered separately in the risk assessment. International sources of information on susceptible populations are provided in Table 7. In addition, several other considerations must be considered to estimate the effects of exposure, such as, measuring cumulative (i.e., combined exposure to multiple chemicals through one exposure source such as water) and aggregate (i.e., exposure to a single chemical through multiple routes, such as water, food and air) exposure. Exposures can be measured directly or estimated from exposure models or generalized from existing data (WHO/IPCS, 2021). Of the three methods, estimating exposures from existing data is often the

simplest approach. However, such data are often unavailable or not entirely representative of the exposure scenario of interest. Measurements, on the other hand, usually provide the most accurate and relevant data, but are the most time and resource intensive, precluding their use for many risk assessments. Exposure models can be used to provide estimates of exposure from a variety of sources. For modelling (drinking) water chemicals, exposure models require information about the concentration of a chemical in (drinking) water, the period over which individuals are in contact with the chemical and the route of the contact such as ingestion or dermal. Some of the models that can be used for probabilistic exposure assessment for substances in (drinking) water are listed in Table 8.

Table 7: *International sources of information on susceptible populations.*

Document	Link
Principles for evaluating health risks to progeny associated with exposure to chemicals during pregnancy (EHC 30)	https://apps.who.int/iris/handle/10665/39375
Principles for evaluating health risks from chemicals during infancy and early childhood: the need for a special approach (EHC 59)	https://apps.who.int/iris/handle/10665/39088
Principles for evaluating chemical effects on the aged population (EHC 144)	https://www.who.int/publications/i/item/9241571446
Principles for evaluating health risks in children associated with exposure to chemicals (EHC 237)	https://apps.who.int/iris/handle/10665/43604
Summary of principles for evaluating health risks in children associated with exposure to chemicals	https://apps.who.int/iris/handle/10665/44533
Identifying important life stages for monitoring and assessing risks from exposures to environmental contaminants: results of a World Health Organization review	https://doi.org/10.1016/j.yrtph.2013.09.008

Step 4. Probabilistic hazard characterisation and probabilistic exposure estimation of chemicals in (drinking) water: Probabilistic analysis of uncertainties can be performed in a Microsoft Excel spreadsheet (available via: APROBA_PLUS-V1.14_TEMPLATE_1.xlsx (live.com), assuming that all uncertainties can be reflected by statistically independent lognormal distributions. Table 9 provides a list of different guidance documents, and tools for PRA. In this document we will elaborate upon the application of the APROBA-Plus tool for PRA of (drinking) water chemicals and will further illustrate its applicability for risk assessment of herbicide simazine in drinking water (see the case study in Appendix II).

Uncertainties in a PRA can be combined either by a full probabilistic analysis or an approximate probabilistic analysis. In a full probabilistic analysis, uncertainties are not restricted to lognormal distribution and is the most accurate and flexible approach. However, no general, user-friendly software package is currently available, and the assessors have been developing their own software, which in general includes Monte Carlo simulations, which is a data demanding and time-consuming method. For this reason, approximate probabilistic analysis is a helpful alternative in many cases, including (drinking) water. It is based on lognormal distributions and is much faster and easier to apply. However, it relies on the available PoD and exposure estimates, which may need to be generated first if not available and may take time. Simulations obtained by approximate probabilistic analysis indicate that the resulting confidence bounds are a reasonable approximation to those obtained in the fully probabilistic approach (WHO-IPCS, 2017). Since a large number of new chemicals are registered each year, and a relatively small fraction of them are thoroughly tested (Kaserzon et al., 2018), the use of approximate probabilistic analysis is recommended for rapid PRA of (drinking) water chemicals to avoid potential risk to human health. A prototype software (Microsoft Excel spreadsheet) APROBA tool

(for Approximate PROBabilistic Analysis) was released by WHO in 2017 (WHO/IPCS, 2017) to facilitate the application of PRA by applying lognormal uncertainty distributions to the different aspects of the hazard characterization (e.g. dose-response, interspecies sensitivity), resulting in a probabilistic health-based guidance value. Both can be quickly calculated in a spreadsheet without needing Monte Carlo simulation. The probabilistic health-based guidance value (HBGV) can be compared with a deterministic exposure value (e.g. a mean, a percentile of the population, or an upper confidence bound of the mean/percentile). This approach, however, leads to an unbalanced risk assessment in which uncertainties are made visible in the hazard but not in the exposure (Bokkers et al., 2017).

An extension of APROBA, called APROBA-Plus has been developed by RIVM to bridge this gap by including the option to insert a quick and approximate estimate of the exposure uncertainty. We recommend the use of APROBA-Plus, as it can be practically used for the risk assessment of all substances [including the chemicals of concern in (drinking) water]. The tool compares any available exposure (measured from exposure assessments performed using other models), preferably a high exposure to cover also highly exposed individuals, to the dose which causes a particular adverse health effect in sensitive individuals. Rather than comparing (conservative) point estimates for the effect dose and exposure, the entire uncertainty distribution of both inputs is included in the analysis. The resulting plot transparently visualizes the uncertainty about the distance between hazard and exposure (see case study) as described in the next step. A detailed procedure for using APROBA – Plus tool (available via: https://www.rivm.nl/en/aproba-plus) can be found in the publication by Bokkers et al (Bokkers et al., 2017).

Step 5. Probabilistic risk characterization: Having conducted the probabilistic hazard characterisation and after including the exposure (using APROBA-Plus tool) for the chemical (of concern), the risk is visualised by three ellipses (see appendix, Figure 9) by showing the relative distance between the concentrations to which humans are exposed and estimated levels at which toxicological hazard may be considered. Ellipses in this graph were based on three scenarios in which respectively 90-99%, 45-55% and 0.001-10% of all simazine is removed by conventional drinking water treatment processes. On the x-axis the variation and uncertainty in exposure level (in µg/kg body weight per day) is shown, while on the y-axis uncertainty in hazard level (in µg/kg body weight per day) is shown. The location of the ellipse indicates whether there is a risk (in the red area, i.e. the distance between exposure levels and hazard levels is small or is even overlapping) or not (when located in the green area, i.e. the distance between exposure levels and hazard levels is relatively large). Ellipse shape and size indicate the uncertainty in hazard and exposure. While the range in hazardous levels (y-axis) in our case study is solely based on uncertainty in derivation of hazard levels (e.g. due to the use uncertainty factors based on acute-to-chronic extrapolation or interspecies extrapolation), the range in exposure levels is solely dependent on variation in removal rates.

Step 6. Sensitivity analysis: The primary objective of the sensitivity analysis is to determine which variables and uncertainties most strongly influence the risk estimate. In the present document, the APROBA-Plus tool provides the output with respect to the relative contribution of the individual aspects to overall uncertainty (the shape of the ellipse indicates if hazard or exposure contains more uncertainty). The key goal is to provide a comprehensive risk characterization that is scientifically credible and sufficient for risk decision making. The time and effort required to achieve various levels of complexity should be weighed against the value of the information provided. Additionally, if a variable is specified as influential in the sensitivity analysis, this does not automatically mean that a distribution has to be developed for this variable. If the risk assessor feels that data are simply not sufficient to derive a distribution, then a plausible point estimate can be used. The risk assessor should be aware of a possible problem arising from using point estimates in the absence of data adequate to support a distribution. If a variable has the potential to significantly impact the risk outcome, and a very high-end or low-end point estimate is used in the PRA, this has the potential to right-shift or left-shift the final distribution of risk. Even though there might not be enough data to develop a distribution of variability for an influential variable, it would be prudent to communicate the importance of this data gap to the risk decision makers, and it is recommended to run multiple simulations with several plausible input distributions for that variable. Communication of this uncertainty may be used for decision-making whether additional data should be collected to better define the variable (USEPA, 2014).

Table 8: Models for exposure assessment in (drinking) water.

Models	Description	Link
PROcEED	PROcEED can be used to perform probabilistic reverse dosimetry[3] calculations for estimating a distribution of exposure concentrations likely to have produced biomarker[4] concentrations measured in a population	https://www.epa.gov/chemical-research/probabilistic-reverse-dosimetry-estimating-exposure-distribution-proceed
MCRA	Monte Carlo Risk Assessment can be used to calculate the intake of chemicals through food and drinking water in a probabilistic way, considering the variation in consumption and concentration data and the uncertainty in these input variables.	Monte Carlo Risk Assessment (MCRA) | Rivm
CARES	CARES (probabilistic model) can estimate aggregate and cumulative exposure and risk across multiple routes: food, water, and residential for pesticides.	https://caresng.org/about/

***Table 9**. Guidance documents for PRA of chemicals.*

Sources	Description	Link
Guidance documents		
enHealth	This guidance document provides information on basic concepts in environmental health risk assessment. Specific guidance is provided for several topics related to exposure assessment including assessing exposure from multiple routes and sources of exposure, probabilistic exposure modelling, and biomonitoring.	https://healthinfonet.ecu.edu.au/key-resources/resources/?id=22814
EFSA (2012a)	This document provides guidance on performing basic probabilistic assessments for dietary exposure to single and multiple active substances. A checklist of key issues to consider when preparing a report on probabilistic exposure assessment and several case studies is also included.	https://www.efsa.europa.eu/en/efsajournal/pub/2839
USEPA (2001)	The Risk Assessment Guidance for Superfund (RAGS): Volume III: Process for Conducting Probabilistic Risk Assessment (Part A) provides guidance for using Monte Carlo analysis to characterize variability and uncertainty in human health and ecological risk assessments.	https://www.epa.gov/risk/risk-assessment-guidance-superfund-rags-volume-iii-part
USEPA (2004)	Chapter 31 of ATRA Volume I provides an introduction to conducting probabilistic risk assessments. The advantages of this approach, methods, and considerations for presenting the results of a probabilistic risk assessment are presented.	https://www.epa.gov/fera/air-toxics-risk-assessment-reference-library-volumes-1-3
Tools		
APROBA – Plus	APROBA – Plus a probabilistic tool to evaluate and express uncertainty in hazard characterization and exposure assessment of substances	**https://www.rivm.nl/en/aproba-plus**
Monte Carlo Simulation	Most commonly used method for probabilistic modelling that performs risk analysis by building models of possible results by substituting a range of values for any factor that has inherent uncertainty.	https://www.ibm.com/cloud/learn/monte-carlo-simulation https://doi.org/10.1111/j.1539-6924.2007.00887.x

[3] Reverse dosimetry, sometimes called dose reconstruction, is an estimation of environmental exposures that is consistent with measured biological monitoring data (Ruiz & Fowler, 2015).

[4] Biomarkers are biochemical indicators that can be used to monitor biological changes in response to toxins or other stimuli.

3 Human Exposure Assessment for drinking water quality

3.1 Introduction to Human exposure assessment

Humans are inevitably exposed to different chemicals from a variety of sources including air, water, and food. In the context of chemicals in (drinking) water, these substances predominantly originate from point sources such as industries and sewage treatment plants, and diffuse sources including agricultural runoff and storm water. Unlike point sources of emission, which are regulated to some extent, other sources of concern, such as accidental or deliberate contamination of water supplies are less predictable and frequent (WHO, 2017a). The exposure assessment process is important in identifying and quantifying exposure to a substance(s) of concern that occurs or is expected to occur in the human populations (WHO/IPCS/ILC, 2000). It is a prerequisite for human health risk assessment and for deciding whether exposure and risk mitigation measures and regulations are needed.

In the exposure assessment, both the magnitude (how much) and the route of exposure are critical characteristics for determining potential adverse effects to exposed populations. In addition, the frequency (how often), duration (how long), and characteristics of the exposed population (e.g., life stage, health status) need to be considered. These exposure characteristics depend on the environmental emission source, transport, fate, and persistence of the contaminant, and the activities and behaviour of individuals who come in contact with the contaminants (ECHA, 2013; ECHA, 2016; USEPA, 2019). In addition, exposure may occur to a single contaminant via multiple exposure routes (e.g., inhalation, ingestion, or dermal), called aggregate exposure or to multiple contaminants where interactive processes may be involved (cumulative exposure) should be considered. In general, during exposure assessment, the concentration of chemicals to which an individual is externally exposed, referred to external exposure, is measured. However, external exposure does not necessarily equate to the amount of chemical intake, i.e., internal exposure. Bioaccumulation and toxicokinetic processes such as absorption, distribution, metabolism and excretion (ADME) play an important role and should be taken into account. Exposure assessment can also provide information on changes in exposures over time (by measuring exposure trends) (USEPA, 2019). There are many options available when selecting the best approach for conducting an exposure assessment. Table 10 summarizes the general components of exposure assessment. The choices may vary depending on the assessment goal and available information.

Often, a risk assessment is established as a tiered approach and is also applicable in a drinking water context. A tiered approach starts with worst-case assumptions followed by stepwise refinements up to more realistic scenarios. Tiered approach may also be used when sufficiently detailed exposure data are not available. If the conclusion is “not of concern,” a more detailed (higher-tier) risk assessment for the chemical with respect to the effects/population under consideration does not have to be conducted. On the other hand, if the result show that a substance is “of concern”, the assessment can be refined using increasingly more realistic exposure conditions to reach a final conclusion (ECHA, 2003). In exposure assessments, exposure can be differentiated between retrospective (past) or prospective (future) exposures (Borghi et al., 2020). In either scenario, collection of exposure data, either measured or predicted using models, is essential to correctly evaluate any risks associated with the specific concentration and exposure duration of the chemical of interest.

Table 10. *Components of exposure assessment (modified from: USEPA, 2019). An exposure assessment is often composed of information on exposure levels, stepwise tiers, target population, modelling approach, and stressors. The choice of components and their type, will determine the outcomes in accordance with the research objective, available resources and specific context of the exposure assessment.*

Components	Description	Type	Output
Exposure levels	Determines the fundamental design of the exposure assessment	Direct measurements	The quantified intensity of the contact of a person with a chemical in the exposure medium over an identified time period.
		Indirect estimation	Uses available information on concentrations of chemicals in the exposure medium and information about when, where and how individuals might contact the exposure medium.
		Biomonitoring	Measures the amount of a stressor in biological matrices, e.g., blood. Biomonitoring data aggregate exposures from all routes and pathways
		Exposure modelling	Conceptual or mathematical representation of the exposure process that provide estimates of the amount of a substance to which individual or populations are likely to be exposed.
Tiers	Considers the resources and the acceptable level of uncertainty	Ranges from screening-level assessments that are rapid and use few resources but are highly uncertain to very complex assessments that minimize uncertainty but are resource intensive	Complex exposure estimates can be generated for actual environmental conditions or prospective or retrospective scenarios.
Target population	Determines how the population is described	behaviour	A distinguishable set of behaviours or locations that lead to exposure.
		composition	Provides information on the broader context of exposure for a selected population, including variability within that population or intrapersonal variability.
Modeling approach	Determines how the assessment is conducted	Deterministic	Use point estimates as inputs to exposure equations or models. This approach most often is screening level.
		Probabilistic	Uses statistical distributions for input variables and characterizing the conditions and better account for the uncertainty and variability in influential input variables.
Stressors	Determines how stressor (substance) is considered	Exposure to single stressor, single source, single pathway	Considers single agent/stressor, single source, single pathway
		Aggregate exposure	Considers multiple exposure pathways to a single agent / stressor
		Cumulative exposure	Considers multiple pathways and multiple agents / stressor

3.2 Current developments in exposure assessment

Over the past decade, there has been significant progress internationally in assessment of chemicals, marked by the publication of various guidance documents, frameworks, and research papers (e.g., ECHA, 2014; EFSA 2018; Meek et al., 2011; Kortenkamp, 2019; WHO, 2017a, 2017b). In 2021, an important initiative to promote next-generation chemical risk assessment methods was launched by the establishment of the European Partnership for the Assessment of Risks from Chemicals (EU PARC). This partnership aims to advance research, knowledge and skills in the field of chemical risk assessment for the protection of public health. A key objective of the PARC project is to increase the knowledge on aggregate exposures and provide tools that facilitate comparison between entry routes

and exposure situations. This aims to increase understanding of the relative contributions of exposure sources and pathways to support effective risk management measures (information available via: https://www.eu-parc.eu/sites/default/files/2023-08/PARC_AD6.3.pdf). In 2022, the European Food Safety Authority (EFSA) launched a project (ExpoAdvance) in close collaboration with European and other international partners. This initiative aims to establish a harmonised methodology and regulatory guidance for aggregated exposure assessments covering all relevant sources and pathways of chemical exposure (EFSA 2022). This step also represents a proactive move by EFSA towards advancing exposure assessment to overcome the existing challenges and ensure consistent and reliable approach to chemical exposure assessment. In the context of the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (No 1907/2006), specific guidance has been developed for the assessment of multiple exposure sources for a single substance. In certain cases, guidance is extended to the assessment of multiple closely related and similarly acting substances, such as different salts of the same metal or a number of closely related derivatives of organic substances. In addition, guidance has been developed to evaluate the health risks from combined exposure to multiple chemicals (EFSA, 2021; OECD, 2018). While this guidance serves as a tool to assess potential adverse effects associated with exposure to known combinations of contaminants, it does not address potential concerns associated with exposure to unknown mixtures. This leaves a gap in our ability to evaluate and address the complexity presented by exposure to (low-level) mixtures of chemicals whose compositions or effects are not yet fully understood or characterised. This emphasises from a (drinking) water perspective the need to develop methods to assess and manage the risks associated with exposure to unknown mixtures in the context of chemical regulations and safety assessments.

Efforts have been made in the European Union (EU) to harmonise exposure assessment methods and tools in individual policy areas that fall under the purview of specific European agencies. Guidance on appropriate exposure measurement and modelling methods can be found in various publicly available documents. In particular, the Guidance on Information Requirements and Chemical Safety Assessment, prepared in response to the EU's REACH regulation, provides a detailed discussion of exposure measurement and modelling approaches (ECHA, 2016a; 2017). The European Food Safety Authority (EFSA) has developed harmonised exposure assessment models and tools for food safety, animal health, plant health, and environmental health (EFSA, 2012; More et al., 2019). An example of one such tool is Pesticide Residue Intake Model (PRIMO), a spreadsheet-based tool for dietary risk assessment of pesticide residues (EFSA, 2018). Other models include the ConsExpo model developed for use in consumer safety assessment by the Dutch National Institute for Public Health and the Environment (RIVM; Delmaar et al., 2017) and the European Union System for the Evaluation of Substances (EUSES) for environmental exposure assessment under REACH and the Biocides Directive (Haug et al., 2017). However, despite various efforts to improve processes and information quality, a common EU scientific framework for exposure assessment is still lacking (Bruinen de Bruin et al., 2021; von Goetz et al., 2019). A scientific framework for exposure science will promote the multiple uses of exposure knowledge across EU chemical policy and improve exposure and risk assessment, management, and communication across EU (Bruinen de Bruin et al., 2021). In this chapter, we present the methodology aligning with the existing practices of exposure assessment specifically focussing on the assessment of individual chemicals in drinking water sources.

3.3 Exposure assessment of chemicals in drinking water

Safe drinking water is essential to human survival and well-being and is therefore of paramount importance for public health. In the Netherlands, the quality of water intended for human consumption is closely monitored from source to tap to ensure its safety (ILT, 2022). However, uncertainty about the potential health effects of unregulated or emerging chemical contaminants necessitates the development of an approach to assess human exposure to such chemicals in water, including but not limited to drinking water. The challenges in this context are multifold and include the uncertainties in detecting low levels of chemicals in water, the presence of complex low-level mixtures, variation in the spatio-temporal exposure, and the availability of exposure data (see Table 11). While some guidance has been developed on the general principles, procedures, and statistical methods for human exposure assessment (ECHA

2016a, 2016b; UNEP/WHO/ILO, 2000; USEPA, 2019; WHO, 2021), a functional, user-oriented procedure for a tiered exposure assessment of chemicals in water is lacking. This chapter aims to address this gap by developing an integrated approach that combines existing and new exposure assessment methods into a single protocol to facilitate exposure assessment for chemicals present in water, considering both retrospective and prospective exposure scenarios. The method can be applied in particular in cases where (new) contaminants are identified in source water or drinking water. Aligned with the subsequent chapters of this report, "less-than-lifetime exposure assessment" and "probabilistic risk assessment" this chapter provides fundamental knowledge and approaches for evaluating exposure to chemicals over different durations.

3.4 Steps and considerations in exposure assessment of chemicals in water

The objective of an exposure assessment is to obtain an estimate of the exposure level that can be compared with the appropriate guideline/limit value for the characterisation of risk. Prior to exposure assessment, it is important to make judgements about intended purpose, scope and the technical approaches that will be used. The assessment begins with defining the exposure scenario and defining the exposed population. In each population, not all individuals are at the same risk when exposed to a given level of contaminants. Therefore, identifying the high-risk subgroups should be considered where possible. Consideration should be given to specific life stages (infants, toddlers, children, pregnant/nursing women, and the elderly). Some subgroups may be highly susceptible (for example due to preexisting health state (e.g., diabetes, immunocompromised, etc.) and/or highly vulnerable (for example, based on geographical location, gender, ethnic group or economic status) and can be considered separately in a risk assessment (USEPA, 2019). Differences in individual susceptibility may be due to differences in toxicokinetic patterns or toxicodynamic interactions (EFSA, 2021; Grandjean, 1992). In the EU Technical Guidance on Risk Assessment Part (I), the exposure assessment should where possible describe the exposure of a (sub)population using both actual and reasonable worst-case exposures (ECHA, 2003).

The specific purpose of a human exposure assessment varies widely, but generally falls into one of two categories: (1) the lower tier exposure assessment (screening level exposure assessment) and/or (2) the higher-tier exposure assessment (advanced exposure assessment). Nevertheless, the number of tiers in the assessment can be adapted to the purpose of the assessment as well as the type of chemical being assessed. Whether or not a higher tier is needed depends on the purpose of the assessment, the availability of data, the quality and quantity of the available data, the level of acceptable uncertainty and statistical methodologies (USEPA, 2019). The steps for a tiered exposure assessment for drinking water quality are illustrated in Figure 8 and discussed in more detail in the following sections. The method involves data collection and method selection prior to the assessment, followed by the assessment itself and an evaluation of whether risk is present (risk characterization). If no significant risk is indicated (at any stage), the assessment process concludes. If a significant risk is indicated at the highest tier, several options (such as implementing additional risk management measures) may be specified depending on the overall purpose of the assessment process. Attention should be paid to whether the exposure has ceased, is ongoing or may be expected in the future, i.e., whether the exposure is retrospective or prospective. A retrospective exposure assessment (REA) involves assessing the exposures that have occurred in the past, and a prospective exposure assessment (PEA) involves assessing the exposures that are ongoing or likely to occur in the future (Borghi et al., 2020; PHE, 2019). In each case, consideration should also be given to the duration of exposure. Exposure duration is the amount of time that an individual or population is exposed to the contaminant being evaluated (USEPA, 2011). Exposure durations may be less-than-life time (LTL) (less than 7 years) or lifetime (more than 7 years, up to 70 - 78 years) (Felter et al., 2011; USEPA 2011; WHO/IPCS, 2021). If the exposure turns out to be LTL, the LTL exposure assessment approach should be followed. Otherwise, lifetime (LT) exposure assessment should be followed (see chapter 1).

Table 11. Challenges of exposure assessment for chemical contaminants in water (modified from: Villanueva et al., 2014). The relevance of each of these challenges may vary depending on the context of the exposure assessment.

Challenges	Description
Low exposure levels	Generally, the (priority) substances found in drinking water are present in low concentrations. Accuracy of analytical measurements in water is particularly important at the low range of exposure.
Chemicals occurring in mixtures	(Drinking) water contains a complex low-level mixture of chemicals, such as, pharmaceutical residues and in some cases disinfection by-products (DBPs). Depending on the individual components of the mixture, an exposure assessment on chemical-by-chemical basis may not be feasible or could result in incomplete exposure estimates.
Spatio-temporal variability	Contamination patterns of drinking water sources are often subject to temporal-spatial variations depending on inputs from contaminant sources. Repeated measurements and distribution of sampling sites covering different water zones are necessary to assess geographic and temporal variations during the relevant exposure period.
Exposure misclassification	Longer exposure periods are likely to result in exposure misclassification i.e., assigning a different duration than the actual one, which is likely to affect the association between exposure and effects and thus underestimate or overestimate the risk. In the case of diseases associated with chronic exposures, data collection should preferably include water use over the duration of an exposure period relevant to the onset of disease.
Lack of monitoring data	This is particularly problematic in evaluating some exposures (e.g., emerging contaminants) and some outcomes due to lack of historical records. Further research is needed to develop validated simulation models that can be used to estimate levels and exposures over the relevant time period.
Lack of validated biomarkers of exposure	Exposure biomarkers can facilitate exposure assessment. However, there are significant challenges in developing biomarkers for the various constituents of concern that are sensitive to typical variations in exposure, reflective of the time periods of interest. Currently available validated biomarkers generally reflect recent exposures and may not be useful for results with latency periods longer than the half-life of the biomarker compound. Exceptions may occur when the time between consecutive exposure events is shorter than the elimination half-life or when exposure can be considered constant within the relevant time window.
Multiple exposure routes (ingestion, inhalation, dermal absorption)	Exposure to various water contaminants can occur through multiple pathways. For example, some DBPs can be incorporated through inhalation, dermal absorption, and ingestion. For such contaminants, exposure by all possible routes should be evaluated to obtain the most accurate estimate of health risk. Since water is an important exposure medium for oral, dermal and inhalation exposure (in form of vapours or aerosols), an additional challenge is the lack of information on exact amounts of consumed water.

3.4.1 Lower-tier exposure assessment

The lower tier[5] (Tier I) is a screening-level exposure assessment based on a small amount of (readily) available monitoring data and conservative/default assumptions to estimate the highest exposure (high-end exposure) as a realistic worst-case scenario, particularly for the most sensitive subpopulation (individuals at highest level of risk) such as children (USEPA, 2003). Screening-level assessments often use a deterministic approach based on point estimates which are single exposure values (USEPA, 2019). Acquiring comprehensive data on contaminants in source water, whether surface waters or groundwaters, can be challenging due to potential incompleteness, scarcity, or unavailability of data, particularly concerning emerging contaminants (Khan, et al., 2022).

Monitoring (measured) data plays an important role in providing information on the actual occurrence of a chemical of interest in water sources used for drinking water production (Michel et al., 2022). The Water Framework Directive (EC, 2000; Directive 2000/60/EC) mandates the availability of monitoring data on the chemical status of surface and groundwater in most EU member states. However, when using monitoring data, it is important to carefully assess if

[5] Some organisations use different terminology. For example, OECD considers three tiers, preliminary, refined and comprehensive assessments.

the data accurately reflects the real exposure scenario (ECHA, 2016c). Information on sources of measured data is provided in Table 12. If monitoring data are not available, a screening-level model (more details below) can be used to estimate the environmental (surface water/ground water) concentrations and exposures via drinking water. Under REACH, monitoring exposure data are not available for most identified uses of chemicals. Therefore, exposure and regulatory risk assessment in Europe are largely based on computational models (Schlüter et al., 2022). There are some screening-level models used by USEPA and other stakeholders in a variety of applications, including drinking water, to estimate concentrations of contaminants in human exposure assessments (USEPA 2023). For example, E-FAST (Exposure, Fate Assessment Screening Tool) is a screening-level model (publicly available from USEPA) that provides conservative estimates of chemical concentrations in water resulting from the releases of industrial chemicals to estimate human exposure by ingestion of contaminated water (Massarsky et al., 2022; USEPA, 2010). E-FAST calculates the concentration of contaminant in source water (consequently drinking water) assuming that untreated water is consumed as a sole drinking water source on a daily basis (USEPA, 2010). This can be considered as a worst-case situation, as in practice source water is treated to comply with quality standards in place to preserve drinking water quality. In specific cases, chemical contaminants remain in drinking water after treatment, or may enter the drinking water distribution system. In such cases, Environmental Protection Agency Network Evaluation Tool (EPANET) can be used. EPANET is explained in more detail in proceeding section.

Screening assessment results have a high degree of uncertainty (for example, due to incomplete data) and do not describe variability (which can be better described with more data in a higher-tier). Therefore, screening-level results are only used for a first approximation to rapidly determine the exposures that are of less concern to human health. The number of tiers in an exposure assessment is flexible and can be adapted to the purpose of the assessment as well as the type of chemical to be assessed. The USEPA's Environmental Fate and Effects Division (EFED) has defined four assessment tiers for drinking water based on the level of effort, the amount of data considered, the spatial scale, and the certainty in the estimated chemical (pesticide) concentration (USEPA, 2020). If no risk is expected despite the high level of conservatism in the lower tier, it is acceptable to end the exposure assessment at this stage. If the assessment indicates that there is a potential health risk at the estimated exposure levels, or if the results do not adequately support decision-making, the assessment can be refined, or exposure and risk mitigation measures taken.

Table 12. *Sources and types of human exposure assessment data for drinking water quality assessment (modified from: USEPA, 2019).*

Type of exposure data	Source of exposure data	Type of exposure measurement
(Drinking) water monitoring data for first and/or higher tier exposure assessment	Location-specific water sampling Local, state, and federal agency studies (for example USEPA data EU Local, regional or national monitoring databases (for example, Rijkswaterstaat in the Netherlands) (refer to Drinking Water Directive (recase), Article 12 and Annex II on monitoring Peer-reviewed scientific literature	Fixed-location monitoring (to establish long term trends at specific sampling locations and identifying changes in existing conditions. Short term monitoring (to establish short term trends) Source monitoring (to track the release rates in water, ensure regulatory compliance, identify disposal options)

3.4.2 Higher-tier exposure assessment

A higher-tier exposure assessment is an advanced and comprehensive approach, that is capable of addressing complex (realistic) exposure scenarios and provides more reliable results compared to lower-tier assessments. In a higher-tier exposure assessment, both deterministic and probabilistic data can be used as input (USEPA, 2003). The deterministic approach used in higher-tier exposure assessments differs from that in lower tier assessments, by using more comprehensive chemical specific data, sophisticated models (higher-tiered models) with higher-precision, sampling, and analysis techniques. The advantages of higher-tier exposure (either for retrospective or prospective)

assessments is that it can provide more refined exposure estimates. The goal is to obtain a more detailed insight in the exposure distribution, including the contribution of different sources, pathways, and the routes of exposure (aggregate exposure). This can also help to determine which risk management measures are required. Using probabilistic methods, a probabilistic statement can be made on the likelihood that effects may occur, or which fraction of a population is likely to be exposed to levels leading to adverse health effects.

There are various models that can be used for the exposure assessment of surface water bodies or drinking water. EPANET is a hydraulic and water quality calculator that tracks the flow of drinking water and its constituent concentrations throughout a drinking water distribution system. EPANET can evaluate a wide range of chemical reactions, including the movement and fate of a reactive material as it builds up or decays over time. The calculated exposure levels can be used to estimate a potential health risk (see section 3.4.5). If no risk is identified, it is acceptable to end the exposure assessment at this stage. If the assessment indicates an appreciable health risk cannot be disregarded, risk management measures should be suggested. A workflow for a tiered exposure assessment of chemicals in (drinking) water is given in Figure 8.

3.4.3 Screening level approaches in practice

Under normal conditions the drinking water supply is very safe in the Netherlands, with multiple barriers against contaminants. However, under certain conditions a substance may enter the system. The unwanted substance can enter the drinking water supply at various locations, e.g. at the source water, at the production location post treatment, in the drinking water distribution system (DWDS, transport, distribution or connection pipes), or in the drinking water installation (pipes, or taps). Depending on the location of contamination, different methods can be used. The basic steps for the exposure assessment are:

1. Determine the amount of the contaminant entering the system
2. Determine the concentration of the contaminant; take into account
   (a) dilution
   (b) treatment efficiency (if the contamination location is pre-treatment)
   (c) fate during distribution (chemical-physical reactions)

3. Determine the number of people that are exposed to the contaminated water
4. Determine the dose. The dose for the oral route is the amount of water consumed, multiplied by the concentration of the chemical. The inhalation route (e.g. through aerosols formed during showering) and the dermal route (with sometimes relatively long exposure duration during bathing or swimming) in most circumstances contribute much less to exposure and therefore are generally regarded less relevant in case of drinking water. However, there are certain substances such as volatile organic substances (VOCs) which have high vapour pressure and solubility (e.g., trichloroethylene, trichloromethane, chloroform, skin permeable DBPs) for which these exposure routes should be considered (Levin et al., 2013; Weisel and Jo, 1996).

Table 13 gives some examples of these basic steps in the exposure assessment, depending on the contamination location and the required screening level (tier). When the water source is contaminated, measured concentrations in the source water plus an estimate of the treatment efficiency, assuming no changes during distribution, 100% of the people in the supply area are being exposed and a consumption of 2 litres of drinking water per person per day is a tier 1 assumption. When there is a reason to believe that the contaminant will be partly absorbed by sediment and settle in the DWDS, and potentially at a later moment can be resuspended again, it may be needed to account for the settling (and resuspension) of the sediments and use a tool such as Aquarellus (van Summeren et al., 2022), which is a higher screening level. When the contamination occurs in the DWDS (e.g. permeation from contaminated soil into a pipe) it is important to realize that only a part of the population in the supply area will consume the contaminated drinking water. One approach is a rough estimate (e.g. 30%, Tier 1, based on the knowledge of the distribution system). However, the number of people that are affected may also depend on the time of the contamination (during the night or during the morning peak). A hydraulic network model (e.g. EPANET) (Rossman, 2000) may be used to

improve the estimate of the population that is affected (tier 2). When the contamination occurs in the home (e.g. lead from pipes or taps), a measurement can help estimate the maximum value (measure after stagnation, Tier 1) or average value (take several random daytime sample [RDT] samples, tier 2), or location specific values (proportional sampling, tier 3). When measurements are not time and work intensive (e.g. when the exposure assessment needs to be done for more than 100 homes), a hydraulic model of the drinking water installation with detailed demand patterns is an option to determine drinking water concentrations. Such a model will also allow for very specific consumption modelling. This model has been applied to determine the exposure to lead from lead pipes and appliances in the home [Dash et al., 2022].

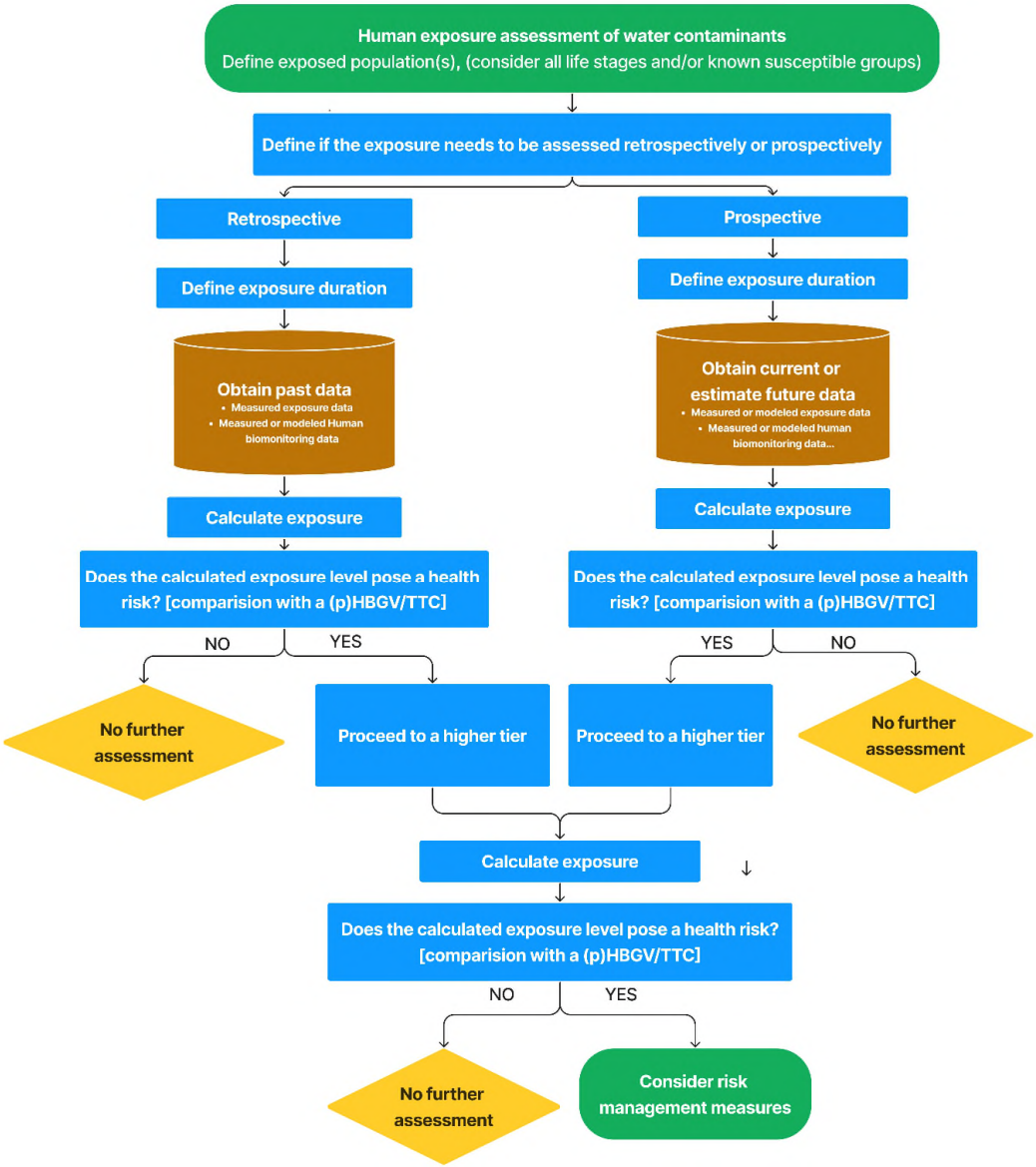

Figure 8. *Workflow for a tiered exposure assessment of chemicals in (drinking) water.*
(p)HBGV = (provisional) Health based guideline value; TTC = Threshold of Toxicological concern; ***Green*** *= Start and end of steps;* ***Blue*** *= Process;* ***Brown*** *= Data collection;* ***Yellow*** *= Decision.*

Table 13. Examples of models that can be used for exposure assessment at various screening levels (T1 lowest tier, T2, T3 and T4 as examples of higher tiers). Models and assumptions depend on the contamination location. Sources: Blokker et al., 2018, 2021; Dash et al., 2022; Hijnen et al., 2011, 2012; Mons et al., 2007; van Summeren et al., 2022. QSAR = quantitative structure-activity relationship; WTP = water treatment plant; DWDS = drinking water distribution system; RDT = Random daytime sample; WC = water closet

<table>
<tr><th>Contamination location</th><th>Concentration in drinking water</th><th>Treatment</th><th>Fate during transport & distribution</th><th>Population affected</th><th>Consumption</th></tr>
<tr><td>At the source (before treatment)</td><td>T1: measured data (in the source, after treatment or in the distribution system)
T2: source estimate + dilution (volume of water)</td><td>T1: log removal estimate
T2: log removal estimate per treatment step
T3: Treatment efficiency per treatment step using QSAR (based on chemical structure of substance)</td><td rowspan="3">T2: processes during distribution (adsorption, reaction, settling, resuspension),
T3: Aquarellus (as T2, but location specific, based on hydraulic network model)</td><td>T1: 100% in supply area</td><td rowspan="3">T1: 2 Litre per day
T2: probability distribution of daily intake
T3: as T2, and discern age groups</td></tr>
<tr><td>At WTP or in transport mains (after treatment)</td><td rowspan="3">T1: measure data
T2: source estimate + entrance process (leaching, permeation) + dilution (pipe dimensions)</td><td rowspan="3">not applicable</td><td rowspan="2">T1: estimate % downstream
T2: Hydraulic network model (e.g. EPANET)
T3: as T2, but with stochastic demands</td></tr>
<tr><td>During distribution (in DWDS)</td></tr>
<tr><td>In drinking water installation (home)</td><td>T1: max value
T2: RDT value
T3: proportional sampling or use hydraulic network model (of the home) to calculate concentrations at consumption point (part is flushed through w.c. or shower)</td><td>T1: 100% of people in the home + estimate of number of homes
T2: model adults and children separately</td><td>T1-T3: as above
T4: model consumption with hydraulic model + demand</td></tr>
</table>

3.4.4 Higher tier methods for retrospective and prospective exposure assessment

For the retrospective exposure assessment (REA), exposure concentrations can be determined using measured (drinking) water monitoring data. However, assessing past exposures to many contaminants may be challenging due to limited historical data availability, especially in case of CECs. An additional method to estimate exposure is by exposure reconstruction in a higher-tier assessment. Exposure reconstruction involves the estimation of external exposures. Reconstruction of exposure (i.e., reverse dose modelling) relies on the type of internal body measurements (e.g., measurements of biomarkers) to estimate exposure dose (e.g., daily intakes) rather than external measurements (USEPA, 2012). These exposure levels can be compared to regulatory exposure guidance values or no-effect levels in toxicity studies to determine potential risks (Clewell et al., 2008). Studies have highlighted the potential adverse impact of exposure misclassification on the human health risk assessment process, which is likely to affect the association between exposure and effects and thus underestimate the risk, thus emphasizing the importance of conducting high-quality exposure and dose reconstructions (Blair et al., 2007; Borghi et al., 2020; Grandjean et al., 2005).

For a prospective exposure assessment (PEA), current and future exposure levels can be determined using monitoring data or can be estimated through exposure modelling. Determining the internal dose in relation to specific exposure levels should be considered (if possible). The internal dose is a more direct measure for the exposure because it accounts for bioavailability[6] (Hermens et al., 2007). The internal dose can be measured using forward dosimetry to determine the actual dose to which an individual is exposed internally. The forward dosimetry approach utilises human/animal PK data or PK modelling to estimate reference biomarker concentration in relevant matrices. Similar to reverse dosimetry, forward dosimetry also uses PK models to link external exposures to biomarker concentration to evaluate the health risks and set priorities for risk assessment (USEPA, 2017). After obtaining exposure levels, the exposure rate can be determined as a part of the effort to characterise the risk. The method for calculating exposure rate is described in the publication of Gurusanker et al. (2017).

3.4.5 Exposure calculations

When calculating exposure values for oral routes of exposure, different values can be obtained depending upon the duration of exposure under evaluation and the type of health effect (cancer or noncancer). These include the average daily dose (ADD) (mg/kg bw-day) and the lifetime average daily dose (LADD). ADD is calculated for the characterisation of potential noncancer risk (when the contaminant has not been proven to cause cancer) and based on the duration may be chronic ($ADD_{chronic}$, long-term exposure lasting 7 years or more), sub-chronic [$ADD_{sub\text{-}chronic}$, short-term and intermediate exposures lasting several days and less than 7 years) and acute (ADD_{acute}, very short exposures including instantaneous exposures and those lasting up to 14 days) (MADEP, 1995). In the present study, exposures lasting upto 7 years are referred to as less-than-lifetime (LTL) exposures and the exposures lasting more than 7 years are referred to as lifetime (LT) exposures (see chapter 1).

LADD is calculated for carcinogenic risk (when the contaminant has been proven to cause cancer. The difference between these exposure measures is the averaging time (AT). The LADD is used for cancer assessments where the LADD is usually described in terms of lifetime cancer risk, even though the exposure does not occur over the entire lifetime. In these cases, AT is replaced with lifetime and the value is assumed to be 70 years (USEPA, 2003)[7]. The evaluation of exposure for the dermal route is based on dermal absorbed dose (DAD) (USEPA 2004). The method for estimating the exposure rate in a sub-chronic exposure scenario via ingestion and dermal routes is described in TEXT BOX 5. It must be noted that there is a difference in what the values for oral and dermal routes mean in terms of

[6] Bioavailability is a fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs.

[7] In E-FAST, the acute exposures averaging time is 1 day, while the chronic non-cancer exposure averaging time is 57 years.

exposure. In the case study presented in this chapter, the calculated oral exposure refers to 'external exposure' (intake), whereas the dermal exposure refers to 'internal exposure' (uptake). In the oral exposure, the contaminant still needs to go through toxicokinetic and toxicodynamic processes until it reaches the systemic blood circulation to then be active to exert effects on biological targets. In the dermal exposure, it is being implicitly assumed that the contaminant circumvents these processes by entering the circulation 'directly' and becoming immediately bioavailable.

TEXT BOX 5

$$\text{ADD or LADD}_{\text{oral}} = \frac{C_w \times IR \times EF \times ED}{Bw \times AT}$$

Where:

ADD	*= Average daily dose (intake) [mg/kg bw-day]*
LADD	*= Lifetime average daily dose [mg/kg bw-day]*
Ingestion	*= amount of substance consumed via oral intake*
C_w	*= Concentration of target chemical in water [mg/L]*
IR	= Ingestion rate of water [default intake = 2L/day adult (70 kg bw), 1L/day children (12 kg bw)
EF	*= Exposure frequency i.e. number of exposure events over the length of time [days/week or year]*
ED	*= Exposure duration is the length of time over which exposure occurs [days or weeks or years]*
BW	*= Body weight [kg] [default BW = adult (70 kg), children (12 kg), bottle-fed infants (5 kg)]*
AT	*= Averaging time (days) is the period of time over which the exposure is relevant for health risk characterization*

$$\text{DAD}_{\text{dermal}} = \frac{DA\ event \times SA \times EV \times EF \times ED}{Bw \times AT}$$

Where:

DAD	*= Dermal absorption dose [mg/kg-day]*
Dermal	*= amount of substance absorbed through contact with the exposed surfaces of the skin e.g., during showering*
DA_{event}	*= absorbed dose per event (mg/cm^2/event) (USEPA, 2004)*
SA	*= (Average) skin surface area available for contact [Cm^2]* *[children (7400 cm^2) and adults (17750 cm^2)] (Muniz- Bustamanteet al., 2022)*
EV	*= Event frequency [events/day]*
EF	*= Exposure frequency i.e. number of exposure events over the length of time [days/week or year]*
ED	*= Exposure duration is the length of time over which exposure occurs [days or weeks or years]*
BW	*= Body weight [kg] [default BW = adult (70 kg), children (12 kg), bottle-fed infants (5 kg)]*
AT	*= Averaging time (days) is the period of time over which the exposure is relevant for health risk characterization*

For non-carcinogens:	Acute exposure	AT = 1 day (by default)
	short-term and subchronic exposure	AT = ED x days/week
	[Chronic Daily Intake (CDI)]	AT = ED x 365 days/year
For carcinogens:	Lifetime	AT = (70 years) x 365 days/ year

These equations are based on equations and parameters provided in EPA EFH (EPA 2011) and EPA RAGS Part E (EPA 2004).

Depending on the underlying questions of the exposure assessment both or only one of these exposure routes are included.

Note 1: The averaging time (AT) used in calculating ADD for non-carcinogenic and LADD for carcinogenic risk estimates is usually different. For non-carcinogenic chemicals, the average exposure during the contact with a chemical is generally the relevant exposure duration for risk assessment (e.g., AT = ED × 365 days). However, for cancer risk assessment, the average duration is set to a lifetime, which is usually assumed to be 70 years (or 25,550 days) in risk assessments (WHO/IPCS, 2021) or 78 years (USEPA, 2011; ATSDR, 2018). The difference between is based on the different mechanisms for cancer and non-cancer agents.

For Inorganic chemicals or highly ionised organic chemicals DA_{event} can be calculated as follows:

$DA_{event} = FA \times Kp \times C_w$

For organic chemicals, USEPA provides two equations as shown below:

If $t_{event} \leq t^*$, *then*

$$DA_{event} = 2 \times FA \times Kp \times C_w \frac{\sqrt{6 \times \tau \times t\,event}}{\pi}$$

If $t_{event} \geq t^*$, *then*

$$DA_{event} = 2 \times FA \times Kp \times C_w \left[\frac{t_{event}}{1+B} + 2\tau\,(1 + 3B + 3B^2 / (1 + B^2)\right]$$

Where:

DA_{event}	*= absorbed dose [mg/cm²/event]*
FA	*= Fraction of 1,4-dioxane absorbed = 1 (unitless) (USEPA, 2004).*
Kp	*= Dermal permeability coefficient of target chemical in water [cm/h]).*
C_w	*= Concentration of chemical in water (measured or modelled value) [mg/cm3]*
τ	*= Lag time per event (hr/event)*
Π	*= 3.14*
t_{event}	*= Event duration [hr/event]*
t^*	*= time to reach steady state [hr] = 2.4 τ*
$t_{event} \leq t^*$	*= for short-term exposures where the duration of the exposure is shorter than the time it takes to reach steady state*
$t_{event} \geq t^*$	*= for longer-term exposures where the duration of the exposure is longer than the time it takes to reach steady state*
B	*= Permeability coefficient (Unitless).*

Guidance on these equations is detailed in Section 5.3.2 - Estimating DA_{event}, from USEPA Dermal Exposure Assessment: Principles and Applications (USEPA, 1992).

$MRL_{absorbed\ dose}$ or $RfD_{absorbed\ dose}$ = (Oral MRL or RfD) x ABS_{GI}

Cancer Slope $Factor_{absorbed\ dose}$ = Oral Cancer Slope Factor / Abs_{GI}

Following formula is used to convert the absorbed dermal dose to an equivalent administered dose

Administered Dermal Dose (ADD) = DAD / ABS_{GI}

Where:

ABS_{GI} represents the absorption of the chemical through the gastrointestinal tract following ingestion (EPA, 2004).

The full dermal absorption equation can be written as follows:

$$AAD_{dermal} = DA_{event} \times SA \times EV \times EF \times ED / (BW \times AT \times ABS_{GI})$$

For most chemicals, the absorbed dermal dose is the same as the oral administered dose because chemical is assumed to be 100% absorbed through the GI tract, thus ABS_{GI} equals 1. Therefore, no adjustment from absorbed dermal dose to administered oral dose is needed for organic chemicals. For inorganic compounds 100% GI absorption is not the case. Therefore, the absorbed dermal dose should be adjusted using the chemical specific ABS_{GI} adjustment factors listed in EPA RAGS, Part E, Exhibit B4 (ASTDR, 2018; USEPA 2004).

3.4.6 Risk characterisation

Risk characterisation integrates information from the exposure and effect assessment process and contributes to a conclusion about the nature and extent of the risk associated with the exposure to a chemical during a given exposure period. To evaluate the potential of non-carcinogenic health hazards of a chemical, a Hazard Quotient (HQ) can be

calculated by dividing the duration-specific (acute, intermediate, or chronic) exposure dose (i.e. ADD or DAD) with available non-cancer health guidelines such as RfDs (see TEXT BOX 6) (Bleam, 2012). For carcinogenic effects, risk characterisation depends on the mechanisms of carcinogenicity and the relationship between dose and carcinogenic response. Different approaches have been used to characterise the risk for toxic effects that are considered to have a threshold or non-threshold. For carcinogenic chemicals, an oral slope factor (TEXT BOX 7) can be used for oral exposure of chemicals, for example via drinking water (or food) to calculate a (increased lifetime) cancer risk (CR) that may occur from the LADD (see TEXT BOX 7). Information about the classification of the contaminant in terms of carcinogenicity can be obtained from the US National Toxicology program (NTP) (available via: https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc); USEPA Integrated Risk Information System (IRIS) (available via: https://www.epa.gov/iris) and the International Agency for Research on Cancer (IARC) (available via: https://www.iarc.fr/). For the characterisation of the risk of genotoxic and carcinogenic substances, the Margin of Exposure (MOE) approach is used, as it is not appropriate to derive a health-based guidance value (HBGV) due to the nature of these effects (i.e., the lack of a threshold of effects). In addition, the MOE approach is also used when the data are insufficient to derive a HBGV (EFSA, 2016). MOE is defined as a ratio of reference points such as benchmark dose (lower confidence limit) (BMDL) or no-observed-effect-level (NOAEL) to the estimated human exposure level or dose. BMDL is considered a more appropriate reference point because it is not scientifically valid to establish a NOAEL for genotoxic carcinogens (mediated via a DNA reactive mode of action) as there may be no threshold in the dose response relationship. The BMD approach is a preferred method by USEPA for assessing a dose response relationship (USEPA, 2012). Other authorities such as the European Food Safety Authority (EFSA) also use the BMD approach for food safety risk assessment (EFSA, 2016). When toxicity data are absent or limited, the 'Threshold of Toxicological Concern' (TTC) approach can be considered as an alternative for the MOE approach[8] (FASFC, 2018). The method for risk characterisation is illustrated in the case-study on 1,4-dioxane.

TEXT BOX 6: Risk characterisation of non-carcinogens

(a) HQ = ADD / RfD

Where:
HQ>1 implies significant non-carcinogenic health risk,
HQ≤ 1 implies that exposure may not lead to non-carcinogenic health risk

Or

(b) Calculate MOE if the data are inadequate
MOE = BMD10 (or NOAEL)/Estimated exposure dose (ADD or DAD)
MOE ≥ 100 is generally considered to be protective

Or

(c) Use TTC in absence of chemical specific data
ADD > TTC : risk unacceptable
ADD ≤ TTC : risk acceptable

$HI = HQ_1/RfD_1 + HQ_2/RfD_2 + \ldots + HQ_n/RDV_n$

Where:
HI = Hazard Index
HQ_n/RfD_n = Hazard Quotient of "n" number of chemicals in a mixture
HI>1, implies significant non-carcinogenic health risk,
HI≤ 1 implies that exposure may not lead to non-carcinogenic health risk

[8] the TTC approach is not applicable to high potency carcinogens (i.e. aflatoxin-like, azoxy- or N-nitroso-compounds), inorganic substances, metals and organometallics, proteins, steroids, substances that are known or predicted to bioaccumulate, nanomaterials, radioactive substances, and mixtures of substances containing both known and unknown chemical structures (EFSA, 2016).

TEXT BOX 7: Risk characterisation for carcinogens

(b) Calculating lifetime cancer risk associated with lifetime (LT) exposures

(1) Carcinogens with non-mutagenic mode of action

$$ILCR = D \times CSF$$

(2) Carcinogens with mutagenic mode of action

$$ILCR = \frac{D \times CSF \times ADAF \times ED}{LT}$$

(c) Calculating lifetime cancer risk associated with less than lifetime (LTL) exposures

$$ILCR = \frac{D \times CSF \times ADAF \times ED}{LT}$$

ILCR	Increased lifetime cancer risk
CSF	Cancer Slope Factor* [mg/kg-day]
D	Exposure dose [mg/kg bw-day]
ED	Exposure duration [years]**
LT	Lifetime [years] (considering 70 years average expectancy)
ADAF	Age dependent adjustment factors (10 for children 0 < 2 years; 3 for children to < 16 years; = 1 for children ≥ 16 and adults.

To obtain the overall risk for a 70-year period that (initiated at birth), the risk is calculated for each age group and exposure periods and then added together to obtain the total. It is also possible to compute cancer risk can also be calculated for any exposure duration combined with the doses for the target age group (USEPA, 2011).

Cancer risks will be considered negligible where the estimated cancer risk is 1-in-100,000 ($\leq 1 \times 10^{-5}$) (WHO, 2022) or 1-in-1,000,000 ($\leq 1 \times 10^{-6}$) (van der Aa et al., 2017). The risk exceeding 1-in-10,000 ($\geq 1 \times 10^{-4}$) is considered unacceptable (USEPA, 1992; Nag & Cummin, 2022).

**The cancer slope factor (CSF) converts estimated daily intake averaged over a lifetime of exposure directly to incremental risk of an individual developing cancer (USEPA, 1989). For carcinogenic chemicals, the US Environmental Protection Agency (USEPA) has an oral slope factor (or an inhalation unit risk). Oral slope factor can be used for oral exposure of chemicals, for example via drinking water (or food) to calculate an increased excess Lifetime Cancer Risk (ILCR) that may occur from the LADD. This number indicates the number of additional cancer cases that could be expected in a population of one million people exposed to a given concentration of pollutants and a given level of intake during a lifetime of 70 years.*

*** Example: less-than-lifetime (LTL) exposure of 6 months = 0.5 years*

(c) Calculate MOE if the data are inadequate

MOE = BMD10 (or NOAEL)/Estimated exposure dose (ADD or DAD)

MOE banding
MOE > 1,000,000 : highly unlikely to be a concern
MOE 10,000 - 1,000,000 : unlikely to be a concern
MOE < 10,000 : may be a concern

Or

(d) Use TTC in absence of chemical specific data

3.5 Discussion

In a retrospective risk assessment strategy, one is limited to adopt responsive actions when confronted with unavoidable past exposure events (reactive approach), whereas in a prospective risk assessment strategy, one can also choose to adopt anticipatory actions to avoid future exposure events (proactive approach). Risk quantification is often the result of a reactive approach (action in response to), from which an exposure-based Hazard Quotient (HQ)

is estimated. In the present study it is emphasised that the HQ can also be used as a target risk value in a proactive approach (action in anticipation to), from which protective exposure limits can be derived. This approach can be of particular interest to water managers in search of pragmatic tools for risk prevention, mitigation, or reduction.

Under unknown future exposure scenarios, a prospective approach is most desired to design a well-informed risk management plan. However, the reliability of this approach may be reduced if (1) input information is mostly reliant on experimental animal studies without supplementary data from new animal-free approach methodologies, human biomonitoring programs and epidemiological investigations, (2) little is known about the environment (e.g., availability and quality of water sources, seasonal variability, aging infrastructure, contaminants of emerging concern) and (3) the target population composition and behaviour is not characterised as this may overlook vulnerable sub-population groups (e.g. children, breastfeeding women, immunosuppressed individuals). In order to address the uncertainties associated with worst-case assumptions, adopting measures closely resembling an actual exposure scenario can help substantiate and provide important input, such as human biomonitoring data. Therefore, integrative human exposure *in silico* models combined with human biomonitoring data collected from relevant human tissue samples, are a valuable tool in estimating potential risk under various plausible environmental conditions and ahead of new human exposure events. The contributions of these complementary approaches to drinking water quality research will allow to further understand how drinking water contaminants contribute to the human exposome and adverse health effects (Schullehner et al., 2023).

In this report, a risk assessment method has been presented and illustrated in the context of potential effects of drinking water contaminants to human health. This was done from a retrospective and, particularly, a prospective viewpoint. Safe drinking water is essential for everybody. Nevertheless, the parameters established to estimate human exposure to water contaminants are often based on selected population statistics (e.g., body weight). This makes the models more widely applicable but may compromise their utility and contextual relevance, as these are not tailored to capture the variability and uncertainty across, for example, sub-population composition, vulnerable groups, and local exposure conditions. Humans can be exposed to a multitude of contaminants through different exposure pathways, behaviours, and concentrations that can vary substantially over space and time. Therefore, local and regional water companies and health authorities are consistently vigilant about water quality to ensure that human health is sufficiently protected. In order to further improve the contextual reliability and relevance of the risk values derived in the present study, aspects such as physical- and behavioural-specific data may be considered as input parameters which are not considered in the present study. This can be further refined to address local and regional water managers concerns, by the provision of locally relevant drinking water usage and customer group profile information. In addition, simultaneous exposure to several chemicals at low concentrations is common in drinking water, and the complex low-level mixture of chemicals can vary in time and concentration (WHO, 2017a). Therefore, a detailed description of the occurrence of (new and existing) chemicals in different water supplies is important to assess any risks to human health. In addition, the ease of monitoring and thorough removal from drinking water are also important considerations in exposure and risk mitigation and can be considered for follow up study.

The exposure assessment methods presented in this report collectively contribute to a comprehensive yet semi-detailed exposure assessment. These methods provide the basis for a more robust and informed research in the domain of water quality limit setting and future research related to drinking water quality. However, it is important to point out that the applicability of these methods depend more on the nature of the research question. These methods can serve as a tool to form informed opinions and improve our understanding in assessing human exposure to contaminants in drinking water. In addition, these methods allow us to tailor our investigations and responses, ultimately leading to a more nuanced view of water quality and its implications on public health.

4 General discussion

The increasing presence of anthropogenic substances in water systems poses significant challenges to human health and the environment. Despite technological advancements, effectively addressing the health risks associated with anthropogenic substances in water systems remains a challenge. The evolving landscape of chemical use and pollution requires constant refinement and improvement of risk assessment methods. Therefore, integration of new scientific knowledge, improved data quality and availability, and accessibility of risk assessment processes is crucial in this regard. In this context, we followed the external developments in toxicological and health risk assessment in this project, to be able to advise drinking water companies with an advanced knowledge base. The main objective was to update our existing knowledge and gain new insights relevant to human hazard and risk assessment. To achieve this, we were actively involved in ongoing (inter)national projects that are at the forefront of knowledge development of (innovative) risk assessment methods (see Appendix IV). Our collaborative efforts also extend to working with academic and industrial partners within consortia. By participating in these activities, we ensure that we keep abreast of the latest advancements in the field and thus contribute to an informed advisory role. In addition, we prioritised the dissemination of the interim results of this project externally. This involved (re)presenting KWR at an (inter)national congress focused on toxicology and risk assessment. This proactive engagement not only ensured to keep our knowledge aligned with the latest developments but also facilitated knowledge exchange with the experts in the field.

In recent years, there have been several relevant developments in the field of hazard and risk assessment that have the potential to contribute to future-proof drinking water practises. Building on these recent findings, new developments are foreseen to further improve water quality assessment and efficiently meet the demand for safe drinking water in a time of constant water quality challenges. An important activity in this report was to reflect on developments from and outside the BTO and their implications, and to provide an outlook on initiatives of interest for future research (see Appendix V). As part of this activity, the BTO and KWR reports issued in the period 2018-2023 were screened for their content on toxicology and risk assessment. Research that met this criterion have been summarized in this chapter. This look ahead builds upon previous BTO research and external developments and summarises the research directions for the coming 6-year period of the BTO (and beyond) that will contribute to better risk assessment of chemicals in water.

In addition to the advancements highlighted in the current report, there are other research directions that need to be explored to address the challenges posed by anthropogenic substances in water systems. An important area for future investigation is the gathering knowledge for development of a risk assessment methodology specifically tailored to chemical mixtures, which was not considered in the current study. Understanding the combined effects of multiple micropollutants in water is essential for the accurate assessment of health risks. Furthermore, it is essential to investigate internal exposure doses for human exposure assessment, as the present study focused exclusively on external doses. The inclusion of internal exposure assessments through Physiologically Based Kinetic (PBK) modeling, will provide a more comprehensive understanding of the potential health effects associated with exposure to chemicals via different routes, including drinking water, inhalation and dermal contact. By considering both external and internal exposure doses, a more accurate risk assessment can be performed to effectively protect human health. Moreover, considering both external and internal exposure doses facilitates the development of targeted risk reduction strategies tailored to specific exposure routes and sensitive populations. Incorporating these recommendations into future research efforts will contribute to the ongoing refinement and improve risk assessment methods and ultimately improve our ability to mitigate the health risks posed by contaminants in water systems.

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5 Appendices

I Appendix: Less-than-lifetime (LTL) risk assessment case – study

Illustrative case-study: Lead

Pb contamination of drinking water is a problem of public concern, and regulators across the world are focussed on taking actions in the interest of public health (ATSDR, 2020; Lim et al., 2012). The method for the LTL risk assessment of chemicals in (drinking) water is illustrated with a case study on modelled fluctuating concentrations of lead (Pb) in drinking water. Several other suitable case studies were suggested by experts from various drinking water utilities. Inclusion of more than one case study was beyond the scope of this research.

I.I Identification of chemicals in (drinking water) (step 1)

Low-level Pb exposure has long been associated with adverse health effects, particularly in children. However, health concerns in adults also exist (ATSDR, 2020). According to the latest technical report by WHO, a proactive approach should be taken to identify, assess and manage Pb in drinking water (WHO, 2022). This includes understanding Pb sources in drinking water, monitoring Pb levels in drinking water (including in supplies known or suspected to contain Pb materials) and adopting appropriate procurement and installation programmes to prevent the introduction of Pb into new water systems. Tap water from old Pb water pipes in the premise plumbing (in-house installation) accounts for a large portion of total daily Pb exposure of the people living in these houses. Even in newly built homes with new plumbing and faucets, the brass fittings can lead to undesired concentrations of Pb in drinking water, this can be most prominent in the first few months of use (Elfland et al., 2010).

To assess whether a consumer is exposed to undesirable amounts of Pb, sampling protocols play a crucial role. Sampling protocols appear in various forms and each protocol typically has its own objective (for example, to address Pb exposure at a community scale, Pb exposure at a household scale, presence of Pb releasing components, localisation of Pb releasing components). To test the effectiveness of the various sampling protocols in a controlled environment, simulations of a premise plumbing system were performed using EPANET. EPANET is a software application used to model water distribution systems and as a tool to understand the transport and fate of drinking water and its micropollutants in distribution systems. In this case, it was applied to a domestic drinking water system. Advantages of this method (modelling) include control and knowledge of the location of Pb-releasing components and Pb dissolution behaviour, the ability to control water demand patterns as well as the ability to accurately measure lead exposure (Dash et al., 2022). It should be noted that the modelling has its own simplifications and some aspects that occur in reality (such as the occurrence of particulate Pb) may be missing in the simulations.

I.II Exposure assessment (step 2)

Data for the exposure assessment were obtained by modelling the flow of dissolved Pb in water using EPANET (Dash et al., 2022). In this case, a typical (fictitious) Dutch house with water usage spread over three floors was considered, and the Pb exposure in an average family with two children was estimated (see page 5-7 in KWR 2022.075). The starting point of the simulations was to define the indoor plumbing system. For this purpose, the lengths, and diameters of the pipes as well as the points-of-use were defined. Information on the point of use, and composition of the household (number of adults and children, attitude towards water usage) was fed into SIMDEUM which is a stochastic drinking water demand model (Blokker et al., 2017). SIMDEUM generates water demand patterns for various points-of-use based on the input information. The output from SIMDEUM is coupled back to the EPANET model. The leaching of dissolved Pb from various pipes is modelled.

Using the modelling framework, consumption was evaluated for a household on a weekly basis, for a total period of 20 weeks to illustrate the method. In the simulation considered for Pb exposure, Pb is assumed to leach from the pipes (in total one metre long and 32mm in diametre). It is assumed that the water from the distribution network contains no lead. The plumbosolvency (Pb concentration at equilibrium) is assumed to be 110 µg/L, whereas the Pb dissolution rate is assumed to be 0.115 µg/(m^2s). These values are known to be dependent on parameters such as water chemistry and temperature (Mededeling nr. 096, KIWA). However, these factors were considered as constants and not considered further in this study.

In the present case study, modelled Pb levels in tap water varied (fluctuated) between 2.4 – 5.4 µg /L (average 3.8 µg /L) (fluctuating exposure, see Figure 1) over a period of 20 weeks at the kitchen tap (for cold water). The average Pb concentration computed using the modelling framework includes all events (drinking water, cooking food, rinsing dishes, washing hands etc) and no further disaggregation has been performed. We applied a conservative approach and used the peak concentration of 5.4 µg /L (assuming that the concentration remains same throughout 20 weeks). Exposure is estimated for two groups; children (at extra risk) and adults (as the general population), following the USEPA method and the publication of Alidadi et al., (USEPA, 2011; Alidadi et al., 2019) with some modifications [e.g., default body weight is based on value selected by EFSA (EFSA, 2012a)]. The differences in the exposure doses between children and adults are based on the difference in their body weight (see Table 13).

I.III Hazard assessment (step 3)

The adverse effects of Pb are well known, and it is probably the most extensively studied heavy metal. Studies have reported the presence of various cellular, intracellular, and molecular mechanisms behind the toxicological manifestations of Pb in the body (Gillis et al., 2012; Shvaciy et al., 2022; Virgolini & Aschner, 2021). Pb is known to induce neurological, respiratory, urinary, and cardiovascular disorders due to immune-modulation, oxidative, and inflammatory mechanisms (ATSDR, 2020; Balali-Mood et al., 2021). Childhood exposure to Pb is associated with long-term decreases in intelligence quotients (IQ) (Halabicky et al., 2022). The evidence for the carcinogenicity of Pb in humans is inconclusive because of the limited number of studies, and the failure to adequately account for potential confounding variables. Previously, Pb and inorganic Pb compounds were classified in Group 2B (possibly carcinogenic to humans), whereas organic Pb compounds were classified in Group 3 (not classifiable as to its carcinogenicity to humans). The most recent IARC evaluation resulted in an upgrading of inorganic Pb compounds to Group 2A (probably carcinogenic to humans) whereas organic Pb compounds remain in Group 3 (IARC, 2022; Rousseau et al., 2005; WHO, 2016). However, the IARC Working Group noted that some of the organic Pb is metabolized into ionic Pb, which is expected to have same toxicity as inorganic Pb (Rousseau et al., 2005). Based on the Drinking Water Directive (DIRECTIVE (EU) 2020/2184, EU, 2020) and the World Health Organisation (WHO, 2016), the permissible limit of Pb in drinking water is set as 10 µg/L. It should be noted that the guidance value (GV) of Pb (10 µg/L) is designated as provisional on the basis of treatment performance and analytical achievability and is no longer health-based. Concentrations are required to be maintained as low as reasonably possible (ALARP), with the objective of meeting a target value of 5 µg/L (WHO, 2016). However, Pb is not an essential element (elements that are necessary or beneficial for health), and there is no safe level of Pb exposure (Flora et al., 2012; Vorvolakos et al., 2016; WHO, 2022).

I.IV Risk characterisation

Here we estimate both the non-cancer risk of Pb based on the ADD calculated from the (modelled) exposure data and cancer risk (as Pb is classified as Group 2A by IARC). We used hazard quotients (HQ) to estimate the non-cancer health risk of Pb in (drinking) water. The calculation for HQ requires information on the exposure concentration of Pb in drinking water and the RfD for Pb that is likely to be without an appreciable risk of harmful effects during a lifetime exposure. Exposure concentrations were obtained by modelling the flow of dissolved Pb in tap water. In recent assessments, the authorities do not recognise a safe level for Pb exposure, therefore, there is no recommended reference dose (RfD) / health-based value for Pb. However, different oral RfDs have been reported in the literature (1.4×10^{-3} to 4×10^{-3} mg/kg bw-day) (Aendo et al., 2019; Guo et al., 2018; Nag & Cummins, 2022). In our study, the

HQ for Pb was calculated based on the lowest (precautionary) RfD of 1.4×10^{-3} mg/kg bw-day (1.4 µg/kg bw-day). To estimate the carcinogenic health risk of Pb in (drinking) water, we used the oral cancer slope factor for Pb. EPA has not developed an oral slope factor for lead because of the many uncertainties, some of which may be unique to Pb. We used the oral slope factor of 0.0085 mg/kg-day derived by OEHHA (OEHHA, 2023). The results obtained based on the measured exposure concentrations (adults: 0.15 µg per kg bw-day, children: 0.45 µg per kg bw-day) and the oral CSF were compared with the already established risk limit of 1-in-1,000,000 (≤ 1 x 10-6) (van der Aa et al., 2017). The results showed unacceptable cancer risk in adults in an assumed short-term exposure scenario of 20 weeks in both age groups. Furthermore, the risk was higher in children. However, it must be considered that this case study is based on a worst-case scenario in which the maximum measured concentration of Pb in the treated water was used in the risk characterisation. The present case study was used to illustrate the method and not intended for comprehensive risk assessment (i.e., considering variability and uncertainties associated with spatiotemporal contexts, population-specific behaviours, age-specific anthropometrics, vulnerable sub-population groups). However, in a real life scenario, if an unacceptable risk is identified, it is recommended to refine the risk assessment to reach a final conclusion on the risk. This could include more comprehensive exposure assessment that take into account various factors, such as the internal body concentrations variability and uncertainties associated with spatial and temporal context, population-specific factors, and vulnerable populations.

Table 13. *Risk characterisation in two age groups based on non-cancer and cancer effects.*

Exposure duration	Population	Exposure type	Exposure dose	Non-Cancer effects		Cancer effects	
	Age group		(mg/kg/bw-day)	HQ	Risk	ILCR	Risk
LTL	Adults	ADD_{oral}	1.5×10^{-4}	<1	acceptable	6.99×10^{-6}	unacceptable
	Children	ADD_{oral}	4.5×10^{-4}	<1	acceptable	6.29×10^{-5}	unacceptable

Note: LTL = less than lifetime; LT = lifetime; ADD = average daily dose; DAD = dermal absorption dose; LADD = lifetime average daily dose; HQ = hazard quotient (calculated only for non-cancer effects); CR = cancer risk; - = non cancer effects do not apply.

I.V Discussion

The main objective of developing a method for LTL risk assessment was to characterise risks of realistic chemical exposure scenarios that represent exposure over (very) short or intermediate periods.[9] A modelled 20-week exposure to lead (Pb) in a typical Dutch household was used as a case study to illustrate the application of our method for LTL risk assessment of (drinking) water (assuming no lead exposure occurs before or after the 20-week period), because in some parts of the Netherlands and beyond, houses (and buildings) contain Pb pipes, and consequently there is a risk of leaching of Pb from Pb-containing components/materials (WHO, 2022). The composition of the indoor water distribution system of houses and buildings can have an impact on the water quality at the tap and determine the potential Pb exposure. The indoor water distribution system of a house is in principle not the responsibility domain of the drinking water utilities but of the house owner and is of concern for the drinking water utilities. One of the strengths of this case study was the innovative exposure assessment using a modelling framework in a typical (fictitious) Dutch house via (drinking) tap water (an added value of our modelling approach is that it shows the concentrations at shower and could thus also serve to determine the exposure through inhalation of aerosols or dermal contact) (Dash et al., 2022).

The concentration of Pb in (drinking) water depends on the type of material, general water chemistry, and temperature. However, these factors were not considered in this study and can be addressed in future research (risk management). In addition, the average Pb exposure includes all events (drinking water, cooking food, washing dishes, washing hands, etc.). In the present case study, no further disaggregation was made but this can be addressed in future research, in which the contribution of oral and dermal exposure can be addressed. Furthermore, an important

[9] Exposure modelling was initially carried out for all Dutch water utilities, outside the BTO, later on, the next step was carried out within the BTO). There was also a predecessor, which was done for the Ministry of Infrastructure (I&W) (KWR 2021.004).

criterion to be considered is the time of exposure (e.g., if exposure in the morning is higher than in the afternoon). The WHO currently recommends a parametric value of 10 µg/L (or as low as reasonably practicable), while in the future (by 12 January 2036), a stricter standard of 5 µg/l is required to be met (Directive (EU) 2020/2184). The modelling framework presented in the case study is of particular interest to Dutch water utilities that want to know if copper pipes with Pb soldering and/or brass components or any specific components in the in-house distribution system can lead to the exceedance of the forthcoming stricter standards.

In the present case study, the health risk was estimated based on a risk threshold, although it is generally assumed that there is no safe threshold for Pb exposure (Vorvolakos et al., 2016). HQ was used to characterise the non-carcinogenic risk of Pb at a modelled (drinking) water concentration of 5.4 µg/L and a duration of 20 weeks. HQ was lower in adults (0.11) than in children (0.32). Nevertheless, both values are lower than the safe level of 1, indicating no potential human health effects at this concentration and duration of exposure. The difference in the HQ is assumed to be due to the difference in body weight, which is 70 kg for adults and 12 kg for children. The cancer risks estimated in the present study were all above the acceptable level of 1×10^{-6} in both population groups (van der Aa et al., 2017). Compared to the adults, the risk was higher in children. In the present study, a maximum measured concentration of Pb in treated water is used for characterizing its risk to human health, under a worst-case scenario approach. While this approach is methodologically sound, it should be emphasized that this represents an extreme and unlikely exposure scenario. In this case study, the health risk assessment for the carcinogenic and non-carcinogenic effects is not considered adequate to allow calculation of a (LTL) GLV for Pb in drinking water. These values only provide an indication of the levels at which adverse health risks may be considered when evaluating lead exposure in drinking water for LTL scenarios. However, expanding modelling to encompass additional exposure scenarios can provide more insight into possible health risks. Based on the assessment, management decisions and/or mitigatory measures can be taken. Overall, our results demonstrate that LTL risk assessment can be useful for evaluating the potential short-term presence of chemicals in water intended for human consumption. Based on the results of the LTL risk assessment, risk mitigation can be considered, such as avoiding water distribution components that have an impact on the exposure and using materials that meet the minimum requirements for materials in contact with water intended for human consumption, as specified in the Drinking Water Directive (Directive (EU) 2020/2184).

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II Appendix: Probabilistic risk assessment (PRA) case-study

Illustrative case-study: Simazine

The objective of this fictional case-study on simazine is to illustrate the application of PRA method for the human health risk assessment of a chemical contaminant in (drinking) water.

II.I Chemical identity

Name:	Simazine
CAS registry number:	122-34-9
Synonyms:	6-chloro-N, N'-diethyl-1,3,5-triazine-2,4-diylamine; Gesatop; Princep, Simanex
Molecular formula:	C7H12ClN5
Molecular weight:	201.66
log Know:	2.18

II.II Problem formulation

Simazine is a member of triazine family, a group of chemicals commonly used as broad-spectrum herbicides due to their inhibition of electron transfer in photosynthesis (Elmore & Lange, 2008; Grasselli et al., 2018; Qian et al., 2014). These herbicides have been used extensively in the United States, Europe, and Australia for more than 50 years (Breckenridge et al., 2016). Compared to other herbicides, triazines are more water soluble and can leach from soils into surface and ground waters; therefore, contaminating sources for drinking water production which can be a concern both for human and animal health. Simazine has been banned in most European countries since 2004. It has not been detected anymore in surface and ground waters of Dunea, PWN and Waternet in the last five years (personal communication) . However, it was reported the second most detected pesticide in surface waters and ground waters in different regions worldwide not very long ago (Grasselli et al., 2018; Sai et al., 2015). Conventional treatment processes have been reported to be less effective in removing simazine from (drinking) water supplies, however, improved treatment methods such as a combination of activated carbon and ozone have been shown to be effective in its removal (Health Canada, 1989; Aldeguer Esquerdo et al., 2020).

II.III Hazard identification

Human and animal health concerns resulting from simazine are due to its potential endocrine disrupting effects, that can lead to reproductive disorders (Grasselli et al., 2018). While carrying out an investigation on the hazard simazine (or any other chemical of concern) poses, it is important to seek further information from the emitters (industries, companies, etc) and (local) authorities as to what else can be of concern (e.g., its transformation product simazine-2-hydroxy). Data on the effects of simazine can be obtained by looking in the (inter)national guidelines, databases (ECHA, eChemPortal, TOXNET, IPCS, IRIS, USEPA, ATSDR, HSDB, IARC, CompTox, PPDB) and websites of recognized authorities in the field of health protection (including RIVM, EFSA, USEPA and EU committees) for toxicological data as well as relevant scientific literature. According to WHO (WHO, 2022), simazine does not appear to be genotoxic in mammalian systems. Studies have shown an increase in mammary tumours in the female rat but no effects in the mouse. IARC has classified simazine in Group 3 (not classifiable as to its carcinogenicity to humans).

II.IV Gathering information on exposure scenarios

Information needs to be collected related to ways people can come into contact with simazine, how much exposure is likely to occur and for how long the exposure is likely to occur. For illustration, let us assume that the main source of simazine exposure (to the most sensitive population) is via (drinking) water and the mean detectable concentration of simazine in different river basins is ≤ 0.03 µg/L (0.00003 mg/L). Three removal efficiencies are considered: (good

removal (90-99%), moderate removal (45-55%) and bad removal (0.001-10%) for simazine in the treatment processes.

II.V Hazard characterisation

Table 14 shows the inputs on the (critical) endpoint, the type of study in which the critical effects were observed, and the protection goals that define the target human dose (HD_M^I). In this context, "M" denotes the magnitude of effect on individuals, and "I" signifies the incidence goal. For example, HD_{05}^{01} represents the dose at which 1% of population would experience an effect of 5%. For a detailed description of the hazard characterization and guidance on selecting the appropriate input, the readers are referred to WHO-IPCS (2017) and the publication of Bokkers et al (2017). In the present illustration, we use the same study for input values as used by WHO for deriving the drinking water guideline value for simazine (WHO, 2003). Weight changes, effects on haematological parameters and an increase in mammary tumours identified as critical endpoints in a chronic study in the rat are used as input parameters (NOAEL= 0.52 mg/kg bw/day = 520 µg/kg bw/day; allocation to water = 10% of TDI; weight = 60 kg adult; water intake = 2 litres/day) (WHO, 2003; 2022). The protection goals (Target benchmark response M[10] and the incidence goal[11] I) are set to 5% and 1%, respectively. The coverage (WHO- IPCS, 2017), reflecting the probability that the probabilistic RfD is small enough (i.e., smaller than the true HD_M^I), is set to 95%. After applying these values, a probabilistic RfD of 0.00052 mg/kg bw/day (0.52 µg/kg bw/day) can be derived. This reflects the human dose at which, with 95% coverage (confidence), 1% of the population would have a ≥5% reduced body weight. Or, in other words, the human dose at which 99% of the population would experience a decrease in body weight of less than 5%. In Table 15, the probabilistic inputs related to various hazard characterization aspects are listed and Table 16 shows the contribution of each source of uncertainty on the total uncertainty in hazard characterization provided by APROBA- Plus tool based on the information in Table 14 and 15. Table 16 shows that the uncertainties are mostly related to NOAEL and intraspecies differences.

[10] Benchmark response is a predetermined change in response (BMR). Normally, the default BMR is 5% or 10% change in the response rate of an adverse effect relative to the response of control group depending on whether response data are continuous or quantal (dichotomous).

[11] Incidence goal is a fraction of the population not being protected from that magnitude of effect.

Table 14: Parameters used in the APROBA-Plus tool associated with an experimentally derived NOAEL for rats used as input in the probabilistic risk assessment.

Inputs related to study, endpoint and protection goals		
DESCRIPTION	**INPUTS**	**COMMON VALUE(S)**
End-point	Decreased weight in rats	Case-specific
Data type	Continuous	Case-specific
Data route	Oral	Case-specific
Study type	Chronic	Case-specific
Test species	Rat	Case-specific
Body weight test species (kg)	0,400	0,4
Human median body weight (kg)	60	60
Target BMR (= *M*, user input for BMDLs only)	5%	5%
Population incidence goal (= *I*)	1%	5%, 1%, 0.1%, 0.01%
Probabilistic coverage goal	95%	95%
PoD type	NOAEL	Case-specific
PoD value	520	Case-specific
BMDU (User input for BMDL PoDs)		Blank if PoD is NOAEL/LOAEL
PoD units	µg/kg body weight per day	µg/kg body weight per day
Deterministic overall AF	1000	Case-specific
Deterministic RfD	0,00052	Calculated

II.VI Exposure assessment

As mentioned above, the exposure assessment is based on a range of simazine concentrations in (drinking) water sources of 0.03 µg/L. Assuming three different ranges of removing efficiencies and applying the default body weight of 60kg, an allocation of 10%[12] (WHO) and assuming an intake of 2 litre of drinking water per day, the resulting concentration in (drinking) water will result in a human exposure of :

$$(0{,}03/60/0.1) * 2 * E_r$$

in which Er refers to the lower and upper bound removal efficiencies for three scenarios (good removal of Simazine (90-99%), moderate removal (45-55%) and bad removal (0.001-10%). This yields the following intakes: of 8.6 x 10^{-9} - 8.6 x 10^{-8} µg/kg bw/day (good removal), 7.7 x 10^{-7} - 8.6 x 10^{-7} µg/kg bw/day (moderate removal) and 3.7 x 10^{-7} - 4.9 x 10^{-7} µg/kg bw/day (bad removal).

Table 15: Inputs related to variability and uncertainty related to the hazard characterization of Simazine.
LCL= Lower Confidence Limit; UCL = Upper Confidence Limit

Inputs related to adjustment, variability and uncertainty			
HAZARD CHARACTERIZATION ASPECT		INPUTS	PROVISIONAL VALUE(S)
PoD	LCL	0,52	Calculated from inputs
(Modelled BMD uncertainty)	UCL	0,52	Calculated from inputs
NOAEL to BMD	LCL	0,07	0,07
(NOAEL or LOAEL only)	UCL	1,57	1,57
Interspecies scaling	LCL	3,68	3,68
(Allometric for oral)	UCL	5,49	5,49
Interspecies TK/TD	LCL	0,33	0,33
(Remaining TK & TD)	UCL	3,00	3,00

[12] As this case study is based on the WHO drinking water guideline for simazine (WHO, 2003). Therefore, we use the same input values (60kg body weight, 10% allocation factor) as used by WHO and not the standard values (70kg body weight, 20% allocation factor) used by RIVM.

Duration extrapolation	LCL	1,00	1,00
	UCL	1,00	1,00
Intraspecies	LCL	2,24	2,24
	UCL	41,88	41,88
Other aspect #1	LCL	1,00	1,00
Carcinogenicity	UCL	10,00	1,00

Table 16: Contribution of various sources of uncertainty to the total uncertainty in the probabilistic risk assessment of Simazine.

Intermediate calculations for uncertainty analyses	% Contribution
ASPECT	to overall Uncertainity
PoD	--
NOAEL to BMD	34%
Interspecies scaling	1%
Interspecies TK/TD	17%
Duration extrapolation	--
Intraspecies	30%
Other aspect #1	19%
Carcinogenicity	

II.VII Risk characterisation and sensitivity analysis

From the graph (Figure 9), the uncertainty ellipse for the target human dose and exposure lies towards the green area, with the uncertainty in HD_M^I playing a bigger role than variation/uncertainty in exposure levels. Thus, the chances are that the protection goals are met to a major extent. For the protection goals to be completely met, the eclipse should be fully in the upper green left corner. One option would be to perform a BMD modelling on the original dose-response data (if available) rather than the NOAEL, as this was found to be a major source of uncertainty (accounting for 34% of the overall uncertainty) in the estimated target human dose. If the data set is not suitable for such modelling, additional experiments that allow BMD modelling could be a priority. Having this information available could result in a shift of the entire ellipse upwards into the upper green area from which would be concluded that the protection goals are very likely to be fully met.

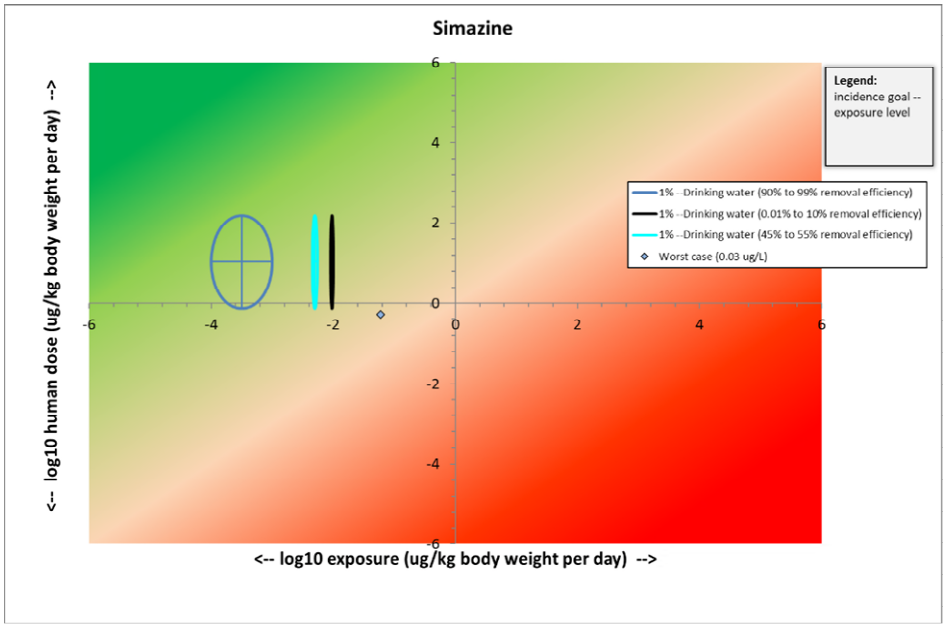

Figure 9: *Graph depicting the probabilistic risk assessment of Simazine, comparing human exposure (in µg/kg body weight per day) to hazard (in µg/kg body weight per day). Three scenarios are taken into account: one in which 90-99% of all Simazine is removed (left ellipse) through conventional drinking water treatment, one in which 45-55% of all Simazine is removed (middle) and one in which only 0,01-10% (right) of all Simazine is removed.*

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III Appendix: Human exposure assessment (HEA) case-study

Illustrative case-study: 1,4 - dioxane

This case study serves to illustrate the human exposure assessment method presented in chapter 3 using 1,4-dioxane in water as an example. 1,4-dioxane was selected as it is classified as a Substance of Very High Concern (SVHC) under Article 57 (a) and (f) of REACH and a probable human carcinogen (ECHA, 2012; Tang & Mao,2023) and it has occasionally been measured at relatively high concentrations in the surface water (Meuse & the Rhine River) used for abstraction of drinking water (KWR, 2019). In addition, 1,4-dioxane is easily distributed in the aquatic environment due to its high affinity to water and is difficult to remove from raw water by drinking water purification processes as well as from wastewater by conventional wastewater treatment processes (Ginsberg et al., 2022; McElroy et al., 2003; Tang & Mao,2023).

III.I Substance information

1,4-dioxane (CAS number: 123-91-1) is a synthetic heterocyclic organic compound, consisting of a six-ringed structure, and classified as an ether. It is a colourless, volatile liquid at room temperature with a faint sweet odour, similar to that of diethyl ether. It is miscible with water, most organic solvents, aromatic hydrocarbons, and oils (NCBI, 2022). Apart from 1,4-dioxane, there are two more isomers, 1,2-dioxane and 1,3-dioxane that are rarely encountered in the environment. 1,4-dioxane is a widely used organic solvent in the chemical industry, in insecticides and herbicides, and as a stabiliser for chlorinated solvents (particularly 1,1,1-trichloroethane) (ATSDR, 2012; NCBI, 2022). It also occurs as a by-product in various chemical reactions used to produce polyesters, soaps, and plastics [e.g., polyethylene terephthlalate (PET)] (Mohr, 2010).

1,4-dioxane dissolves completely in water, even at high concentrations and does not readily evaporate, making its removal difficult using traditional water treatment systems (Lee et al., 2021). Based on scientific evidence of serious effects of 1,4-dioxane on the environment, its high persistency, and its mobility in the aquatic environment, together with its classification as a Carcinogen category 1B (carcinogenic potential for humans largely based on animal evidence), it is a priority substance in regulatory risk assessment. Under EU's REACH regulation, 1,4-dioxane is classified as a substance of very high concern (SVHC) (Article 57(f) of regulation 1907/2006) (ECHA, 2021) as well as by the Dutch National Institute for Public Health and the Environment [Rijksinstituut voor Volksgezondheid en Milieu (RIVM)] [13]. As a result of its wide industrial use, it is reported to be a common constituent of certain industrial wastewaters and has also been found up to 80 µg/L, 40 µg/L and 1 µg/L in groundwater, surface water, and drinking water, respectively (ECHA, 2021; Hale et al., 2022; WHO 2022). Due to its frequent occurrence in (drinking) water sources, it was put forward by the Dutch Association of River Water Companies (RIWA) as a drinking water relevant compound and included in the list of candidate substances for the monitoring program for 2014 of the International Commission for the Protection of the Rhine (ICPR/ICBR, 2011; RIVM, 2012). Given the prevalence of 1,4-dioxane in Dutch (drinking) water sources, there could be a potential health risk if these concentrations survive the treatment and penetrate drinking water. Therefore, continued monitoring and reporting of 1,4-dioxane concentrations in raw and treated water is mandatory in the Netherlands.

In contrast, 1,4-dioxane is unregulated by the United States Environmental Protection Agency (USEPA) and there is no enforceable maximum contaminant level (USEPA, 2017), while the World Health Organization (WHO) and several other countries have proposed (health-based) guidance values for 1,4-dioxane in drinking water. These values span a wide range of concentrations (3 - 50 µg/L) and are subject to considerable variation, due to the differences in

[13] Totale ZZS-lijst | Risico's van stoffen (rivm.nl)

extrapolation methods from high doses (animal studies) to the much lower doses expected in humans (e.g., via ingestion of contaminated drinking water) and lack of epidemiological studies (Ginsberg et al., 2022). In the Netherlands, the current drinking water guideline value for 1,4-dioxane is 3 µg/L (RIVM, 2018). In a recent development, RIVM has issued its opinion regarding the derivation of drinking water guideline value for 1,4 dioxane, revising the guideline value to 38 µg/L (available via: https://www.rivm.nl/documenten/rivm-opinie-over-afleiding-drinkwaterrichtwaarde-14-dioxaan-cas-123-91-1-door). This health-based value can be considered as an advisory value for policy makers regarding the indicative drinking water guideline value for 1,4-dioxane which is officially communicated on the RIVM website "Risico's van stoffen" (accessible via: https://rvszoeksysteem.rivm.nl/).

III.II Exposure assessment of 1,4-dioxane in (drinking) water

In the present case study, the first step was to acquire exposure data for 1,4-dioxane present in surface and ground water. This data was sourced from REWAB which is an acronym for (in Dutch) "Registratie opgaven van Drinkwaterbedrijven", translated as "Registration of Declarations of Drinking Water Companies". REWAB serves as the designated system utilised by Dutch drinking water companies to record data for monitoring and reporting the quality of drinking water. As per REWAB records, analysed for the years 2018-2022, in raw surface water, 1,4 dioxane was found in about 90% of the 12 measured locations above reporting limit (0.1-0.3 ug/L). The yearly median over all locations was around 0.24 ug/L. Yearly maxima over all locations are around 1 ug/L but went up to 1.8 ug/L in 2018. In raw groundwater, 1,4 dioxane was found in about 4.5% of the 155 measured locations above reporting limit (0.1-2 ug/L). The yearly median was at reporting limits and was 1 ug/L (2018-2021) and 0.3 ug/L (2022). Yearly maxima were the outliers between 1.1 and 4.8 ug/L. Following the final treatment step, 1,4-dioxane was detected above the reporting limit (0.025-1 ug/L) at approximately 5.5% of the 217 locations. Acknowledging the observed variability in the measured concentrations, we adopt a conservative approach, assuming a worst-case scenario in which human exposure to 1,4 dioxane via drinking water is considered at 1 ug/L. This concentration represents the highest measured concentration of 1,4-dioxane in the treated water. In this hypothetical scenario, it was presumed that there were no changes in the concentration of the 1,4-dioxine in the distribution network. To assess the exposure level of a given population, it is important from a public health perspective to identify high-risk groups. Only two population groups were selected in this case study because including more than two categories was out of scope of the project. The categories include children (as a sensitive group based on the water consumption to body weight ratio and vulnerable development stage) and adults (as the general population). All water consumed by adults and children was assumed to originate from a single source. The exposure assessment considered oral and dermal exposure via drinking water at durations up to 1 year (LTL) and of a lifetime (LT). Exposure via inhalation during bathing or showering due to 1,4-dioxane's low volatility (vapor pressure of 38.1 mm Hg at 25 °C; Henry's Law constant = 4.8 X 10^{-6} atm-cu m/mol at 25 °C), and during the consumption of food, may occur under various specific circumstances. However, these were not the focus of the present case-study and may be explored in future. The results are shown in Table 17.

III.III Risk characterisation

In the present case study, the non-carcinogenic and carcinogenic health risk associated with 1,4-dioxane exposure via drinking water were estimated. The HQ was used to characterise the non-carcinogenic risk of 1,4-dioxane at a measured (drinking) water concentration of 1 µg/L as a realistic exposure scenario. The carcinogenic risk was determined using the oral cancer slope factor for 1,4-dioxane. The HQ was lower than the acceptable level of 1, both in adults and children. This indicates no potential human health effects at the concentration of 1 µg/L, and an exposure duration of one year. The duration of one year was chosen to illustrate LTL exposure, during which contamination persists for only one year. Although 1,4-dioxane is classified as a presumed carcinogen to humans, and a non-genotoxic mechanism is expected, the carcinogenic mechanism remains unclear (Ginsberg et al., 2022; ECHA, 2021b). Nonetheless the more conservative (health protective) non-threshold dose extrapolation approach was applied by calculating the additional cancer risk arising from the measured exposure concentration of 1 µg/L. The results obtained based on the measured exposure concentrations and the oral CSF (0.1 mg/kg-day) were compared

with the already established risk limit of 1-in-100,000 (≤ 1 x 10^{-5}) (WHO) or 1-in-1,000,000 (≤ 1 x 10^{-6}) (NL). The risk exceeding 1-in-10,000 (≤ 1 x 10^{-4}) is considered unacceptable (USEPA; 1992 Nag and Cummin, 2022). The results showed acceptable cancer risk in adults in an assumed sub-chronic exposure scenario of one year. When extrapolating it to determine the added risk of developing cancer over a lifetime exposure no cancer risk was indicated as the estimated lifetime cancer risk was within the acceptable excess risk distribution range of (10^{-6} – 10^{-4}). However, it must be considered that this case study is based on a worst-case scenario where the maximum measured concentration of 1,4-dioxane in the treated water was used in the risk characterisation. Typically, the 1,4-dioxane concentrations in the treated water are lower or below the detection limit. The present case study was used to illustrate the method and not intended for comprehensive risk assessment (i.e., considering variability and uncertainties associated with spatiotemporal contexts, population-specific behaviours, age-specific anthropometrics, vulnerable sub-population groups).

Table 17. Risk characterisation in two age groups based on duration and type of exposure.

Exposure duration	Population	Exposure type	Exposure dose	Non-Cancer effects		Cancer effects	
	Age group		(mg/kg/bw-day)	HQ	Risk	CR	Risk
LTL	Adults	ADD_{oral}	2.86 x 10^{-5}	<1	acceptable	4.08 x 10^{-8}	acceptable
		DAD_{dermal}	5.71 x 10^{-8}	<1	acceptable	8.16 x 10^{-11}	acceptable
	Children	ADD_{oral}	8.33 x 10^{-5}	<1	acceptable	1.19 x 10^{-7}	acceptable
		DAD_{dermal}	1.82 x 10^{-7}	<1	acceptable	3.72 x 10^{-11}	acceptable
LT	Adults	$LADD_{oral}$	4.08 x 10^{-6}	<1	acceptable	5.83 x 10^{-8}	acceptable
		$LADD_{dermal}$	8.16 x 10^{-9}	<1	acceptable	1.17 x 10^{-10}	acceptable
	Children	$LADD_{oral}$	1.19 x 10^{-5}	<1	acceptable	2.6 x 10^{-10}	acceptable
		$LADD_{dermal}$	2.6 x 10^{-8}	<1	acceptable	3.71 x 10^{-11}	acceptable

Note: LTL = less than lifetime; LT = lifetime; ADD = average daily dose; DAD = dermal absorption dose; LADD = lifetime average daily dose; HQ = hazard quotient (calculated only for non-cancer effects); CR = cancer risk

III.IV Discussion

The present case-study provides a method for risk characterization to assess the non-carcinogenic and carcinogenic health risks associated with 1,4-dioxane exposure via drinking water. The risk posed by 1,4-dioxane in drinking water via oral and dermal exposure was estimated. The HQ values for adults and children were found to be below the safe threshold level, indicating that there are no apparent immediate (non-carcinogenic) health effects associated with 1,4-dioxane exposure at the selected concentration and duration. The cancer risk estimated in the present study were all equal or below the acceptable level of 1 x 10^{-6} for any of the studied scenarios (van der Aa et al., 2017). A worst-case presumption on exposure was made in the derivation of these risk quotients associated with 1,4-dioxane, leading to the conclusion that the risk variability across more realistic scenarios may be even lower. While it provides valuable information, there are critical aspects to consider regarding the risk assessment and its implications for human health. In the present study, a maximum measured concentration of 1,4-dioxane in treated water is used for characterizing its risk to human health, under a worst-case scenario approach. While this approach is methodologically sound, it should be emphasized that this represents an extreme and unlikely exposure scenario. In reality, 1,4-dioxane concentrations in treated water are often lower or below the detection limit. The study makes use of health-based thresholds to assess non-carcinogenic and carcinogenic risks. This should be considered in the assessment of non-carcinogenic risks. The study primarily focuses on oral and dermal exposure routes and neglects the potential risks associated with inhalation exposure during activities like bathing or showering. Given 1,4-dioxane's semi-volatility, a more inclusive assessment may account for all potential routes of exposure considering the local-specific water usages by sub-populations as to provide a comprehensive understanding of the risks. In the present study, monitoring data from REWAB was used, and a worst-case scenario was assumed. To assess dermal exposure risks, the study uses an oral reference dose due to the absence of a specific dermal reference dose for 1,4-dioxane. This introduces

uncertainty into the estimation of dermal exposure risks, and therefore uncertainty about the potential implications for the present risk assessment.

In conclusion, while the presented case study provides valuable insights into 1,4-dioxane exposure risks through drinking water, it is essential to communicate the limitations and assumptions underlying the risk assessment clearly. Additionally, the study's conclusions should be considered in the context of real-world exposure scenarios, variations in risk thresholds, and the potential for inhalation and dermal exposure. Further research and a broader dataset could improve the accuracy and generalizability of risk assessments for 1,4-dioxane and drinking water contaminants of emerging concern.

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IV Appendix: External developments

In the current project, external developments in the field of toxicological and health risk assessment were actively followed and are summarised below.

IV.I Project VHP4Safety: Towards chemical safety assessment based on human data

Chemical safety assessment, designed to minimize the risks of chemical exposures to human health and the environment, has traditionally been based on animal testing. Testing on vertebrate animals is not part of the safety assessment of water samples. Nevertheless, the assessment of the safety of drinking water sources has often relied on existing (human or animal) data. Data obtained from cell- and tissue-based test systems (*in vitro* bioassays) and/or computer models (*in silico* methods such as QSAR and read-across) are used for prioritization steps and screening purposes.

In recent years, extensive progress has been made in the development and validation of non-animal new approach methodologies (NAMs) (*in vitro* and *in silico* methods), as a key strategy to accelerate and improve the prediction of adverse effects of chemicals on human health (traditionally based on animal studies). A new proposal in this direction is the Virtual Human Platform for Safety Assessment (VHP4Safety)[14,15] project, which has the mission to improve the prediction of the potential harmful effects of chemicals and pharmaceuticals based on a holistic, interdisciplinary definition of human health, to be represented in the Virtual Human Platform, and fast-track the transition from animal-based testing to innovative animal-free safety assessment. The Virtual Human Platform integrates data on human physiology, chemical characteristics and perturbations of biological pathways, in an inclusive and integrated manner that also considers human-relevant scenarios to discriminate vulnerable groups (such as patients, children, and elderly), chemicals with sector-specific utilities (pharma, consumer products and chemical industry), and different regulatory requirements and stakeholder needs.

VHP4Safety addresses the societal prerequisite of the transition to animal-free NAMs for safety assessment, by integrating various scientific disciplines in the consortium and working with many stakeholders towards implementation and societal acceptance of an approach to chemical safety assessment that is based on human physiology rather than animal data. One such stakeholder is KWR, providing unique insights about the overlaps between human relevant NAMs for chemical safety assessments and safeguarding the quality of drinking water. Since mid-2021, KWR collaborates in the VHP4Safety project with leading scientific groups from Dutch universities, university medical centers, public health institutes and applied research organizations, with practical and theoretical expertise spanning the technological, biological, chemical, medical as well as the social sciences. Together with collaborating partners, KWR also ensures the active involvement of various academic, regulatory, industrial and societal partners in the project throughout the entire safety assessment knowledge chain. In this way, the consortium brings together the necessary and complementary expertise to build test and evaluate the relevance and reliability of the Virtual Human Platform for Safety Assessment to use in a water quality context, with a keen interest in a more future-proof drinking water practice and public health.

The project includes three research lines (Figure 10) of which KWR follows the developments in research lines 1 and 2, and is actively involved in research line 3: implementation. Activities in this research line are:

[14] VHP4Safety – the Virtual Human Platform for safety assessment project NWA 1292.19.272 is part of the NWA research program 'Research along Routes by Consortia (ORC)', which is funded by the Netherlands Organization for Scientific Research (NWO). With a budget of over 10 million Euros, the project starts on June 1, 2021 and will last for the duration of 5 years. The project is coordinated by Utrecht University (Juliette Legler), together with the Dutch National Institute for Public Health and the Environment (RIVM) and University of Applied Sciences Utrecht (HU).

[15] vhp4safety.nl/

- Technology assessment, to stimulate active involvement of all relevant stakeholders in society to take into account their needs, interests and values in the virtual human platform design and performance.
- Acceptance for safety assessment, to promote the acceptance and determine the positioning of the virtual human platform in the transition to animal-free safety assessment.
- Training and education, to stimulate capacity building inside and outside the consortium, including internationally.

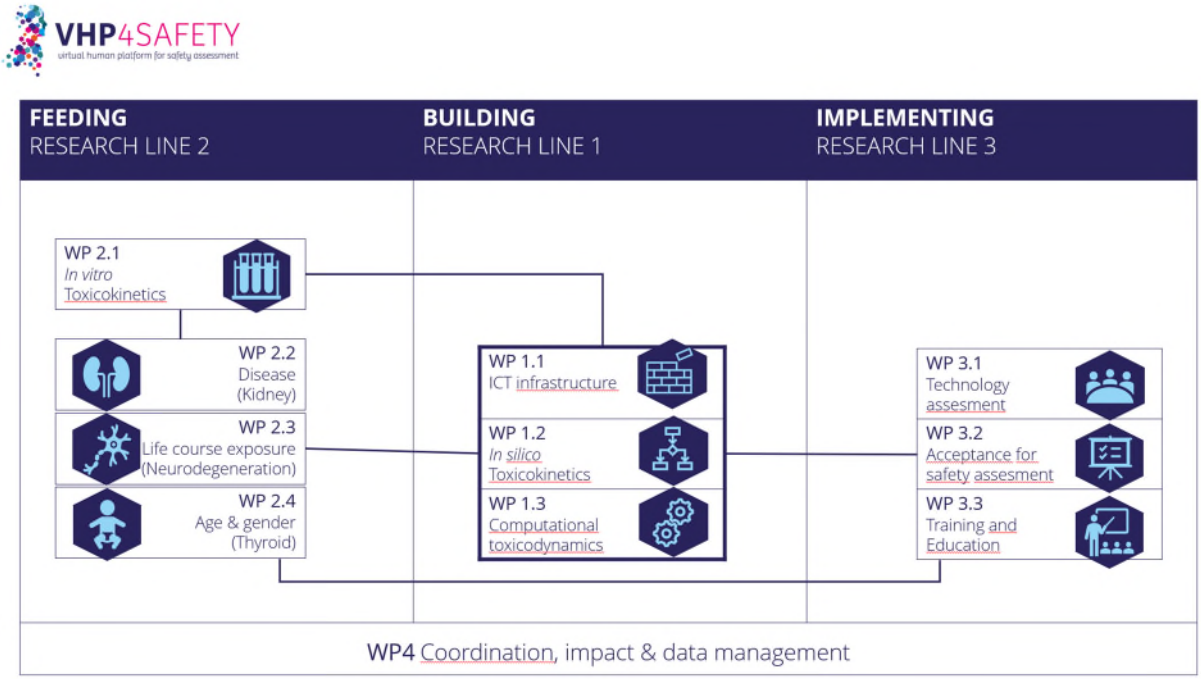

Figure 10: Research lines of VHP4 Safety project.

IV.II Project AFARA

Endocrine disrupting chemicals (EDCs) are harmful to humans and animals. The European Commission has created legislation for the identification of EDCs, but animal tests are still needed to determine these substances. Apart from the ethical concerns, the relevance of these animal tests to humans is questionable and tests are not available for all EDC-related effects. Current testing strategies are therefore not suitable for identifying all EDCs. Several innovative, non-animal testing models for testing EDCs have been developed, although these are not always accepted by science, regulatory authorities, and society at large.

At the beginning of February 2023, the project AFARA (Animal-Free Assays for endocrine disruption: from science to Regulatory Acceptance) was launched. The goal of AFARA is to facilitate the pathways for regulatory acceptance of existing models for endocrine disruption that are both animal-free and human-relevant. Over the next five years, it is aimed to bridge the gap between science and regulation with the main goal to facilitate implementation and acceptance of existing non-animal and human-relevant models for endocrine disruption. It is foreseen that in further research, the implementation of these models for water quality research will be evaluated. The results of this project will also be aligned with ongoing projects such as the NWA-ORC Virtual Human Platform for Safety Assessment (VHP4Safety) and the European EURION projects like GOLIATH. The project continues until 2028.[16]

[16] Text adopted from Funding for implementation and acceptance of animal test free research - News - Utrecht University (uu.nl) and Sciencrew

IV.III Project MOMENTUM

Micro- and nanoplastic particles (MNPs) can be found nearly everywhere: in air, in food and in water. MNPs have accumulated in our environment and concentrations are expected to increase over the coming decades, due to the increased and continuous plastic production worldwide and the persistent nature of these particles. Researchers have been worried about the potential harmful effects of MNPs in the environment for a long time. Most studies, however, have been focusing on the risks of these particles to marine life, while potential effects on human health have been studied less extensively.

The ZonMw/Health Holland MOMENTUM project [17] aims to unravel the human health effects of MNPs (micro- and nanoplastics) and propose solutions to minimize their potential health impact, by combining efforts from academia, governmental institutions and industry. During the initial two years of the MOMENTUM project, reference MNPs were both generated and tested. It seems that MNPs might exert a disruptive influence on our immune system. The MOMENTUM project is also delving into the specific types and quantities of MNPs to which we are exposed. In MOMENTUM, KWR is involved in multiple working packages with a focus on the analysis, characterization and risk assessment of MNPs. In work package 6, KWR is working with a consortium of (inter)national academic and industrial partners on a roadmap for safety and risk assessment of MNPs and solutions for exposure prevention. Due to the unique characteristics of plastic particles, this requires a different approach than for chemical substances. Early data on the actual exposure of humans to MNPs and human health hazards of MNPs appeared insufficiently reliable for risk assessment upon close examination. Due to the complexity of MNP risk assessment, it is important to first prioritize research needs and questions. Together with RIVM, KWR drafted a road map, identifying the most important needs and questions related to MNP risk assessment, based on a workshop with researchers from academia, government and industry. One of the main outcomes is that more research is needed on the effects of chronic MNP exposure (including the harmonization of MNP analysis) and copresence of other particles, pathogens and compounds in environmental systems to place the potential impact of MNP in perspective. The project continues until August 2024, after which KWR will participate in a work i package related to MNP risk assessment in a follow-up project, MOMENTUM 2.0.

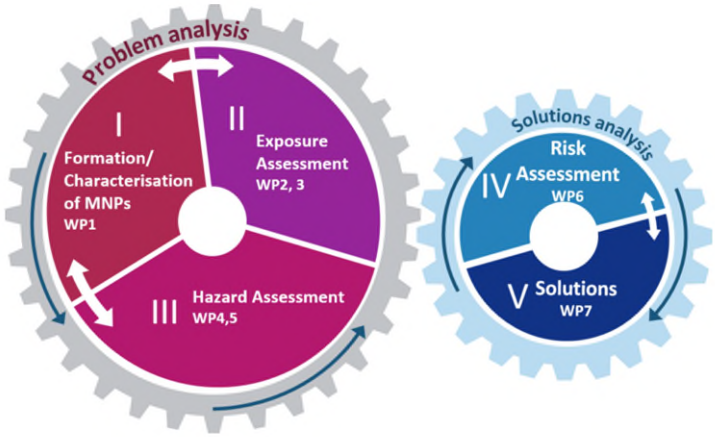

Figure 11. MOMENTUM project structure (https://momentummicroplastics.nl/)

IV.IV Project on multivariate analysis to explore relationships between detected chemicals and their toxicological effects (JSPS)

Chemical analysis and bioassays have been implemented to investigate water quality and assess the toxicity of chemicals in the water. Chemical analysis facilitates detection and measurement of both target and non-target

[17] momentummicroplastics.nl/

compounds in water samples, with high-resolution mass spectrometry enhancing the ability to identify a wide range of chemicals. Complementing this, bioassays including various bio-analytical tools can provide a comprehensive assessment of the overall effects of the chemicals in a sample. A diverse array of bioanalytical tools has been developed to capture specific cellular responses, and a battery of the assays has been used to evaluate several endpoints in previous studies. Bioassays offer a distinct advantage over chemical analysis as they can reflect the effect of undetected chemicals and the combined effects of various chemicals. While both methods have found application in numerous studies, it remains uncertain to what extent toxicological effects can be explained solely by detected chemicals, particularly when considering their combined effects. To address this research question, we have investigated a method to explore the relationships between chemicals and cellular responses. This work is a part of a project led by a research fellow of the Japan Society for the Promotion of Science (JSPS), hosted at KWR. The JSPS project, titled "Development of a Predictive Method for Toxic Effects of Chemicals in the Aquatic Environment Using Machine Learning Algorithms" focuses on exploring relationships between bioassay data and chemical concentration data to develop a tool to predict toxicity. This approach can be extended to the assessment of toxicological risk and ecological risk by collecting data sets with each endpoint. The project will be continued until March 2026.

Preliminary analysis based on data from *in vitro* assays suggests that the detected concentrations of each chemical in a water sample may offer valuable insights for estimating the combined toxicity of chemicals in the sample and this estimation has the potential to reduce the need for additional bioassays. However, the predictive power of the models requires refinement. Once improved, this methodology might operate more effectively where data on chemical concentrations have been accumulated. By using knowledge of the toxicity of individual chemicals, it would be possible to predict the combined toxicity in a water sample without performing additional bioassays, contributing significantly to the screening of toxicological effects. Furthermore, interpreting these models can aid in identifying important chemicals that may be strongly associated with bioactivity, allowing prioritization of chemicals to be monitored. However, additional research would be required to extrapolate the predicted effects by this method to effects at different biological levels, especially in the context of human health risk assessment.

IV.V Partnership for the Assessment of Risks from Chemicals (EU project PARC)

The European Union research and innovation programme PARC (Partnership for the Assessment of Risks from Chemicals)[18] was established in order to improve the risk assessment and sustainability of chemicals. The PARC project will strengthen the scientific basis for the assessment of risks from chemicals and make next-generation risk assessment possible in order to improve the protection of human and environmental health. The project is part of the European Union's Horizon research and innovation programme (2021-2027).

The European chemicals strategy – the EU Chemicals Strategy for Sustainability – outlines the ambitions needed to provide a clean habitat free of pollution from undesirable chemicals in the environment. The PARC project is part of this strategy, and the aim is to further the implementation of that strategy. The project brings risk assessors and policymakers together, with scientists, so that the methods required can be developed faster, and so that data and knowledge will be more easily available.

In collaboration with the European Environmental Agency (EEA), European Food Safety Authority (EFSA) and European Chemicals Authority (ECHA), the knowledge questions are being mapped out in order to make it possible to interpret the risks of chemical substances and to provide the appropriate solutions. The project will strengthen the scientific basis for the assessment of risks from chemicals and make next-generation risk assessment possible in order to improve the protection of human and environmental health.

The principal objectives of the PARC project are:

- to develop the scientific knowledge needed to face current and future challenges in the area of chemical safety.
- to supply those responsible for assessing and managing the risks of chemical exposure with new data, methods, and innovative tools.
- to strengthen the networks of actors who specialises in the different scientific fields that contribute to risk assessment.

[18] https://www.eu-parc.eu/

KWR's involvement in the PARC project is contributing themes that are relevant for water quality, and drinking water quality in particular. The areas that KWR will address specifically are monitoring and exposure, risk assessment and data management.

KWR will work specifically in the PARC project on:

- Contributing to the ongoing development of wastewater-based epidemiology for monitoring human exposure to environmental pollutants. This includes the development of analysis methods to detect and measure exposure biomarkers in wastewater and link them to population studies and, ultimately, health endpoints.
- With other partners, KWR is working on integrated approaches for testing and assessing potentially adverse effects of substances. This will involve the use of new approaches such as *in vitro* assays/bioassays and *in silico* methods. The topics reviewed are DNA damage, endocrine disruption, specific organ toxicity and estimating relevance for humans.

KWR is contributing to the refinement or development of physiologically based pharmacokinetic (PBPK) models for prioritised individual compounds and mixtures of compounds. The activity focuses on chemicals about which there is limited information, the different sensitivities in physiological processes.

IV.VI International Congress of Toxicology (ICT 2022)

At the International Congress of Toxicology (ICT2022) in Maastricht –The Netherlands (19-21 September 2022), a joint conference by EUROTOX and the International Union of Toxicology (IUTOX) and the Dutch Society of Toxicology (NVT), many inspiring sessions and presentations brought the KWR delegates to the state of the art in toxicology. It also allowed the possibility of connecting with peers and collaborative partners (such as in PARC, MOMENTUM and VHP4Safety), discuss new project ideas and make new global connections. Some key findings and realizations are listed below.

The water sector may have use for the various innovative approaches presented at the ICT2022 to predict the toxicity and safety of compounds in the water. This is important as we are often confronted with chemicals for which toxicological information is scarce or lacking. Approaches come from different domains. Particularly in drug discovery, it is important to predict the toxicity of drug candidates early in the development process. For regulatory decision-making, it is crucial to assess the safety of industrial products. It was clear from the conference that, for predictions of chemical toxicity, read-across, Quantitative Structure-Activity Relationships (QSARs), machine learning or deep learning ('artificial intelligence', AI) are used. These are based on either chemical properties, or high-throughput measured biological properties such as in toxicogenomics approaches (expression of genes, proteins, metabolites), or cell structure changes.

The Health and Environmental Sciences Institute (HESI) is a non-profit institution whose mission is to collaboratively identify and help to resolve global health and environmental challenges through the engagement of scientists from academia, government, industry, NGOs, and other strategic partners. One of their topics is using AI for drug discovery. These techniques can be of help for the drinking water sector to estimate risks of compounds in water for which toxicological information is not yet available. However, a lively debate highlighted the possibilities as well as the pitfalls of using AI in toxicology. Efforts are currently directed mainly toward making machine and deep learning models self-implementing and more intuitive. This is a welcome development because, at the moment, the methods act as black boxes.

The concept of "Key Characteristics" was discussed in several presentations. This concerns a definite list of possible main characteristics (such as 'is electrophilic' or 'modifies cellular differentiation') of groups of chemicals that have a particular adverse effect (such as carcinogenicity, neurotoxicity, liver toxicity, etc.). With the help of the Key Characteristics, mechanistic evidence can be collected from scientific literature in order to link a chemical to (potential) adverse effects by mechanism. This approach can be used complementary to the constituents in 'adverse outcome pathways' (AOP), which describe the events from molecular initiating events (by a chemical) to the adverse

outcome. In the discussions, it was often made clear that mechanistic evidence is increasingly welcomed and required by regulatory authorities. In a talk organised by the 'Committee on Toxicity' and the 'Committee on Carcinogenicity' subgroup SETE (Synthesis and integration of Epidemiological and toxicological Evidence) it was explained how two types of evidence for toxicology (animal studies and human studies) could be integrated. Their approach is a visual representation of both types of the evidence (Committees on Toxicity and Carcinogenicity, 2021). This can help to estimate uncertainty in toxicological risk assessment between both types of evidence and links with the proposed follow-up of the trend alert on the use of epidemiological studies to derive risk limits (BTO 2022.058).

It was clear from several presentations that toxicity testing should not stop with the compound of interest but should always expand to possible metabolites. Textile dyes, for instance, are notorious for transforming or breaking into similarly toxic or even more toxic metabolites, potentially also in drinking water treatment. Defining a 'metabolic space' for compounds puts possible problematic metabolites forward. The necessity to assess the impact of metabolites through prediction was the object of various research, which pointed out the importance of comparing different methods to characterize the toxicological profile of "new" chemicals and their acute or chronic exposure to humans.

The Chemicals Strategy for Sustainability adopted by the EU in 2020 aims at a toxic-free environment by 2030. This goal requires a horizontal intervention in legislation, not only in the highly regulated pharmaceutical and industrial market, and the adoption of the precautionary principle at all levels. A transdisciplinary approach that uses different sources of information and various expert judgments is deemed necessary to assess the wide variety of chemicals found in the environment.

The KWR delegates contributed to the conference with talks on combined use of bioassays and QSAR for water-relevant substances (BTO 2023.015, Hoondert et al. 2022) and combined research of genotoxicity bioassays in the circular economy (Reus et al. 2022, Reus et al. 2023) and trigger values for genotoxicity bioassays (BTO 2023.053 Reus et al. 2022). In addition, there was a poster on the bioassay-track for evaluating chemical water quality as defined in the 'knowledge impulse water quality' research program (Pronk et al. 2022), and a poster that illustrated an *in silico* approach for predicting and prioritizing the hazard assessment on the expected health-related impact of transformation products derived from pesticides during drinking water treatments (Ferrario et al. 2022). As part of the current BTO project, a poster was presented on required advanced risk assessment frameworks to address current challenges in risk assessment related to water quality, including less than lifetime exposures to chemicals via drinking water (Majid Shaikh et al. 2022). Lastly, the expected impact of climate change as well as of climate solutions on water quality, how this could be addressed using risk-based monitoring approaches and an overview of EU climate impact projects was presented on a poster (Dingemans et al. 2022). This was an eye-opener to many toxicologists working in different sectors, which was the intended impact.

Several resources that might be relevant for water quality assessment were mentioned. For example the (https://envirotoxdatabase.org/) which holds toxicity data of individual chemicals on species. The data of USEPA's ECOTOX database is included in the EnviroTox database. It is yet unclear how this overlaps with the NORMAN ecotoxicity database. The National Center for Computational Toxicology (NCCT) presented several online resources. One of these are the Opera-models for predicting chemical traits (like $\log K_{ow}$) based on chemical structure. Furthermore a source for structural alerts for chemicals was introduced, 'Toxalerts', that can be accessed via the online chemical database with modelling environment 'OCHEM'. These can be used to link chemicals with such structural alerts to (for example) toxicological endpoints, or removal efficiencies. The NCCT showed the USEPA WEB-ICE tool. This tool uses data of surrogate species that behave similarly in their (acute) response to a multitude of chemicals to a species of interest to boost data availability. This can be used to make species - rich SSD curves which could be implemented for the derivation of HC5 for bioassays in the SFT2 (De Baat et al. 2022). This system can also support in the collection of data for enough different (surrogate) species for evaluating a chemical in a regulatory context.

Abstracts of the ICT2022 can be accessed via the following link: Toxicology Letters (elsevierdigitaledition.com)

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V Appendix: Reflections and look-ahead

In this section, a review and outlook of the methods presented in the main report are presented to assists in preparing emerging topics and the relevance of proposed methods in the light of evolving scientific developments.

V.I Effect-directed monitoring using *in vitro* bioassays

Bioassays give insight in potential risks without information on identity and concentrations of the chemicals present, which make bioassays a powerful, efficient tool to assess potential hazards of complex mixtures such as water samples (see TEXT BOX 8 below). Bioassays have been increasingly applied for water quality assessment in several BTO and DPWE projects during the past years, including the reoccurring robustness research for the four Dutch dune water utilities (Dunea, PWN, Waternet and Evides) (Bertelkamp et al. 2020a, Schriks et al. 2016, Wols et al. 2021, Hoondert et al., in prep), investigation of wastewater treatment efficacy (Bertelkamp 2020b, Hofman-Caris et al. 2023) and drinking water treatment efficacy (Heringa et al. 2011, Timmers et al. 2022). Specific projects focussed on the development of methods and interpretation, such as the comparison of genotoxicity bioassays (Hockin et al. 2018, BTO 2023.052 Reus et al. 2023, Reus et al. in prep).

TEXT BOX 8. BIOASSAY model systems

Risk is a result of hazard and exposure. Hazard assessment aims at identification of the potential adverse effects of a chemical. If no experimental data (on a specific toxicological endpoint) are available from databases or literature, toxicity data for individual chemicals or mixtures can be obtained by performing *in vitro* or in *vivo* experiments (bioassays). For human health hazard assessment in water quality monitoring, *in vitro* (cell-based) bioassays are used rather than regulatory *in vivo* bioassays, because of ethical considerations concerning the use of vertebrates and for practical and economic reasons. *In vivo* bioassays with invertebrates using e.g. water flea (*Daphnia*) or algae, however, are frequently used for environmental water quality monitoring.

In vitro bioassays make use of bacteria, mammalian cells or yeast. *In vitro* bioassays for measuring several biological effects are available, including general cell toxicity (cytotoxicity), DNA damage (genotoxicity), endocrine disruption, oxidative stress and effects on metabolism. As there are many more biological effects, many more *in vitro* bioassays exist, but not (yet) prioritized for use in water quality monitoring. More background information on bioassays and their use in water quality assessment can be found in the e-book *Biological tools in water quality assessment* (Escher et al. 2021).

The biological effects measured with relevant *in vitro* bioassays represent modes of action for human and environmental health effects. A response in a specific bioassay indicates the presence of chemicals that are able to induce that specific response. However, it remains difficult to translate bioassays responses to exact human and environmental health risks. Effect-based trigger (EBT) values provide a threshold indicating when a human or environmental health risk cannot be excluded, and further research is warranted. The development of EBT started about a decade ago with investigation of hormonal activity in drinking water and its sources, using highly sensitive *in vitro* CALUX bioassays allowing the detection of estrogenic (Erα), androgenic (AR), progestogenic (PR), and glucocorticoid (GR) activities (Brand et al. 2013). Recently, EBT were (re)established for the same and additional *in vitro* CALUX bioassays using a different approach (BTO 2020.053, Béen et al. 2021). This resulted in (new) EBT values indicating a potential human health risk of the ER CALUX, anti-AR CALUX, AR CALUX and GR CALUX and preliminary EBT values for the PR CALUX and polycyclic aromatic hydrocarbon (PAH) CALUX. The same approach has been applied to derive EBT values for genotoxicity bioassays (Ames fluctuation test, umu test and p53 CALUX) and oxidative stress (Nrf2 CALUX) resulting in preliminary EBT for these bioassays that need further optimization (BTO 2023.052). EBT

values for CALUX bioassays indicating a potential environmental health effect have been developed by Van der Oost et al. (2017a). The health consequences of some biological effects are yet not well understood. An example is oxidative stress, which can be measured with Nrf2-CALUX and AREc32 bioassays. It is known that oxidative stress can affect various cellular components, including DNA, but the damage can also be reversible. The Nrf2-CALUX and AREc32 are proposed in the basis set bioassays NL (SFT2). At the moment these results can be interpreted, but do not yet consider the difference between reversible and adverse effects.

The availability of bioassays for different endpoints require guidance for end-users to select appropriate bioassays for answering their research needs. KWR participated in the DEMEAU project that was performed within the European Union Seventh Framework Programme and aimed at selecting a panel of bioassays that is relevant for (drinking) water quality assessment (Schriks et al. 2015). These results were built upon within BTO research identifying critical aspects in implementation including availability of a standardized protocol, guidance on interpretation of the results, optimization of sample pre-treatment and possibility for automatization of bioassays (BTO 2017.008). For the application of bioassays on surface water, the Smart Integrated Monitoring (SIMONI) strategy was the first bioanalytical tool to be applied in surface water quality monitoring programs in the Netherlands and included the implementation of EBT values (Van der Oost et al. 2017a, Van der Oost et al. 2017b). In a Global Water Research Coalition (GWRC) project partners Veolia, Suez, Griffith University, Helmholz Centre for Environmental Research – UFZ, GWRC and KWR have spent the past few years evaluating methods and tools for effect-based monitoring. Here, a decision-making tool was developed that groups bioassays into three test batteries based on assay sensitivity, with test battery selection depending on the sampling campaign context (e.g. drinking water) and purpose (e.g. assessment of treatment efficacy) (Neale et al. 2021). A comparable approach where water context is taken into account in the bioassay strategy, is the basis set of bioassays (SFT2) that has been defined in the project Toxicology as part of the Dutch Knowledge impulse water quality (KIWK) project, a collaboration between the Dutch government, provinces, water authorities, drinking water companies and knowledge institutes, including KWR (SFT2, 2022). EBT values derived by Béen et al. (2021) (BTO 2020.053) for the interpretation of bioassay results have been implemented in this strategy and this meets one of the critical aspects regarding data interpretation that was identified in earlier BTO research (BTO 2017.008). In addition, a guide for conducting biological effect monitoring specific for advanced treatment of wastewater treatment plant effluent has been developed by experts in the field of water quality assessment using bioassays (Ecofide 2023).

Bioassays can (also) play a strong role in directing further research. By using methods such as effect-directed analysis (Brekelmans et al. 2021, Houtman et al. 2020) and evaluation of available (experimental) databases such as ToxCast, observed bioassay responses can be linked to specific substances that are of interest for further research, including risk assessment and if needed, mitigating measures. As part of the NORMAN network, KWR is leading a project to establish a database of toxicity data obtained from *in vitro* bioassays, particularly for environmentally relevant chemicals. This database will serve as a useful source of bioassay data for risk assessment. Previous BTO research demonstrated that by using existing bioassay databases, results from non-target screening (NTS) can be integrated with bioassays for an estimate of the chemical water quality, which facilitates prioritization for further research, including chemical identification, targeted analysis, and risk assessment (BTO 2017.053, BTO 2019.002). A strategy for the performance of bioassays has been applied on NTS data (BTO 2021.013) and will be further investigated in future BTO research. Although the combined interpretation of NTS and bioassay is promising in principle, some challenges need to be considered, such as the possibility of a lack of activity in a bioassay, precluding to make a direct link with concentrations in water. Previous BTO research demonstrated, for example, that targeted chemical analysis of the sum parameters of PAH's, chlorophenols, aromatic amines and halogenated aliphatic carbons from the Dutch Drinking Water Directive cannot be replaced by the conduct of ToxCast/Tox21 bioassays, because the chemical groups investigated appeared to be not or only to a less extent active in the considered bioassays (BTO 2018.075). This may, however, be different for other groups of compounds and bioassays.

It is expected that bioassays will become increasingly important in water quality assessment with the availability of the SFT2 and the guide for conducting biological effect monitoring specific for advanced treatment of wastewater treatment plant effluent (Ecofide 2023), and that they will be more and more combined with *in silico* tools and chemical analysis and interpreted together. New research should focus on development, implementation and use of bioassays for complex toxicological endpoints such as reproductive toxicity, (developmental) neurotoxicity and immunotoxicity for which standard bioassays are currently lacking. Collaboration with experts from academia and research institutes on these topics is essential to join forces and strengthen the implementation of new bioassays, also for the drinking water practice. Test batteries for (developmental) neurotoxicity and other complex biological effects are likely to include a battery of two or more *in vitro* tests and should be developed in close collaboration with other organisations.

Data on activity of individual compounds in bioassays (ToxCast, NORMAN Bioactivity Database) can be useful to define optimal testing strategies as most biological effects require more than one bioassay in a testing strategy. This data-driven approach is complementary to previous research in which different genotoxicity bioassays were applied to water samples by conducting experiments in practice (BTO 2023.052; Reus et al. 2023, DPWE Genotoxiciteit). New bioassays and test batteries will also require research on data interpretation in a water quality context. In addition, there are existing bioassays in current test batteries for which no EBT value is available yet. Interpretation of bioassays should be further facilitated, either by applying existing methods (BTO 2020.053, Béen et al. 2021, Van der Oost et al 2017a) to derive EBT for new bioassays, or by application of alternative methods depending on the available data.

There is also a need to understand the uncertainty of the occurrence of possible adverse effects in humans based on bioassay data. The recognition from previous BTO research (BTO 2022.058) that (new) risk limit values tend to end up lower, for example if they originate from epidemiological data, requires understanding of epidemiological data interpretation. Collaboration with authorities and experts on this topic is essential to get insight into possibilities and consequences of new and alternative methods to substantiate drinking water requirements and to prepare for future discussions. Consortia that were established previously including AFARA, MOMENTUM, PARC, VHP4Safety (see appendix V) will continue for the next few years, and formation of new consortia on water quality relevant topics is foreseen.

V.II In silico predictions of toxicity

If experimental data are not available for a chemical of interest, its potential toxicity can be predicted using *in silico* tools. These are software tools based on the identification of (quantitative) structure activity relationships [(Q)SAR] and read-across using experimental data from structurally similar chemicals. An overview of available *in silico* tools and how to implement this in risk assessment has been made within previous BTO research (BTO 2018.030) and was recently updated with a selection of tools and insights that were considered most practically applicable for water quality assessment (BTO 2023.015, Reus et al. 2022). *In silico* tools can also be used for prioritization of further research, e.g. in ongoing robustness research for the dune water utilities toxicity based on QSAR predictions were weighed in decisions on chemical selection (Wols et al. 2021). In the past years, *in silico* tools, including the approach for toxicological risk assessment developed within previous BTO research (BTO 2018.030, Baken et al. 2018), have been implemented in water quality risk assessment in practice for individual substances (Baken et al. 2016, *unpublished data* WQS 2022) and for hazard assessment of substances identified from chemical measurements (BTO 2018.023, BTO 2023.084). The BTO project presented in this report focused on methodologies for risk assessment that address specific questions or exposure scenario's and that could follow-up the basic approach (BTO 2018.030) for a refined risk assessment, e.g. in case there is uncertainty about the assessment or if the assessment requires a more accurate exposure scenario. Within BTO, *in silico* approaches have also been developed for specific purposes, e.g. for toxicity assessment of persistent mobile organic contaminants (PMOC) (BTO 2023.060), substances of very high concern (SVHC) (BTO 2023.in prep; Wols et al. 2023), and VO Water quality footprint (BTO 2023.011).

In silico tools are continuously under development to improve the predictions, including for complex biological effects such as neurotoxicity (BTO 2024.016, Majid et al., 2024). New versions of available *in silico* tools are issued on a regular basis, in which underlying databases are updated. Previous projects on *in silico* tools (BTO 2018.030, BTO 2023.015) focused on individual compounds, but *in silico* tools can be useful for hazard assessment of complex mixtures as well. This requires the development of methods for the integration of data obtained with bioassays, chemical analysis and *in silico* tools in a practical setting to demonstrate the applicability of this approach for the drinking water practice. It is aimed for to expand the network on *in silico* toxicology, e.g. via the NORMAN network. In addition, custom models can be developed as demonstrated in previous work (BTO 2023.060, BTO 2024.010, Wols et al. 2023) and combined with expertise on NTS *in silico* tools. Starting from 2024 a new BTO project will focus on the combination of bioassays, chemical analysis and *in silico* tools, with the aim of developing an integrated approach to assess water quality.

V.III Complex toxicological endpoints

Complex biological effects such as neurotoxicity (adverse effects to the nervous system) and immunotoxicity are difficult to assess with *in vitro* bioassays and *in silico* tools due to the complexity of these effects (many cellular processes involved). Adequate information on the neurotoxic and immunotoxic potential of chemicals is essential, because it is known that water-relevant substances can have these effects (e.g. plant protection products, per- and polyfluoroalkyl substances). As standardized bioassays for neurotoxicity are currently lacking, models that could be included in a testing battery for water quality assessment were investigated (BTO 2020.035, Reus et al. 2020). One neurotoxicity bioassay, the acetylcholine esterase (AChE) inhibition assay, has recently been implemented as part of the SFT2[19], but with such a complex biological effect such as neurotoxicity, it is foreseen that a battery of multiple bioassays would be required for a proper hazard assessment (BTO 2020.035, Reus et al. 2020). In addition to the models studied in BTO 2020.035, two promising methods have recently been evaluated, consisting of one custom *in silico* approach and one bioassay using planarians for neurotoxicity assessment in water quality monitoring (BTO 2024.016; Majid et al. 2024). The project envisions pilot studies as a follow-up project, in which a test set comprising water-relevant substances and water samples will be studied. This initiative aims not only to validate and refine the methods but also allows testing of additional endpoints, such as genotoxicity, carcinogenicity, and developmental toxicity of water relevant substances.

Besides challenging aspects of hazard and risk assessment based on complex biological effects, the complexity can also be related to the nature of the compounds under assessment. The past years, micro- and nanoplastics (MNP) in the water cycle have been studied. These compounds require a specific hazard and risk assessment approach due to different characteristics (taking not only the chemical structure, but also the size and shape into account in the assessment, as well as microbiological aspects) (Hoondert et al., 2022). Together with a consortium of experts in the MOMENTUM[20] project, KWR contributes to the development of a risk assessment approach for MNP, including analytical and risk management methods.

Since water contains a complex low-level mixture of substances, realistic exposure patterns also consist of mixtures developments on mixture toxicology should be closely followed to apply for mixture risk assessment in a water quality context. In the context of exposome, it is important to acknowledge that other external exposure factors beyond the general external environment that can influence human health can also be considered.

[19] The AChE inhibition assay is listed as 'experimental' in the SFT2, indicating that this assay has potential to be added to the basis set bioassays and/or can be used for additional insight/confirmation.

[20] | Universiteit Utrecht | Homepage (momentummicroplastics.nl)

V.IV Use of human data for toxicological assessments

In addition to cell-based methods and animal studies, epidemiological (human) data may also provide useful toxicological information for risk assessment. Human data can be considered a golden standard to predict health risks for a human population. There are indications that health risk limits are increasingly being derived from epidemiological studies and that this may be leading to new, lower risk limits (PFAS, nitrate[21]). On the other hand, there are also recent examples of risk limits derived from animal data that ended up lower based on new insights from new studies (bromate, bisphenol A[22]). The water sector may thus be increasingly confronted with lower guideline values, which has consequences for their analytical measurement and water treatment strategies (BTO 2022.058).

Although human data may be a golden standard for human hazard and risk assessment, (sufficient) good quality data is not available for most chemicals. At the moment, (regulatory) risk assessment still mostly relies on animal studies, as *in vitro* assays are not accepted for all biological effects. For genotoxicity a tiered approach is accepted, starting with two or three *in vitro* assays, which are followed-up with one or more *in vivo* studies only if a positive *in vitro* response has to be confirmed. The drawback of this current *in vitro* testing strategy is the high number of misleading positives[23]. Driven by the development of new approach methodologies (NAM), including the p53 CALUX, the current genotoxicity testing strategy is now being revisited by KWR and other institutes within the EU Partnership for the Assessment of Risks from Chemicals (PARC)[24]. In addition, for other biological effects the development of *in vitro* assays and testing strategies is continuously ongoing, and it is expected that more and more *in vitro* assays will be regulatory accepted. This is not only for animal welfare reasons, but also for a better prediction e.g. by using human cell-based assays related to relevant adverse outcome pathways. An ongoing development is the Virtual Human Platform for Safety Assessment (VHP4Safety)[25], aiming at integrating data on human physiology, chemical characteristics, and disruptions of biological pathways, in an inclusive and integrated manner. Various scientific disciplines in the consortium, including KWR, are working towards implementation and societal acceptance of an approach to chemical safety assessment that is based on human data rather than animal data. With a human-based risk assessment platform that allows the evaluation of regulated and new substances, VHP4Safety has the potential to also contribute to a more future-proof drinking water practice. In a related project (ALARA), the process of acceptance and implementation of animal-free models in the human safety assessment of substances is studied, with a focus on endocrine disrupting chemicals (EDCs)[26].

V.V Use of models and big data to improve exposure and hazard assessments

Next to hazard assessment, information on realistic exposure is essential for an adequate risk assessment. If a chemical is hazardous without individuals being exposed, the associated human health risk is low or negligible. On the other hand, if a compound is less hazardous but individuals are highly or chronically exposed, there may be a human health risk. Concentrations of substances in water, air and food can be determined chemically, but this does usually not reflect the internal exposure of a substance (i.e. the concentration of the substance in cells and tissues of an organism) due to toxicokinetics (absorption, distribution, metabolism and excretion of a substance) and herewith taking into account bioavailability. For adequate risk assessments in a water quality context, internal exposure data is needed. Internal exposure data (e.g. blood concentrations) may be available from conducted experimental studies

[21] For details and references, see BTO 2022.058.

[22] For details and references, see BTO 2022.058.

[23] Kirkland D et al. How to reduce false positive results when undertaking *in vitro* genotoxicity testing and thus avoid unnecessary follow-up animal tests: Report of an ECVAM Workshop. Mutat Res. 2007 Mar 30;628(1):31-55.

[24] Partnership for the Assessment of Risks from Chemicals | Parc (eu-parc.eu)

[25] VHP4Safety (sciencrew.com)

[26] Animal-Free Assays for endocrine disruption – from science to Regulatory Acceptance (AFARA) - Research - Utrecht University (uu.nl)

and databases. However, this information will not be available for all substances. As the rodent studies that are generally used to assess toxicokinetics raise ethical concerns and high costs, it is warranted to investigate the feasibility of using physiologically based pharmacokinetic (PBPK) modeling for water quality questions. PBPK models provide input on internal concentrations in a relatively rapid way and take into account bioavailability, which is more accurate than risk assessments based on intake. PBPK models can also be used for quantitative *in vitro - in vivo* extrapolations (QIVIVE) (Punt, 2022). Currently, there are limited practical examples where these approaches are being applied in a water quality context. However, the potential for their utilization appears promising for further research and implementation, as these offer the advantage of faster and more cost-effective assessments compared to testing.

Omics methods such as (toxico)genomics, transcriptomics, proteomics, and metabolomics are techniques that provide lots of detailed data on gene, protein and metabolite expression in various cellular pathways, which can be linked to adverse biological effects. Omics methods overcome the limitation that bioassays are rather specific for a certain biological effect, yet it also requires expert knowledge on how to interpret the data. The applicability of functional genomics in assessing drinking water quality (BTO 2021.008), neurotoxicity assessment (BTO 2020.035) and environmental proteomics in environmental monitoring (BTO 2021.048) have been investigated previously and could serve as a starting point for further exploration of the use of omics methods in water quality assessment.

Data interpretation, *in silico* tools and PBPK modeling are all examples where data are involved. Over the years, more data will come available, not only for toxicity, but also monitoring data. Bioinformatics and data science will therefore remain extremely important in water quality assessment and will be integrated in any research project.

V.VI Contexts in which toxicological assessments can be applied

Toxicological assessments can be applied in various contexts, such as calamities focusing on individual substances (*unpublished data* WQS 2022), robustness and efficacy of water treatment processes (e.g., Hofman-Caris et al. 2023, Timmers et al. 2022, Wols et al. 2021) and water reuse focusing on water as a complex mixture of chemicals. Water reuse now gains a lot of interest, as the expected increase in water demand and the challenges in availability and quality of drinking water sources, emphasizes the need for new, alternative drinking water sources. To safeguard human health, the quality of new drinking water sources should be checked. For non-potable use, drinking water quality requirements may be too strict, still the water should comply to a standard that is regarded as safe, depending on the application. Research on a strategy for and evaluation of water quality assessment of alternative sources is foreseen in a multi-disciplinary approach within the WiCE program. This project will provide a knowledge base to support future discussions on safe/acceptable chemical and microbial exposure via drinking water and empower the drinking water companies with the knowledge needed to meet the evolving regulatory requirements by enabling them to ensure the drinking water quality aligns with the changing guideline limit values.

Since water generally contains a complex low-level mixture of chemicals and biological contaminants, focusing solely on specific groups of chemicals or pathogens will not be sufficient to safeguard human health. New and future challenges in drinking water quality and health require an integrated approach to monitoring, risk assessment and action in which chemical and microbiological monitoring, hazard and risk assessment are combined (BTO 2022.014). An example hereof is the increased need for water reuse, which is associated with potentially less well controlled health risks resulting from circulating chemical and microbiological risks. Within the Water in the Circular Economy (WiCE) program, the Water Wise concept was developed, the foundation for an easy-to-use, transparent and consistent evaluation framework as a first step towards the quantitative evaluation of the chemical risks inherent to new water cycles (BTO 2020.033). Research and expert judgement on new water cycle-related risks are needed to develop the framework further. Depending on the information fed into the tool and the outcome of the Water Wise risk assessment, the output may include a recommendation to apply bioassays for water quality monitoring. In a recent WiCE project, six *in vitro* bioassays to detect genotoxicity were applied to six samples from different water

cycles. Results indicated that a set of bioassays was able to discriminate between contaminated and less/non-contaminated samples of water cycles. This is a first step towards a strategy for bioassay data interpretation and risk assessment in a water re-use context (BTO 2023.052, Reus et al. 2023).

V.VII Immunotoxicity as an endpoint; on the lookout for CECs

Ensuring the provision of high quality, safe drinking water is essential for public health and well-being. Drinking water companies are faced with increasing numbers of parameters in the drinking water law and rulemaking that are intended to protect the customers from potential risks of existing and emerging chemicals. The EU Drinking Water Directive has recently incorporated PFAS and BPA into national regulations (wetten.nl - Regulation - Drinking water regulation - BWBR0030152 (overheid.nl)). Scientific evidence shows that these anthropogenic micropollutants in water can adversely affect the human immune system (EFSA, 2020; USEPA, 2022). In order to keep up with the developments in immunotoxicology and effectively respond to its implications for drinking water regulations, BTO project Immunotoxicology that started in January 2024 aims to disseminate the information on immunotoxic effects and the health implications of (potential) immunotoxicity induced by chemicals within the water system. Importantly, the project aims to enhance our ability to better predict and interpret immunotoxicological properties of chemicals, specifically in the context of drinking water. Given that immunotoxicity can lead to low guideline values for health as observed with PFAS and BPA, it becomes crucial to lookout for similar substances before they pose challenges to drinking water production. The outcomes of this project will establish a foundation for predicting the immunotoxicity of unfamiliar compounds, thus serving as a warning system for potential contaminants of emerging concern. Additionally, the results will provide the knowledge basis to formulate a practical testing strategy for immunotoxicity in drinking water (sources) by *in-silico* and effect-based methods.

V.VIII Protecting drinking water under different regulations

The drinking water sector faces the requirement to follow stringing standards, prompting the need for a proactive approach. With the guideline limit values expected to continue to decrease and the search for new risk assessment tools and alternative risk assessement principles, there is a clear need for the water companies to : (1) understand the latest advancements in NAM – based limit derivation from a drinking water sector perspective, (2) increase their adaptability and prepareness to potentially important regulatory developments and requirements and (3) strengthen drinking water companies and KWR's position as trustworthy collaborators and actors in the public health debate with keystakeholders (e.g., Ministry of Infrastructure and Water Management,, the Human Environment and Transport Inspectorate and the Dutch National Institute for Public Health and the Environment) about (drinking) water limit setting for contaminants of emerging concern (e.g., PFAS, immunotoxic substances, pharmaceuticals, endocrine disruptors, microplastics, antibiotic-resistant microbes). The current BTO project on "Protecting drinking water under different regulations" that started in January 2024, serves as a proactive initiative to position the drinking water sector at the forefront of regulatory compliance, technological advancement and public health discourse. This will be achieved by studying the developments in (inter)national regulatory risk assessment across domains, in particular food and drinking water safety and evaluate the potential impact of the adoption of alternative risk assessment principles on water quality standard setting and overall risk management.

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