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Characterisation of **toxic pressure** of chemical pollutants in vulnerable areas

Methods and guidance for operational characterisation

RIVM report 2023-0379
M. Faber et al.

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Colophon

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Synopsis

Characterisation of toxic pressure of chemical pollutants in vulnerable areas

Methods and guidance for operational characterisation

The production and use of chemicals can lead to the release of toxic compounds. If these end up in water or soil, they can harm the environment. The more chemicals enter the environment, the greater the so-called toxic pressure. This can harm plants, animals and ecosystems in vulnerable areas, such as nature reserves.

In recent years, there has been growing concern in society about mixtures of chemicals in the environment, such as those emitted by industry, agriculture and households. However, relatively little research has been done on their effects on vulnerable areas.

RIVM has developed a framework to assess what the presence of these chemicals means for these areas. A guideline describes the approach step by step. The approach is suitable for determining toxic pressure in soil and surface water. For both, case studies were conducted to improve and illustrate the approach.

The approach merges three existing methods (chemical analyses, effects of mixtures on plants and animals, and ecological field work) from scientific literature. Practicality has also been considered.

RIVM recommends testing how well the approach and guideline work in practice and to develop them further, for example to determine toxic pressure in groundwater and organisms as well.

Keywords: chemicals, chemical pollutants, toxic pressure, vulnerable area, inventory, guideline, case study

Publiekssamenvatting

Toxische druk van chemische verontreinigingen in kwetsbare gebieden

Aanpak en handreiking voor een beoordeling

Bij de productie en het gebruik van chemische stoffen kunnen chemische stoffen vrijkomen. Wanneer deze stoffen bijvoorbeeld in water of de bodem terechtkomen, kan dat het milieu belasten. Hoe meer stoffen er in het milieu komen, hoe groter de zogeheten toxische druk. Dit kan schadelijk zijn voor planten, dieren en ecosystemen in kwetsbare gebieden, zoals natuurgebieden.

De laatste jaren is er in de samenleving steeds meer bezorgdheid over mengsels van chemische stoffen in het milieu, zoals van stoffen die de industrie, de landbouw of elk huishouden uitstoot. Maar er is nog relatief weinig onderzoek gedaan naar de effecten daarvan op kwetsbare gebieden.

Het RIVM heeft nu een eerste opzet gemaakt van een aanpak om te kunnen bepalen wat de aanwezigheid van deze stoffen betekent voor deze gebieden. In een handreiking is beschreven hoe die aanpak stapsgewijs kan worden ingezet. De aanpak is geschikt om de toxische druk te bepalen in bodem en oppervlaktewater. In het rapport is voor beide een situatie uit de praktijk uitgewerkt.

De aanpak voegt drie bestaande methoden samen (chemische analyses, effecten van mengsels op planten en dieren, en ecologisch veldonderzoek) en is wetenschappelijk onderbouwd. Verder is er rekening gehouden met de praktische uitvoerbaarheid.

Het RIVM beveelt aan te toetsen hoe goed de aanpak en handreiking in de praktijk werken en ze daarna verder uit te werken. Bijvoorbeeld ook om de toxische druk te bepalen in grondwater en organismen.

Kernwoorden: chemische verontreiniging, toxische druk, gevoelig gebied, kwetsbaar gebied, inventarisatie, leidraad, voorbeeldstudie

Scientific Advisory Panel and Acknowledgements

The 'Toxic pressure in vulnerable areas' project (in Dutch: 'Toxische Druk in Kwetsbare Gebieden') was executed by a project team at RIVM, supported by a Scientific Advisory Panel with an interest in and specific expertise with respect to the research. The members of the committee are gratefully acknowledged for their contributions to the various phases of the project, from the collation of methods to the design of the Guidance, and eventually the evaluation of the final product (the present report). This report is meant to be a first version of an operational methodology that can be used in practice to characterise toxic pressure in ecosystems. The members of the panel are listed below.

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The project team further acknowledges the valuable contributions of various colleagues and organisations. Annemarieke Verbout is acknowledged for her contributions as project coordinator, up to July 2022. We thank various colleagues and organisations for providing study data, context and/or support for the case studies, especially Matthijs ten Harkel, Bas Nelemans and Maarten Kantelberg (Province of Noord-Brabant), Wim van der Hulst (Water Authority Aa en Maas), and Gabriel Zwart (Water Authority Limburg).

Contents

Executive Summary

Societal backgrounds

The use of various kinds of chemicals is a characteristic of human societies. Emissions of chemicals to the environment may cause increased toxic pressures on water, sediment and soil. This may also occur in areas that are recognised as vulnerable areas or as having specific protection value.

Project definition and -aims

Various recent developments have triggered a project that was aimed at developing an initial method to characterise the toxic pressure in vulnerable areas and to illustrate the utility of that method by means of two realistic case studies, for the aquatic and the terrestrial environmental compartments, respectively. Those latest developments concern a report on the occurrence of chemical pollution in the form of unintended ambient mixtures by the Dutch Council for the Environment and Infrastructure (in Dutch: 'Raad voor de Leefomgeving en Infrastructuur', RLI), the European Green Deal aspiration of a toxic-free environment, and various new observations on ecological impacts of unintended mixtures in the terrestrial and aquatic environment.

Characterisation of toxic pressure

The Guidance that was developed is intended for experts with a background in environmental chemistry, ecotoxicology and/or ecology (to execute the approach). Moreover, the intention is that the stakeholders at large, responsible for the management of an area or having interests in an area, should be able to understand study reports on the occurrence and magnitude of toxic pressure in a specific vulnerable area that use the new methodology.

Conclusions

The Guidance is designed as a stepwise procedure based on three types of assessment techniques with which toxic pressure can be characterised (Figure 1):

1) Component-based methods – measurements of concentrations of chemicals, followed by a modelling step;
2) Effect-based methods – measurements of effects on sentinel organisms that are exposed in environmental samples, also known as bioassays; and
3) Ecological assessment methods – methods to collect and evaluate ecological field data to identify how toxic pressure affects the local ecosystem.

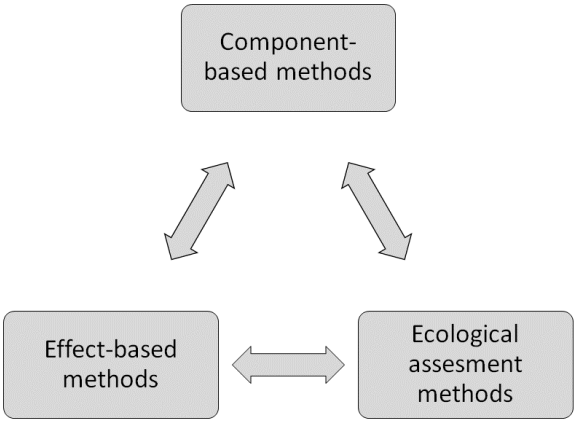


Figure 1 Schematic summary of the combination of three methods to assess toxic pressure. Component-Based Methods are the proposed default approach to start an assessment, and the other methods (Effect-Based Methods and Ecological Assessment Methods) can be employed optionally in combination with Component-Based Methods and as an alternative starting point.

The stepwise procedure can start from a pre-defined default approach, in which the toxic pressure assessment is initially based on chemical concentration measurements. The need for the second and/or third step depends on the specific situation. The stepwise procedure can, however, also be started from one of the two other types of techniques if a specific assessment problem and data already available suggest so.

The Guidance contains a SWOT analysis (Strengths, Weaknesses, Opportunities, Threats) to inform users about the advantages and disadvantages of the various methods suggested in the Guidance. Finally, guidance on how to analyse, collate and interpret study results is provided, as well as some recommendations for further development.

For each of the types of assessment methods recommendations for specific methods are provided for the surface water and soil compartment. For the selection of these methods, their use and their analysis and interpretation we have relied on recent scientific developments in the area of the estimation of toxic pressure of chemicals and on existing methodologies. Examples of the latter are the 'Sleutelfactor Toxiciteit' (Key Factor Toxicity) for the Water Framework Directive and the Dutch soil quality triad method developed to assess the ecological urgency and need for soil remediation.

The case studies showed that the framework can be applied in practice and yields insights to draw conclusions on the presence and magnitude of toxic pressure in the studied areas by the dominant chemical group(s). The case study reporting illustrates how users of the methods eventually can formulate conclusions, for each of the method steps, on toxic pressure in exposed ecosystems in vulnerable areas. The outcome of these cases was used to improve the methods and Guidance and/or to deduce recommendations for their use in the future.

The resulting Guidance should be seen as a first design to characterise toxic pressure in vulnerable areas. In future, the Guidance can be

modified and extended based, among others, on user experiences and new developments.

Limitations

The Guidance for the characterisation of toxic pressure in vulnerable areas presents a first version, at present still with some limitations. The methods collated from literature and in the example case studies are restricted to toxic pressure caused by the presence of toxic chemicals (micropollutants) in surface water and soil. There are no methods yet to assess toxic pressure on, for example, the groundwater compartment, or from substances accumulated in biota such as plants, aquatic organisms (such as fish) and higher fauna. Second, it is important to note that the methods on characterisation of toxic pressure in ecosystems have generally been designed based on methods that are employed using 'generic' species and 'available data from ecotoxicity tests' or bioassays with selected species. Commonly, the latter are not the species that may be of specific concern in the context of nature protection- or biodiversity conservation and restoration policies. Rare and protected species that are relevant for specifically vulnerable areas often belong to the groups for which specific methods are commonly lacking.

Reader’s guide

The report is split into five sections. Section A provides background information on the project topics and states the research goals and methods of this study.

In Section B, the Guidance is introduced. This Guidance gives practical instructions on how toxic pressure can be assessed. The Guidance has been formulated on the basis of:

1. The information that was collected from literature (Section C);
2. The project team’s knowledge; and
3. Feedback from the scientific advisory panel.

Section C serves as a background document with more detailed information on the techniques and approaches described in Section B.

Sections D and E each describe a case study, to illustrate the use and results of the Guidance. The emphasis is on the derivation of conclusions on the characterisation of toxic pressure when applying the different methods. Note that the Guidance has been changed as a result of the case studies, and that steps may have changed accordingly.

Section A – General

This section provides background information on the topic and states the research goals and methods of this study. Section A serves as an overview of these matters and ends with Conclusions and Recommendations that were derived after executing the project. That is, after collating available methods to characterise toxic pressure and formatting them in an operational Guidance, and after using and optimising that Guidance in two case studies. The reader is referred to the other sections for the Guidance (Section B), the available background information (Section C) and the two case studies (Section D and E).

1 Introduction

1.1 Background

The use of chemicals in society results in emissions to the environment, leading to unintentional, increased concentrations of chemical mixtures in water, sediment and soils. Contemporary monitoring data has shown the large spatiotemporal variability of 'chemical pollution' – the overarching term for this phenomenon.
Ambient exposures appear to vary from negligible levels with negligible impacts up to complex mixtures that are present at exposure levels that impact local species assemblages (abundance, composition, functioning) in ecosystem, as compared to a non-toxic situation (e.g. Lemm et al., 2020; Posthuma et al., 2020), often referred to as 'toxic pressure'. Large-scale evaluations, such as by Van Klink et al. (2020), allow us to derive patterns in, for example, insect decline and recovery (here: across the globe, for terrestrial and aquatic habitats, respectively). That study highlighted the importance of local factors to causing impacts, but there is limited attention for the relative role of unintended mixtures in causing impacts or recovery. As it happens, the effects of chemical pollution on biodiversity and in ecosystems are often difficult to establish, given the vast diversity of chemicals and their ambient mixtures.

Upon the design of a novel concept to characterise chemical pollution by means of a metric named 'mixture toxic pressure' (Klepper and Van de Meent, 1997), it became feasible to better address the role of chemical pollution in causing biodiversity decline and restoration. On the basis of using that metric, it was shown that effects of chemical pollution in nature are associated with altered biodiversity and/or loss of specific species (see the review of case studies by Posthuma et al., 2019a), and subsequently with potential adverse effects of societal and economic relevance, for example reduced ecosystem services such as pollination. In reply to observed exposures and impacts of chemicals and their unintended mixtures, the European Green Deal – and as part of that, the Chemical Strategy for Sustainability (CSS) – has formulated a zero-pollution ambition for a toxic-free environment, as its aspirational goal.

Beyond the CSS, specific societal concerns exist on the *chemical pollution in areas of specific societal value and concern* ('protected areas') *or areas recognised as specifically vulnerable*. Opposing expectations have been voiced. For one thing, are chemical pollution levels lower and less impactful there, due to for example a specific, protective status and the associated environmental management in the vicinity of such areas? Or, on the contrary: are chemical pollution levels similar but are species assemblages or individual species in those areas more vulnerable than the 'average' assemblages, and is the impact of the same pollution level in these areas higher as a result?

These matters require a specific approach to chemical risk assessment. That is:

- The area is not 'any area', but an area with a specific protection status;
- The species in the area may be more sensitive to disturbance and chemical pollutants than average species;
- A competent authority may have, or may be bound to achieve, specific nature-policy targets for the area;
- The area may have a specific role for a local population, recreationists or other visitors;
- The area is delineated by some administrative borders, which has influence on how a characterisation of toxic pressure would be executed and reported;
- The area may be surrounded by other areas, where the characterisation of toxic pressure may not be considered relevant, but which may influence the area of interest.

Many of the questions associated with this specific set of conditions are not easy to answer. The key scientific question to be answered for this is: how can chemical pollution with ambient mixtures be characterised in specific protected or vulnerable areas, and does the pollution give rise to a toxic pressure, causing impacts?
Given the observations and concerns on exposure to and effects of chemical pollution in ecosystems, the Dutch government has commissioned a research project on determining toxic pressure in vulnerable areas.

1.2 Goals

On the basis of the societal concern and questions regarding chemical impacts (toxic pressure) on ecosystems in vulnerable and protected areas, three research goals were defined. The goals of this study were:

1. To describe the state of the art, regarding methods to characterise chemical pollution of ecosystems in vulnerable areas, which results in an overview of methods that can be used to characterise toxic pressure;
2. To draft a first Guidance for characterising the toxic pressure of chemical pollution on ecosystems in vulnerable areas, in cases where responsible authorities or stakeholders express a need to do so;
3. To evaluate the scientific and practical validation status of the draft Guidance in case studies (one terrestrial, one aquatic), and to alter (improve) it as deemed necessary.

1.3 Approach

Three different project phases were defined to achieve the goals of the present study.

During the first phase, specific research questions were posed:

On terminology:

1. What is meant by the concepts of 'toxic pressure' and 'vulnerable area'?

On methodology:

2. Which methods are available to characterise toxic pressure on exposed ecosystems?

On practicality:

3. Which set of operational methods is suitable for collecting and interpreting the data needed to characterise toxic pressure on ecosystems in vulnerable areas, and is it feasible to do so?

By means of a literature search, combined with experiences with characterising toxic pressure in realistic cases, this first phase resulted in an extensive collation of options to characterise toxic pressure, if needed.

In the second phase, the information collated from the first phase was used to develop an initial practical draft methodology to characterise toxic pressure in vulnerable areas. As result of this, a proposal for an operational approach (Guidance) was developed, which can be practically used when concerns about specific areas are voiced.

In the third phase, the Guidance was tested, improved and validated by means of two case studies, concerning aquatic and terrestrial pollution, on toxic pressure characterisation in vulnerable areas. The process of testing the draft Guidance initially helped improve the draft Guidance, and the results of that are reported in Section B. Subsequently, the results of the case studies are reported in Sections D and E. The format used aims to illustrate how the outcomes of realistic case studies can be reported in a way that effectively informs stakeholders who are involved with an area under investigation.

Besides using information from literature, a scientific advisory panel was established to give advice on the content and structure of the project phases, on the report, and to improve the Guidance.

Section B - Guidance to characterise toxic pressure of chemical pollution in vulnerable areas

This section of the report provides a practical approach to the use of the available concepts, definitions and methods to characterise the toxic pressure in vulnerable areas. The background to and details of this approach are presented in Section C. The aim is to assist assessors in selecting appropriate methods, employ them in a stepwise manner, and interpret and communicate the results. The resulting Guidance is based on both scientific principles and practical considerations. The latter means that a *default* approach is proposed, but that the *assessor* may deviate from that as they feel is appropriate. The Guidance is explicitly meant to be a first version, which means that it can be improved on the basis of feedback from its users. The Guidance considers the use of Component-Based Methods, Effect-Based Methods and Ecological Assessment Methods, alone or in combination, the types of information that can be obtained from those, and the final interpretation of one or more lines of evidence. The Guidance was designed by the project team and improved on the basis of practical experiences in two Case Studies (reported in Sections D and E) and various rounds of valuable advice by a Scientific Advisory Panel.

2 Introduction to the Guidance

2.1 Goal

This Guidance describes the steps to be taken to characterise **toxic pressure** on ecosystems systematically and efficiently, specifically in **vulnerable areas**. The Guidance sets out to provide a science-based, systematic as well as a practical approach, which expert assessors can use to plan, execute and interpret such characterisations for the ecosystem in such an area or situation of concern. Assessments planned and conducted in line with the Guidance will provide information that can be used as a basis for evaluation with area managers and/or other stakeholders with an interest in a particular vulnerable area, and for decision-making (beyond the scope of this Guidance) if toxic pressure is demonstrated to be the cause of concern.[1]

2.2 Scope

Below, the definitions of toxic pressure, the environment and vulnerable areas are provided, to clarify the scope of the Guidance.

Toxic pressure
Toxic pressure is the pressure on ecosystems (or parts thereof) that is induced by exposure of biota to chemical pollutants and their mixtures. This implies that ecological impacts may occur due to the causal chain between emissions of chemicals to the environment, their fate and behaviour (resulting in exposure concentrations), the exposure of species inhabiting the exposed environmental compartment, and the consequent effects on those species and finally on ecosystems.

In this Guidance, toxic pressure is commonly characterised by focusing on the level of ecosystems, and thus on multiple species, for example when expressing the characterisation as a multi-substance Potential Affected Fraction (msPAF) of species, as this metric has been the basis of defining the term 'toxic pressure'. Other chemical, biological and ecological assessment methods are also considered, but these are often a proxy for, or alternative to, the way to characterise toxic pressure. Their results can be combined in a Weight of Evidence approach.

The ecosystem
In this Guidance, we focus on toxic pressure on ecosystems, paying specific attention to different compartments and endpoints. These include surface water, groundwater, soil, sediment, and biota.

The current Guidance mainly gives instructions on how to determine toxic pressure on ecosystems in surface water and soil and, to some extent, sediment. Methods that have been available so far are less

[1] The organisation of an assessment to characterise toxic pressure in a vulnerable area can encompass not only the technical steps for that (as elaborated in this report), but also a process of organising interactions with stakeholders who have an interest in the area. Various methods can be employed to organise the approaches for stakeholder involvement. See, for example, (1) NEN 5737 of ISO 19204:2017 Soil quality – Procedure for site-specific ecological risk assessment of soil contamination (soil quality TRIAD approach), or (2) Toelichting Maatschappelijke Afweging - Proces (risicotoolboxbodem.nl).

developed or are unavailable for the other compartments. Therefore, the intended use is primarily for the mentioned compartments.

Vulnerable areas
What may be considered as vulnerable areas is discussed in more detail in Section 11.2. A quick scan showed that no common definition for vulnerable areas is available in the scientific literature. A vulnerable area can be any area that is considered vulnerable on the basis of regulatory, scientific, societal and/or practical considerations. This basically means that any area can be considered vulnerable for various reasons.

The quick scan also revealed that there are hardly any risk assessment studies or other studies that specifically focus on the characterisation of effects of chemical pollution on ecosystems, or specific species, in vulnerable areas. Therefore, in spite of the fact that the research for this Guidance was triggered by concerns regarding toxic pressure in 'vulnerable areas', the methods that are described are based on insights that have been collected in generic ecotoxicological studies; they are (thus) generally applicable and can be applied to any selected study area.

Further scope
The current version of the Guidance is explicitly intended to be a first draft, given that its use may result in recommendations for modifications and additions based on the experiences gained by users. The goal is principally to characterise the toxic pressure by all chemicals (or selected groups of substances) in the areas of concern at the level of the multiple-species assemblages present. The Guidance does not yet address the exposure to mixtures of chemicals, and the effects on the complete functioning of an ecosystem (how the biotic and abiotic processes are affected) or on human health. However, the use of complementary Effect-Based Methods (bioassays) and Ecological Assessment Methods is also described.

The Guidance can be used by professionals who are familiar and have working experience with themes such as chemical pollution, environmental quality assessment and environmental risk assessment. Intended users (assessors) are, for example, environmental consultancy companies, research institutes, universities and water boards. Intended end users of the results obtained with the methods are all stakeholders involved in areas with concerns regarding the presence of toxic pressure.

2.3 Structure and content

The Guidance is the result of a background study in which methods were collated and ordered to characterise toxic pressure (Section C). This Guidance describes a stepwise approach that is derived from the available methods. By following the stepwise approach, information is gathered in a structured way, and this leads to a gradual, advanced characterisation of the presence and magnitude of toxic pressure in an area. The stepwise approach was derived on the basis of an evaluation and collation of approaches from (inter)national literature, and feedback

from a scientific advisory panel. In addition, the stepwise approach was reviewed and improved after conducting two case studies.
The Guidance describes five phases, in which a set of information is collected that is needed to characterise the toxic pressure and interpret the findings of the phases (separately or collated):

Phase I	Inventory
Phase II	Problem Definition
Phase III	Research Strategy
Phase IV	Research
Phase IV	Analysis and Interpretation

In Phase I, the societal concern that triggers the use of this Guidance is addressed. This phase helps decide whether characterising toxic pressure is helpful in verifying or eliminating the societal concern and – if not eliminated – which research questions must be answered to address the societal concern.

In Phase II, the problem is defined. This means that a so-called 'conceptual model' is developed in which the area, environmental compartment(s), contaminant(s) of concern and potentially exposed and affected endpoints (species, biodiversity, etc.) are described and summarised in a 'source-pathway-receptor' figure that shows how the presence of chemicals is hypothesised to potentially affect species in the area. The information from Phase II helps design the research strategy (phase III), and is also key to interpreting and communicating results in Phase V.

In Phase III, the research strategy is developed. This includes choosing the study design, selecting the methodologies to assess the toxic pressure, and writing a research plan. The plan commonly describes a stepwise approach, in which the uncertainties that will be encountered (that are largest initially, and that are meant to be reduced in every consecutive step) are listed.

In Phase IV, the research is carried out. All data needed to characterise the presence and magnitude of toxic pressure on an ecosystem in a given environment is collected, in line with the research plan from Phase III.

In Phase V, all data is analysed and interpreted. If applicable, the results of different methodologies are combined. Gathered information can be used to check whether the research questions can be answered. At the end of this phase the user will have characterised the toxic pressure and can present information on sites (within the area) and contaminants or substance groups for which the results show the highest toxic pressure levels. As is common for all risk assessments, the assessor lists (remaining) uncertainties.

In Figure B 1, the complete stepwise approach of this Guidance document is presented. Each step in the scheme is elaborated in the sections below. Text boxes are used to explain backgrounds of steps or to provide illustrative examples. The background section (Section C) provides further information on scientific, conceptual or practical aspects of elements of the Guidance.

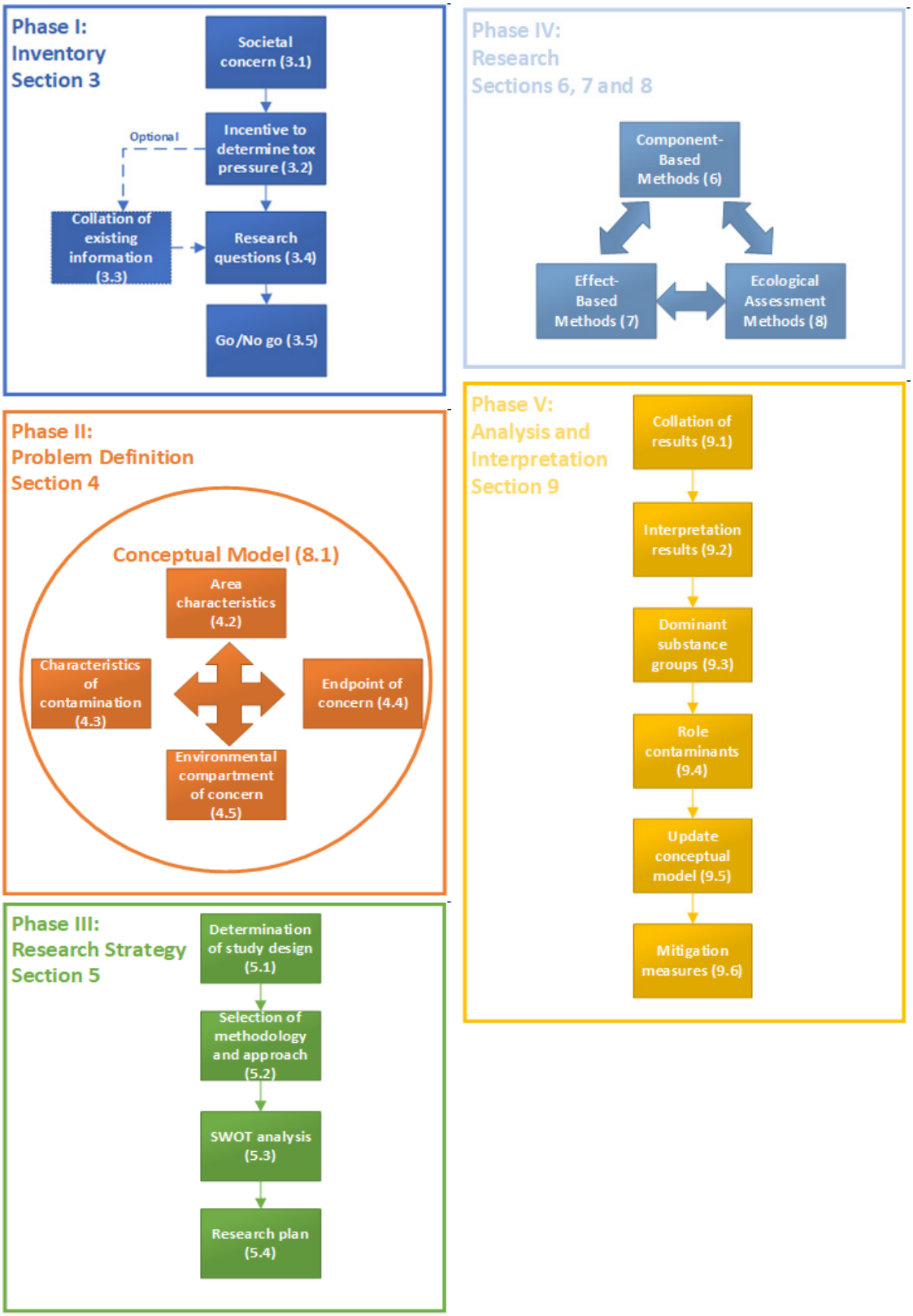


Figure B 1 Stepwise approach to characterise the toxic pressure on an ecosystem in an area of concern. Each Phase is shown in a separate colour and each step is represented by a block in the flow chart. The number of each step refers to the section of the report where the step is discussed.

3 Phase I – Inventory

3.1 Societal concern and incentive to determine toxic pressure

Characterising the toxic pressure on an ecosystem in an area in accordance with this guideline should have a motive. Commonly, the motive is a societal concern, which can be triggered by any relevant consideration. The textbox below provides some examples of societal concerns. Without any such concerns, it is not expected that an assessment is triggered. Different actors could have various motives for a concern on possible chemical pollution.

In this step, the prime focus should be on defining:

- The actors who have the concern;
- The motives for the concern (e.g. emission of chemicals, local pollution, decline of species and/or altered biodiversity);
- Whether initial concerns are more widely resonating;
- The area of concern;
- The hypothesised impacts related to the concern (e.g. decline of a particular species, change of biodiversity).

Ecological characteristics in vulnerable areas can be affected by other factors than exposure to chemicals. In case negative impacts are expected, and there is sufficient reason to assume that the concerns could be validly attributed (totally, or in part) to chemical pollution, one could proceed to Section 3.2. The characterisation of toxic pressure in an area might not be undertaken if actors conclude that impacts and concerns are attributable to other pressures.

Examples of societal concern

- Local residents assume that the biodiversity in a forest nearby has been declining over the years due to chemicals present.
- The numbers of livestock have increased in a municipality and the potential effects of veterinary medicines on animals in a national park nearby is of interest.
- A chemical spill caused a change in colour of a local waterway a few years ago; the current chemical status of the waterway is of interest.
- Land managers of a national park notice that the number of woodpeckers is declining and suspect that the presence of chemicals might be the cause.
- Past activities may have caused soil pollution, which may have spread via groundwater flows to an area of current concern.

3.2 Incentive to determine toxic pressure

In this step, it is determined whether characterising the toxic pressure would provide an answer to the societal concern.

The prime focus is on characterising:

- Whether the concerns may be (partly or fully) related to chemical pollution;

- Whether (and which) other pressures (besides chemical pollution) might be of relevance to (in part) explaining changes in ecosystems.

In case the concerns are fully or partly thought to be caused by chemical pollution, the following step is to collect information that is needed to ultimately confirm (or reject) the societal concerns.

Incentives to determine toxic pressure

Examples where there is an incentive to determine toxic pressure:

- A spill caused a change in colour of the water in a local waterway a few years ago, which confirms that chemicals are, or were, present.
- Local residents assume that the biodiversity in a forest nearby has been declining over the years due to chemicals present. It is not clear whether chemicals are the cause, but it cannot be excluded.
- Chemical monitoring data is available for an area, and various anthropogenic contaminants have been found. However, it is unknown whether effects on the environment are expected.
- An area is being redeveloped from agricultural land to a nature are, and it is unknown whether the presence of contaminants hamper achieving the required state.

Examples where there is no direct incentive to determine toxic pressure:

- The number of fish has been declining in a pond. However, other data shows that the water levels have been dropping over the years due to drought. In this case the effect of drought as the main cause might be more reasonable and should be investigated first.
- Land managers of a national park notice that the number of woodpeckers is declining and suspect that chemicals might be the cause. However, pine marten have entered the national park and their number is rising exponentially. In this case, both toxic pressure and the presence of pine marten could have the effect. It would be most logical to investigate the effect of pine marten on the woodpecker population first.

3.3 Collation of existing information

In this step, it is investigated whether information is available to underpin the societal concern. This step is optional and only applied if the societal concern is based on specific data, for example from monitoring. In that case, the specific data is collected in this step. Otherwise, one could also directly define the research questions to answer to the societal concern (Section 3.4).

Extensive data research is not supposed to take place in this step. However, it is possible that information that was not used to voice the initial concern is already available from elsewhere to underpin or eliminate the concerns. Therefore, a brief inventory of the presence of possibly existing data is helpful. Examples of information available to underpin the societal concern are:

- Chemical data relevant to the area of concern and/or its surroundings;
- Data from ecotoxicity bioassays[2], relevant to the area of concern and/or its surroundings;
- Ecological data relevant to the area of concern or its surroundings.

Data collected in this step can also help later, for example to define the research question(s) in Phase II and may potentially be used to characterise the toxic pressure in Phase III. If possible, it is valuable to also collect data for other areas that resemble the area of concern, as this data may underpin the societal concern.

Data collection

Examples of suitable data are:

- Chemical data: measurements in water, sediment, soil, air and biota (living organisms);
- Bioassay data: data from tests of fish, invertebrates, algae, plants and micro-organisms;
- Ecological data: species richness, relative abundance and biodiversity.

One of the outcomes of this step could be that data is available, but that it is not (entirely) suitable to address the societal concern. Depending on the data, it can still help to define research questions. For example, when the societal concern is "Land managers of a national park notice that the number of deer is declining and suspect that chemicals might be the cause'', and chemical analyses have been conducted in a national park close by, where no effects on the population deer are seen, the data can be used to resemble a reference site.

3.4 Research questions

The inventory of motives for societal concern, as well as of potentially available data for the area of concern or similar areas, provides a first characterisation of the facts regarding the area of concern. In this step, one or more research questions can be posed, with a broad focus in case of relatively broadly underpinned concerns, and a refined focus in case of data-underpinned concerns.

[2] Ecotoxicity data collected by exposing sentinel (test) species in samples of soil, sediment, water or air.

Examples of research questions

Regarding the presence of chemicals:

- Is substance group 'X' present in area 'Y' and to what extent does it exert toxic pressure?
- Which chemicals are present in the soil in National Park 'Z', and to what extent do they exert toxic pressure, alone or as an ambient mixture?
- Is there a specific temporal or geographical pattern for the concentration of chemicals present in area Y, and is it reflecting a toxic pressure pattern?

Analytic (if toxic chemicals are present):

- What is the level of toxic pressure based on the concentrations of chemicals to which organisms are exposed?
- Which substances or substance group(s) dominate the toxic pressure?
- How does the toxic pressure vary over the years?

Regarding interpretation:

- Does the level of toxic pressure relate to an observed effect in a nature area?
- Can the toxic pressure (materialised by the presence of contaminants) be explained by nearby sources or distant sources and transport of contaminants?
- Did mitigation measures implemented in the past reduce toxic pressure?

3.5 Go/No go decision

In this step, it is decided whether it is needed to continue the assessment. The assessment may be stopped if, for example:

- The concerns appear to be fully attributable to non-chemical pressure(s);
- Data (if available) shows that the concerns appear to be unfounded;
- Previous research has already addressed the societal concern.

In case no further steps are needed, motives to cease the assessment need to be summarised. That is, the use of this Guidance ends with a report, summarising the initial concerns, the collated insights of this phase and the reasons why those concerns do not trigger further assessment steps.

If it is decided to continue with the assessment, the information from Phase I is summarised and Phase II can be started.

4 Phase II - Problem definition

4.1 Constructing a conceptual model

A key goal in this step is to create a schematic overview of the problem and its possible cause(s), a 'conceptual model' in expert jargon, combined with an area map. In this case, the 'conceptual model' is based on the so-called 'source-pathway-receptor' principle, which summarises the likely causal chain between the chemicals (the 'source'), the pathways of potential exposure (the 'pathway') and the species/biodiversity/ecosystem exposed (the 'receptor'). The conceptual model can summarise multiple (hypothesised) pathways if relevant, for example when multiple chemicals are present.

The conceptual model gives an overview of all (hypothesised) possible causal chains between sources and effects. A schematic diagram (see Figure B 2 for example) helps visualise the sources and pathways of contaminants, and the hypothesised relative importance of different sources and pathways (by means of the thickness of the arrows), which together imply the presence of toxic pressure. The information in the scheme not only helps develop the research strategy (Phase III), but it is also key to visualising results for interpretation and communication purposes (Phase V). At the start, the thickness of the arrows reflects the *hypothesised* routes of exposure and their relative importance. In the last phase of the Guidance (Phase V) the conceptual model is updated, and the thickness of the arrows must be altered on the basis of the findings with respect to the *established* relative importance of an exposure route. The conceptual model may also be changed in Phase III during data collection, for example if the research plan is adjusted in response to intermediate results.

In order to create a conceptual model, information is needed on the area, sources of contamination, pathways and the endpoints of concern. Before constructing a conceptual model, first collect the information in steps 4.2 to 4.5. While it is recommended to create a conceptual model with all the information provided in these steps, the user of the Guidance is free to develop a model that fits the case.

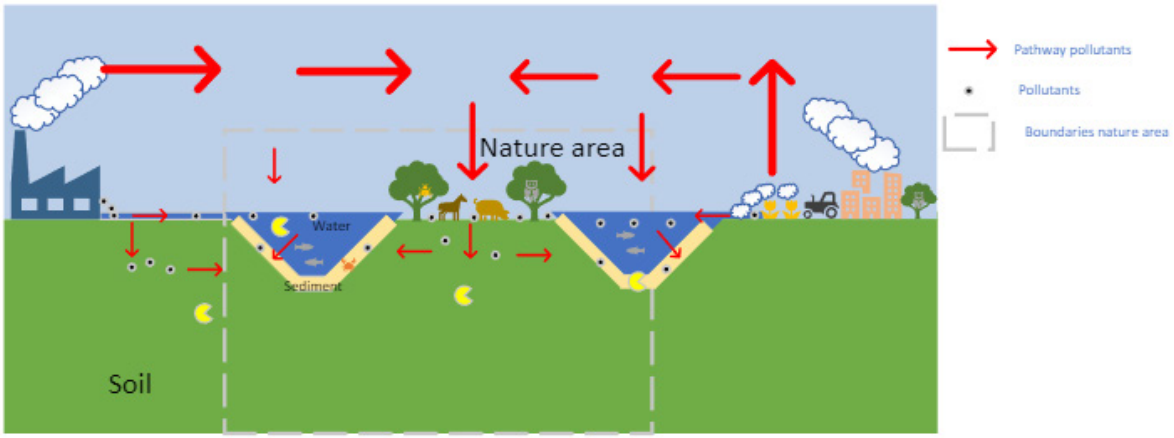


Figure B 2 Example of a schematic overview of the species and ecosystem of a vulnerable area (such as a nature area) subject to pollution, with its own area characteristics, characteristics of contamination, environmental compartments of concern and endpoint of concern (biota). The size and/or thickness of arrows characterises the hypothesised (initial) or established (post-research) relative importance of the elements in a causal chain. Chemicals may be broken down due to the effects of light, microbes or other causes (symbolised by the yellow 'packman'-symbols in the schematic overview).

Conceptual model

A conceptual model helps:

- Define the (suspected) source of the emissions (especially whether there is a need to consider a point source or a diffuse source, as that knowledge is relevant to determine the strategy of sampling);
- Define the emissions (which chemicals are potentially involved, are they subject to environmental breakdown, what is their preferred compartment where they end up, etc.);
- Define the area of concern, and its compartments and characteristics;
- Define the major 'arrows' which link all aspects;
- Define processes that alter exposure or availability of the compounds;
- Define biological phenomena (for example, seasonal bird migration alters exposure from continuous to temporary);
- Define the research strategy (Phase III);

and eventually (Phase V) helps:

- Summarise assessment results by highlighting (through the arrows, and the affected compartments, biota, etc.) the incidence of increased toxic pressure;
- Summarise remaining assessment uncertainties;
- Communicate assessment results, especially by allowing to show the differences between the hypothesised- and the realised (resulting) schematic summary.

4.2 Area characteristics

The second aspect of the problem definition step is to determine the geographical constraints of the case study, so that it is clear to which

area the conceptual model applies (see Box below). This is explored in this step.
Here, maps showing geographical representations of characteristics of the area (e.g. hydrological patterns of water, land usage, wind directions) may be developed that include the surrounding areas. The user describes the area and its surroundings, preferably (also) as a map. Information on the area and surrounding characteristics may help characterise sources and exposure of contaminants; for example, it may help hypothesise that a gradient in exposure is expected or that there are 'hot spots' present for certain contaminants.
General and specific geographical elements that may be assessed in this step are provided below. Note that the listed information is not exhaustive and that not every aspect may be relevant to the specific case.

General characteristics that may be retrieved:
- Geography (location, boundaries, etc.);
- Landscape elements (water, soil, forest, etc.);
- Slopes/geohydrological characteristics (that determine runoff, seepage, groundwater extraction wells);
- (Micro-)climate (temperature, wind direction, precipitation, etc.);
- Economical activities, drainpipes, housing areas, industrial activities, greenhouses, etc. (land use information);
- Optional: specific regulatory status (protected area and associated law).

Water-specific characteristics that may be retrieved:
- Abiotic characteristics:
  - Type of surface water (fresh, brackish and/or saltwater, ditch, river, lake, etc.);
  - Water characteristics (pH, hardness, salinity, etc.);
  - Current(s);
  - Hydrological connections within the area and across the area boundaries, with special attention for potential upstream sources of chemical pollution.
- Ecological characteristics
  - General ecological status (according to Water Framework Directive);
  - Ecological Status for specific 'Biological Quality Elements' (according to Water Framework Directive);
  - Other ecological information of relevance, such as protected species.

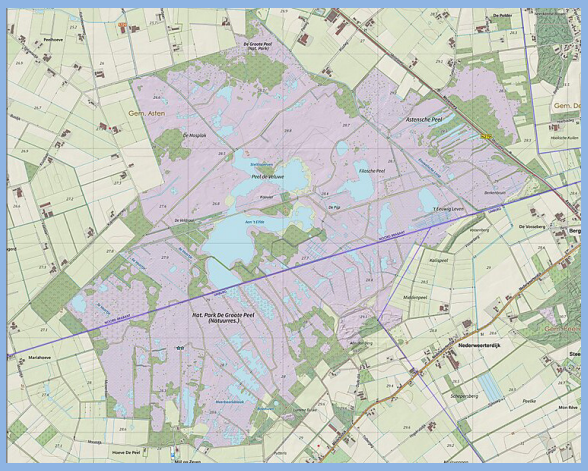
Terrestrial-specific characteristics that may be retrieved:
- Abiotic characteristics:
  - Physicochemical characteristics (e.g. grain size, organic matter, silt, pH);
  - Soil type ;
  - Temperature.
- Ecological characteristics:
  - Ecological information of relevance, such as protected species.

Ecological conditions that may be retrieved:

- Land use
  - Type of vegetation;
  - Other ecological information of relevance, such as distribution of (protected) species.

Example of the characterisation of an area

- In this example, De Groote Peel National Park has been chosen as the nature area of interest.



- On the map, the borders of the area can be seen. The nature area is both a national park and a Natura 2000 area. The EU Birds Directive and Habitats Directive apply to the area. The national park is water-rich and features a high moor area. Information on soil types and local land uses can be retrieved from databases (such as GIS-maps from the Dutch government) and plotted to represent available, detailed information. Information on economic activities in the area and its surroundings could be retrieved from, for example, www.bedrijvenopdekaart.nl. For water and soil-specific characteristics, databases from water authorities or site managers can be accessed (e.g. Dutch water boards, provinces, municipalities). Information on the climate could be retrieved from Dutch weather organisations (e.g. KNMI).

4.3 Characteristics of contamination

With thousands of chemicals in commerce, focusing the assessment is essential, so that those chemicals that may exert a toxic pressure in an area can be identified. Here, it is investigated which contaminants may be present in the area and could be of interest in assessing and/or explaining toxic pressure. It may help to construct a research plan (Phase III), for example in selecting chemical measurements or the selection of bioassays.

To help identify which contaminants may be present, tools may be used that link typical human activities (land uses) with their associated uses

of chemicals (e.g. see the look-up table of the sleutelfactortoxiciteit[3]). Note that, for example in a water body, the local mixture exposure may consist of pollutants from local emissions combined with those from upstream. It should also be noted that chemicals used in the past ('legacy' chemicals) may still be present in an area and could exert toxic pressure; these could also be of interest.

General and specific information on contaminants to be assessed is provided below. Assessing some characteristics may result in a large workload. Note that, although more information leads to better insights into the case, the user can decide how much effort they put into collecting the data.

Characteristics of contaminants that may be retrieved:

- (Presumed) sources (distance to area of concern, point/diffuse source);
- Type and possible identities of contaminants;
- Substance properties (persistence, solubility, volatility, sorption, mobility, bioaccumulation, etc.);
- Emissions (point or diffuse source, quantity, duration), emission points (to be added to the area map) and emission routes (air, water, food, etc.);
- Ecotoxicological information on chemicals likely to be present (e.g. most sensitive species);
- Exposure routes for organisms.

After collecting the information, evaluate whether the available insights suggest a sampling strategy, which might consider any of the following options: (1) the hypothesised toxic pressure is (likely) homogenously or randomly spread over the area of concern; (2) the hypothesised toxic pressure (likely) follows a gradient (from where to where); or (3) the hypothesised toxic pressure is present as 'hot spot' patches. Summarise the hypothesised chemical pollution situation on the map, as a basis for deriving a sampling strategy.

4.4 Environmental compartment of concern

In this step, the compartment of concern is determined. This may pertain:

- A main environmental compartment, e.g. air, soil, water;
- A sub-environmental compartment, e.g. ground water, topsoil;
- A biological compartment, e.g. birds, soil invertebrates;
- Or a combination of the above.

4.5 Endpoint of concern

In this step, the endpoint of concern is selected and defined. The endpoint of concern can be a species, biodiversity, or a functioning ecosystem. Information on the endpoint of concern may help construct the research plan, for example in selecting research methods or contaminants that are of interest to addressing effects on the endpoint of concern. The endpoint of concern may be based on a retrospective line of reasoning – there is chemical pollution and there is an observed

[3] Can be accessed via the website (https://www.sleutelfactortoxiciteit.nl/aan-de-slag/de-pressure-van-dpsir) or directly here: https://www.sleutelfactortoxiciteit.nl/sites/default/files/2023-01/Landgebruik-stoffenlijst%20opzoektabel%20versie%201.xlsx

response that is hypothesised to be attributable to the chemical pollution – or on a prospective line of reasoning. That is, information on, for example, emissions is used to forecast that toxic pressure might be present due to deposition of chemicals in an area. Endpoints of concern are depicted in the conceptual model as schematic end-of-arrow entities.

5 Phase III - Research Strategy and Data collection

5.1 Determination of study design

Here, it is chosen which type of reasoning is used to characterise (evidence of) toxic pressure. There are three options, which are based on observations in the area of concern (and optional reference areas):

1. Characterise toxic pressure by comparing observations in an area to regulatory protective environmental quality standards or other standards or threshold values; this method requires a (regulatory) 'anchor point';
2. Characterise toxic pressure by comparing observations in an area to similar observations made in undisturbed or minimally disturbed references sites; this method is comparative;
3. Characterise toxic pressure as a gradient; this method is comparative but specific, because of the gradient.

In the first case, the outcome is often based on a Risk Quotient (RQ), defined as the ratio of the observed concentration and the protective environmental quality standard, where RQ<1 indicates that the exposure is lower than the protective threshold, so that the toxic pressure (if present) is regulatory accepted, and RQ>1 indicates it is not.

In the second case, results are compared to results from an area that is known to show no or low toxic pressure. In that case, the toxic pressure can be expressed as a relative difference between two sites: one 'potentially contaminated' and one 'pristine' regarding the compounds of concern.

In the third case, results are gathered in such a manner that a hypothesised gradual trend in toxic pressure is revealed. This could be a spatial trend (e.g. from close to a source (high toxic pressure) until further (moderate toxic pressure) and still further away (low toxic pressure), or a temporal tend (e.g. soon after use of a chemical (high toxic pressure) to later (moderate toxic pressure) and still later (low toxic pressure). Here, results may be expressed as absolute toxic pressure (as in the first case) or as relative toxic pressure (as in the second case).

The user can decide which method helps answer societal questions best. Note that the first method can only be applied if standards or other threshold values are available to assess toxic pressure.

It is noted that the characterisation of toxic pressure may also be based on predicted environmental concentrations. In such a case, the research plan employs a selected environmental fate modelling approach, which yields Predicted Environmental Concentrations (PECs) rather than Measured Environmental Concentrations (MECs).

It is further noted that insights gained during the stepwise characterisation of toxic pressure in turn provide novel insights, providing information on selecting one of the other approaches.

5.2 Selection of methodology and approach

On the basis of the collated information in Phase I and II, a research strategy should be designed that can answer the research question and that considers the information on the concern, the hypothesised chemicals of concern, the routes of exposure and the probable effect types, and the spatial pattern of hypothesised exposure levels. The research strategy can be any combination of the following three possible lines of evidence:

I. Component-Based Methods, which use information on concentrations of chemicals to characterise the toxic pressure patterns in an area;
II. Effect-Based Methods, which use information on responses of sentinel species (bioassays) to characterise the toxic pressure patterns in an area;
III. Ecological Assessment Methods, which use exposure-related or impact-related ecological (field) information to characterise the toxic pressure in an area, often in relation to a non- or minimally disturbed reference condition.

In this step, the methodology and approach are selected, as is the sampling scheme. For pragmatic reasons, the sampling scheme considerations are described in Section 6.1 on Chemical-based methods (as this is the default). The user can freely choose to use any combination or sequence of the three lines of evidence. So far, however, most experience in scientific literature has been gained using Component-Based Methods. From current experience, the availability of data that can be used to characterise toxic pressure decreases proportionally from chemical to bioassay to ecological data. Therefore, in practice, the robustness, ease of use and clarity of interpretations on the role of chemical pollution as a driver of impacts decreases from the first to the third method. Therefore we recommend starting with Component-Based Methods.

However, one could also start with another methodology. For example, if there is sufficient evidence for the use of a bioassay battery whilst there is no reason to identify one or more (group(s) of) chemicals of concern, one could choose to start the research by collecting bioassay data (i.e. Effect-Based Methods). If a vast array of (bio)monitoring data is available, one could likewise decide to start with statistical and/or ecological methods to establish a role of chemical pollution in field data (i.e. Ecological Assessment Methods). It may also depend on other aspects, such as the environmental compartment of concern. That is, the data availability for aquatic systems is usually larger than for terrestrial systems, and this information can be used to define the preferred methods.

The decision which methodology and approach to follow can be based on the information collected during the Inventory phase (Phase I) and with

a comparative SWOT[4] analysis of the available methods (see Section 5.3). The research approach eventually decided on is to be written down in a research plan (see Section 5.4).

Research strategies

- Multiple methods are employed to address method-specific uncertainties, such as lack of information on toxic pressure of not-measured chemicals (if chemical analyses are performed). Multiple methods require a combination of the results of various lines of evidence in Phase IV.
- The use of all provided approaches in this Guidance resembles the so-called TRIAD approach, a term first coined by Chapman (1986, 1990) for risk assessment of sediments using a combination of chemical, biological and ecological methods. The TRIAD approach was also adopted in the Netherlands for the risk assessment of contaminated soils. At RIVM, among others, a lot of effort was put into developing a methodology and guidance (e.g. Mesman et al., 2007; Mesman et al., 2011; Rutgers et al., 2008; Mesman et al., 2014), yielding positive experiences with applying the approach to practically evaluate soil contamination (Wagelmans et al., 2010). The approach was also used in the Netherlands for contaminated freshwater and marine sediments and, in a modified version, for fresh surface waters.
- The main difference between the approach in this Guidance document and other TRIAD-type approaches is that here, the various methods are conducted in a sequence, with Component-Based Methods as the preferred initial line of evidence, while in the TRIAD, all methods are conducted. This relates to practical considerations, as a TRIAD approach is more costly and complex than a single line of evidence, whilst the latter may provide sufficient characterisation of toxic pressure for the involved stakeholders. Thus, the choice of one or more methods is not a scientific one, but a contextual one – defined by the stakeholders on the basis of practical arguments.

5.3 SWOT analysis

The different Strengths, Weaknesses, Opportunities and Threats (SWOT) aspects of using chemical monitoring data, bioassay data and/or ecological monitoring data for characterising toxic pressure are presented in Table B 1 - Table B 3. In this step, the collection and interpretation of each type of data can be compared regarding different aspects, such as resources needed, difficulty in execution and interpretation and current availability of methods. Note that this step is optional, but it is informative to determine a research strategy (the first and later optional steps).

Evaluate, for the case under consideration, all aspects and the already available data. Define the research methods, as (1) default: Component-Based methods as the first step; (2) select another type of method as the first step; (3) or select to use the TRIAD approach; and (4) describe whether and how the research steps are planned, and which outcomes are considered to provide a sufficient characterisation of

[4] SWOT = Strengths, Weaknesses, Opportunities, Threats – a SWOT of the various lines of evidence has been made, see next section.

the toxic pressure on an ecosystem to stop a step and the research for a particular case.

Table B 1 SWOT-analysis Component-Based Methods. LOQ=Level of Quantification.

Strengths

- Methods for chemical analyses are highly standardised
- Outcomes are easily interpretable
- Toxic pressure is empirically shown to relate to impacts
- Contribution of individual pollutants can be diagnosed
- Can be applied with chronic and/or acute toxicity data

Weaknesses

- Outcome is only valid for the substances that are included in the analysis method(s)
- Extensive toxicity data is needed
- Outcome is dependent on literature toxicity data of variable origin and quality
- Substances <LOQ are not considered
- Based on model calculations such as Species Sensitivity Distributions (SSDs) and for mixture toxicity (including assumptions)

Opportunities

- Methods are simple and straightforward
- Software is available to quantify toxic pressure (for the aquatic environment)
- Can be expanded to determine toxic pressure for separate taxa (e.g. algae, daphnids, fish)
- Temporal and spatial trends can be characterised
- Suitable for prospective use (e.g. for permits)

Threats

- Chemical analysis methods may not be available or sufficient to measure all relevant (groups of) toxic substances
- Toxicity data may be unavailable for certain substances
- Protective standards for a chemical may differ between jurisdictions and may change over time

Table B 2 SWOT-analysis Effect-Based Methods

Strengths

- Quantify toxic pressure of all chemicals combined
- Demonstrate biologically relevant effects
- Examine effects of 'unknown' chemicals
- A range of standardised methods is available
- Preliminary evidence for association between bioassay responses and ecological status of surface waters

Weaknesses

- Chemicals causing an effect may remain unknown
- Confounding factors like the experimental conditions may co-determine observed effects (less so for cell-line based high-throughput assays)
- Test animals are used for some *in vivo* bioassays
- Test specimens from different batches may vary in sensitivity to the chemicals

Opportunities

- Useful for screening of samples with unknown chemical composition
- Can be used in the absence of chemical analyses
- Bioassays for a specific mode of action are available
- 'Bioassay battery' for aquatic systems available
- Empirical relationship between bioassay responses and both toxic pressure and ecological status (water)

Threats

- Many bioassays available, choice of suitable and relevant bioassays is crucial
- Natural variability of biotic materials not always recognised in mindset of end users
- Interpretation of bioassay data requires thorough explanation

Table B 3 SWOT-analysis Ecological Assessment Methods

Strengths

- Reflects the situation in the field
- High ecological relevance
- Body/tissue residue concentrations of field specimens can be used as evidence for exposure

Weaknesses

- Methods yield statistical associations, not cause-effect proof
- Results may be influenced by other environmental (stress) factors occurring in the field
- Methods are often less standardised
- Requires adequate reference sites of similar typology
- Body/tissue residue may not imply impacts

Opportunities

- Can be combined with chemical analyses and bioassays (TRIAD approach)
- 'Big data' compilations can be used to further substantiate empirical associations between chemical, bioassay and ecological datasets

Threats

- Labour-intensive (costly) and time-consuming
- Many potential methods available, choice of suitable and relevant method is crucial
- When used in isolation the outcome (an observed impact) may be difficult to relate to chemical pressure

5.4 Research Plan

In this step, a research plan is written. The research plan describes the study design and the selection of methods, as well as the motives to select these. The selection should be based on the information gained in Phases I and II.

A research plan can thus account for applying Component-Based Methods is sufficient to answer research questions, but combining two or three lines of evidence may also be accounted for, as each provides complementary information to the others. Whichever the choice, the research plan describes *a priori* which approaches are planned, and why, and via which motives and which step(s)/technique(s) an assessment is stopped in order to describe its conclusion or (eventually) the overall interpretation.

Research plan

- For the present Guidance, the choice is made to propose Component-Based Methods as the basic methodology to determine toxic pressure. The use of Effect-Based Methods and/or Ecological Assessment Methods are optional and may be conducted, for example, when triggered by the results of the Component-Based Methods, if additional information about the toxicity of the environmental compartments in the area and/or the state of the ecology is desired, or when data is already available.
- A research plan may be flexible and may be adjusted on the basis of preliminary outcomes. Flexibility does not imply conducting unmotivated random methods. The research plan must describe how the steps proceed, which methods are to be followed on the basis of which motives, and which information is considered sufficient to draw conclusions on toxic pressure characterisation.

Additionally, the research plan should provide information on the conceptual model, the geographic area, the available data, the measurements and data needed for the research approach, and the interpretation context(s). Information should also be provided on how the research is to be planned and executed. This implies that the research plan should provide insights into if and how existing data is collected (field sampling scheme), how sampling and (chemical or effect) analysis are performed and how results are interpreted, all in relation to the research goals. Also, expected uncertainties regarding outcomes should be noted in the research plan. Guidance on data collection, sampling and analysis, and determination of toxic pressure can be found in separate sections for each line of evidence (Sections 6 to 8). Guidance on the interpretation of data can be found in the description of Phase V (Section 9).

5.5 On sufficient data for characterising toxic pressure

The characterisation of toxic pressure, or its proxy metrics, eventually depends on the sampling efforts that are undertaken, given a selected (combination of) method(s). It is difficult to provide a general rule that clearly defines ‘sufficient data’. However, if available data or data

obtained from an initially planned sampling scheme provides insight into the presence and magnitude of toxic pressure – even with only a few sampling points – then this may be sufficient for some cases (at which point the assessment may stop, providing a conclusion on this data). That is, a 'positive' signal on toxic pressure can be considered sufficient verification of the initially voiced concerns. In contrast, if an initial limited number of samples results in insufficiently clear conclusions, the assessment could proceed with a refined sampling and analysis scheme. Thus, by basing an initial sampling scheme on the conceptual model and on the map, and by further employing the principle of a stepwise approach (towards refinement), the assessment plan can strike a balance between cost/efforts and remaining uncertainties.

6 Phase IV – Component-Based Methods

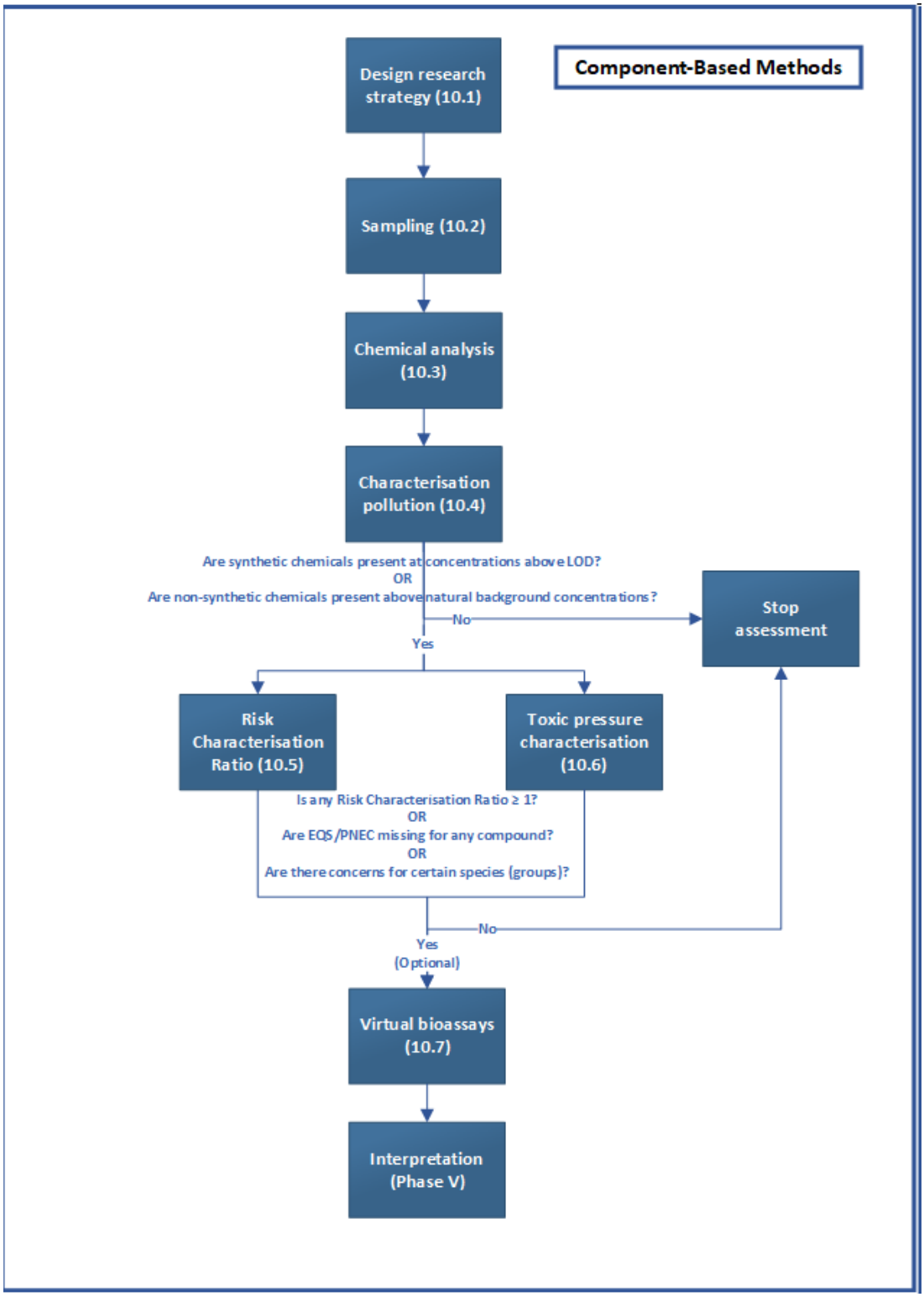


Figure B 3 Steps to characterise toxic pressure or its proxy metrics by means of Component-Based Methods.

Component-Based Methods (Figure B 3) are based on two types of information:

- Information on measured concentrations in the area of concern (potential exposure);
- Information on hazard for each chemical that was measured (toxicity).

The two information sources are combined to draw meaningful conclusions on the absence/presence of toxic pressure, and information on the possible magnitude of the toxic pressure level. The various existing Component-Based Methods are indicated in the scheme. The various methods yield differently specified conclusions (all related to toxic pressure, but in different ways) and it is proposed to use them in a stepwise succession. Later steps thus yield more specific interpretations on toxic pressure, earlier steps result in proxy metrics to evaluate toxic pressure. The proxy metrics are explained below.

6.1 Design research and sampling strategy

If no or insufficient data (see Section 5.5) is available to assess toxic pressure, samples need to be taken in the area of concern to be analysed. There is no clear rule of thumb whether available data is (in)sufficient. A practical consideration could be to use relevant, available data as a first screening method to assess the toxic pressure, and additionally decide on further research, for example on the basis of the results, uncertainties and desired information.

The research strategy should encompass three pillars of information, each with a detailed description:

1. Sampling design;
2. Analytical methods;
3. Data evaluation methods.

6.1.1 Sampling design

When it is decided that (more) new samples are needed, it should be known that various options are available to do so, each fitting particular conditions. It is beyond the scope of this report to describe all possibilities and aspects regarding an appropriate sampling strategy to consider. A good textbook on this matter is provided by De Gruijter et al. (2006).

How, why, when, where and what to sample is not automatically defined but can be informed by the conceptual model and the area map. The question whether results need to have statistical significance determines to a large extent how complicated the sampling design must be. The sampling design, such as the number of samples and spatial and temporal distribution, needs to be designed in such a way that variation in endpoints (e.g. concentration in chemicals) is statistically accounted for. Moreover, it should reflect pre-existing hypotheses on matters such as diffuse pollution, gradient pollution or hot-spot pollution.
Designing such a sample design is difficult and time-consuming. For a proper design, and a complex conceptual model and/or hypothesised pollution pattern, consulting an expert in the area of sampling design is recommended. Knotters et al. (2009) strongly warn against the use of

the term 'representative' when describing a sampling strategy or sampling locations. In their view, it is more important, instead, to provide a detailed description of:

1. The goal of a sampling scheme;
2. The desired quality of the selection of samples; and
3. The design of the sampling scheme.

These define the strategies needed to achieve the desired results.

Important, general aspects to consider are (non-limitative):

- Size and type of the area. Does a whole area need to be assessed or are there concerns regarding a specific part of an area? What kind of area is it, and what is the protection goal? Which environmental compartments are expected to be potentially affected by an increased toxic pressure?
- The (expected) pollutants (type, source, emission routes). Which chemicals are of interest? What kind of sources and emission routes could have polluted the area (in the past)? The visualisation from Section 4.1 helps answer such questions.[5]
- Sampling scheme. Which media should be sampled, and over what time period? Is a single sample sufficient or is a trend series (e.g. a spatial gradient) required?
- Accurate sampling. Which methods should be used to guarantee that adequate samples are taken? Are duplicates sampled? Are samples not contaminated? Is homogeneity ensured? Are all samples and proceedings recorded?

6.1.2 Analytical methods

Before assessors decide to sample, they should be sure that analytical methods are available to sufficiently gain the desired information from the samples.

Important, general aspects to consider are (non-limitative):

- Methods. Which analytical methods are most suitable to detecting the contaminants in the specific media? How should samples be preserved and be delivered to the laboratory? Is sampling performed in line with these methods?
- Availability. Are there laboratories who can perform the measurements? Are they certified to perform these analyses?
- Reliability. The analytical measurement technique(s) need(s) to have:
  - High accuracy (recovery)
  - High repeatability
  - High reproducibility;
- Usefulness. The Limit of Detection (LOD) and Limit of Quantification (LOQ) need to be suitable to assessing the presence and risks of the compounds;
- Other aspects. Other parameters may also need to be analysed. E.g. organic matter as required to determine the bioavailable fraction of a contaminant.

[5] We recommend using the file 'Opzoektabel landgebruik-stoffenlijst' to link certain contaminants to land use.

6.1.3 Data evaluation methods

In addition to the sampling design and the desired analytical methods, it should also be decided how the resulting data is to be eventually processed and interpreted. In case certain analyses and interpretation steps are to be performed, such as statistical analyses or sensitivity analyses, the data might need to meet certain requirements. This could result in adjustments to the sampling design and the requirements regarding the analytical methods.

The above information is not exhaustive, but it gives an overview of aspects that are relevant for all intentions to sample and (subsequently) analyse chemicals. More background and considerations are provided in Section C of this report.

6.2 Sampling

6.2.1 General

In this step, sampling is performed. The research plan (*a priori*) and eventually the report (*a posteriori*) of the assessment must state which sampling strategy is chosen, and why it is (sufficiently) representative of the situation of concern.

Sampled environmental media will contain a mixture of chemicals, with the concentrations and contaminants often unknown to the person who is sampling. These chemicals may be a risk to human health and the environment. As a precautionary principle, safe handling needs to be guaranteed to limit personal risks. This can be achieved by using personal protective equipment (PPE, e.g. gloves and protective glasses) during sampling, but also during processing and analysis. When samples are expected to be heavily contaminated and/or dangerous for health, this should explicitly be communicated to all persons involved in the research. Adequate documentation and the use of clear visuals to allocate these samples is key.

Samples must be properly handled and stored to achieve reliable results. Cross-contamination must be avoided, for example by using clean sampling materials and gloves. Samples should be confined in such a manner that these are preserved. Often, samples are stored in a cool environment to slow down processes that affect the chemical composition, such as volatilisation and biodegradation. Alternatively, samples may also be frozen or dried for storage (e.g. in the case of soil or biological samples).

During sampling, enough environmental media should be collected. Spare media is useful when reanalyses would be needed, when additional analyses are needed to answer unforeseen research questions (or for bioassays, for example) or when (part) of the sample is not useful anymore, due to contamination for instance.

Where possible, standardised methods need to be followed, to guarantee reliable results. Below, several references are provided that help design a sampling strategy. Note that the lists are non-limitative.

6.2.2 *Surface water*

Protocols and guidelines for water sampling are provided by:

- Koninklijk Nederlands Normalisatie Instituut (NEN) (Netherlands): https://www.nen.nl/milieu/waterkwaliteit;
- ISO: https://www.iso.org/home.html;
- Rijkswaterstaat (RWS, the Netherlands): Guidance on surface water monitoring;
- The European Commission: Guidance on Surface Water Monitoring;
- U.S. Environmental Protection Agency (EPA): https://www.epa.gov/sites/default/files/2017-07/documents/surface_water_sampling201_af.r4.pdf

6.2.3 *Sediment*

Protocols and guidelines for soil sampling are provided by:

- Koninklijk Nederlands Normalisatie Instituut (NEN) (Netherlands): https://www.nen.nl/milieu/waterkwaliteit;
- ISO: https://www.iso.org/home.html;
- U.S. Environmental Protection Agency (EPA): https://www.epa.gov/sites/default/files/2015-06/documents/Sediment-Sampling.pdf

6.2.4 *Soil*

Protocols and guidelines for soil sampling are provided by:

- Koninklijk Nederlands Normalisatie Instituut (NEN) (Netherlands): https://www.nen.nl/milieu/bodem;
- Koninklijk Nederlands Normalisatie Instituut (NEN) (Netherlands): NEN 5737:2010 (NEN5737, Bodem - Landbodem - Proces van locatiespecifieke ecologische risicobeoordeling van bodemverontreiniging);
- ISO: https://www.iso.org/home.html
- Stichting Infrastructuur Kwaliteitsborging Bodembeheer (SIKB) (Netherlands): https://www.sikb.nl/richtlijnen;
- U.S. Environmental Protection Agency (EPA): https://www.epa.gov/sites/default/files/2015-06/documents/Soil-Sampling.pdf;
- RIVM: see for example the report on soil biological indicators (Schouten et al., 2003) and the references provided by Mesman et al. (2011) for specific protocols/guidelines.

6.3 Chemical analysis

In this step, the chemical analyses are performed. In many cases, an external company (laboratory) will perform the analyses.

6.4 Method 1: Characterisation of Pollution

The basic method, named Characterisation Pollution, is the basic check on the presence of anthropogenic pollution, serving as a first proxy metric (signal) of toxic pressure. The check is technically limited by the presence of an analysis method for the chemicals identified to be of potential relevance, and the Limit of Detection that is associated with the selected method of analysis. In this step, for each chemical separately, the research aims to check on the presence of concentrations that are attributable to human activities:

- For synthetic chemicals, any concentration >LOD signals the presence of a man-made increase of the ambient concentration;
- For non-synthetic chemicals (such as metals) it is key to discern whether a measured concentration exceeds the natural background concentration. The latter may vary widely across areas due to natural origins, such as iron-rich small streams in the Veluwe area, or zinc and copper-rich streams and riverbeds originating from surface metal ores in the Geul basin.

For part of the non-synthetic chemicals, information on natural background concentrations is available, for example, in the atlas of such backgrounds for metals (Mol et al., 2012).

Measured concentrations that exceed zero (synthetic) or exceed the natural background concentration (non-synthetic)[6] result in the conclusion that chemicals are present due to anthropogenic pollution.

The concentrations may be further interpreted, as to whether they represent a true toxic pressure (an exposure level from which it can be deduced that it implies a degree of possible harm), in various ways.
In case there is no anthropogenic pollution, and if the set of measured chemicals was identified to represent the hypothesised pollution: stop. Report the finding, and the representativity of the measured compounds to support the conclusion.

In case there is anthropogenic pollution, performing Method 2 (Section 6.5) and Method 3 (Section 6.6) simultaneously is recommended.

6.5 Method 2: Risk Characterisation Ratio

The Risk Characterisation Ratio method is the most used proxy to characterise the presence of a toxic pressure. The method consists of calculating a so-called Risk Characterisation Ratio (RCR)[7], on the basis of the measured concentration of a compound (numerator) and the protective concentration (denominator). The denominator is preferably an Environmental Quality Standard (EQS) but can also be a Predicted No Effect concentration (PNEC) of that compound, in case an EQS is not available. Both are regulatory entities that reflect the exposure level below which effects on any endpoint are considered absent or negligible (sufficiently protected).[8] The RCR is calculated as:

- RCR_i = measured concentration$_i$ / EQS_i or
- RCR_i = measured concentration$_i$ / $PNEC_i$

where i = the compound.

If multiple chemicals have been measured, the method proceeds by deriving an aggregate metric, the sum-RCR (Σ-RCR).[9] Note that such aggregate metrics can also be derived for groups of components (e.g.

[6] For some compounds, natural background values have been set. For the Netherlands, these can be found in jurisdiction or on rvs.rivm.nl. In case no natural background value is available, one can assume that the natural background concentration is zero.
[7] In literature, the ratio is also referred to as a Risk Quotient (RQ), based on similar use of data on the numerator and the denominator.
[8] Note that EQS and PNEC may have different statuses and can represent different protection levels, based on the framework in which they have been derived. EQs are often derived by considering different exposure routes, organisms and endpoints, while PNECs are generally derived less comprehensively.
[9] Note that the calculations are also referred to as Toxic Unit calculations, with TU as the abbreviated result metric.

insecticides, herbicides, PAHs, etc.) or for whole mixtures. If the RCR or Σ-RCR are below 1, this means that the toxic pressure level is lower than the regulatory (protective) threshold level and thus regulatory action is not required. In this case, one could stop the assessment once all the chemicals of concern have been measured and conclude that the situation "*represents a case of sufficient protection, according to established regulatory principles"*. Report the findings and conclusions.

Values of RCR>1 or Σ-RCR >1 yield the opposite conclusion: *"the measured concentrations do not represent a case of sufficient regulatory protection according to established regulatory criteria"*.

In short, the steps are as follows:

1. Collect EQS for the environmental compartment of concern
    - Standards can be found on, among others: https://rvs.rivm.nl/onderwerpen/normen (provided by RIVM, the Netherlands), https://webetox.uba.de/webETOX/index.do (ETOX, provided by UBA, Germany), https://substances.ineris.fr/fr/page/9 (provided by INERIS, France) or in the Water Framework Directive (for water)
2. In case EQS are not available for the environmental compartment of concern, collect PNECs
    - (Non-)formal proposed PNECs can be retrieved from, among others, the NORMAN Ecotoxicology Database, from environmental risk assessments of products/substances (pesticides, biocides, medicines, other chemicals) provided by the institutes regulating the marketing authorisation, or in scientific literature;
3. Collect natural background concentrations for non-synthetic compounds
    - Normalise measured concentrations for the natural background concentration;
4. Compare environmental concentrations to EQS/PNECs to derive RCRs
    - An RCR_i can be derived for each contaminant in each individual sample, and for each type of standard
    - Σ-RCR can be derived by summing the RCRs for all contaminants in each sample, and for each type of standard;
5. Collate and interpret all the results (go to Section 9.1);
6. Continue with Method 3 (Section 6.6) to determine the toxic pressure;
7. After performing Method 2 and Method 3, the user can decide whether the current assessments have provided sufficient information for what they desire or need.

Important considerations and notes Method 2

Considerations

- *How to handle contaminants without a protective standard?*
  The assessor may here select from various options:
  - Do not assess these contaminants. Instead continue with Method 3 (or Method 4), collate all information, and derive an overall conclusion on toxic pressure;
  - Collate ecotoxicity information (See Method 4 for sources) on the compounds for which the standard is lacking, and derive a provisional PNEC according to the Guidance documents (for example, in accordance with the Water Framework Directive) used for deriving the formal regulatory standards (or optionally request derivation of a standard by RIVM, especially if a compound is thought to contribute to local toxic pressure). Proceed with the ad hoc PNEC, and make clear that this was done, and which data was used[1];
  - Collate information on protective standards from other sources (such as scientific literature), and use these as 'adopted PNEC'.
- *Which standards to use → National, European, other?*
  For various compounds, the protective standards have been derived in multiple jurisdictions or frameworks, viz. Dutch, European or OECD. Moreover, as scientific knowledge increases, there may be older and more recent PNECs for a compound. It is advised to use the most recent PNECs from any reliable regulatory framework.
- *How to determine RCR for an area instead of for one point?*
  The sampling strategy for an area commonly results in data on multiple chemicals for multiple sites across an area. The RCR and Σ-RCR outcomes can be plotted on the area map (created in a previous step) as point data, or – if feasible – as gradients with spatially interpolated colour shades for different levels of RCR>1 exceedance. Measurements may also have been performed at different moments. It is not possible to derive one RCR for an area, but a geographical visualisation may help demonstrate the current state of an area.

Notes

- An RCR>1 may have implications according to the regulatory framework that applies to the situation. E.g. for the EU Water Framework Directive, RCR>1 implies that efforts should be undertaken to improve water quality until RCR<1. The precise implications are defined in the pertinent frameworks.
- The assessor is responsible for the quality of the used standards and PNECs, meaning that values should not be outdated and from a trustworthy source. Values should never be used blindly and assumed to be correct. Therefore, report all sources of these values.

Note uncertainties, which hold for chemicals for which the LOD is higher than the standard, and for chemicals that might be present but were not taken into account, and the chemicals for which standards are lacking, and describe whether the uncertainty implies that the calculated outcomes underestimate the toxic pressure.

6.6 Method 3: Toxic pressure (msPAF)

The term 'toxic pressure' was originally introduced in association with the quantification of the Potentially Affected Fraction of species for a given environmental concentration, based on a species sensitivity distribution model (SSD, Figure B 4). The toxic pressure is quantified using standardised methods based on a set of laboratory toxicity test data that are used to derive a compound-specific SSD. The toxic pressure per compound in a sample is then derived as the Y-value (potentially affected fraction of species, PAF) given an ambient concentration (X). The method allows to derive a mixture toxic pressure value, expressed as msPAF (multi-substance PAF) for groups of compounds (e.g. insecticides, PAHs, PCBs, etc) as well as for the total of the mixtures.

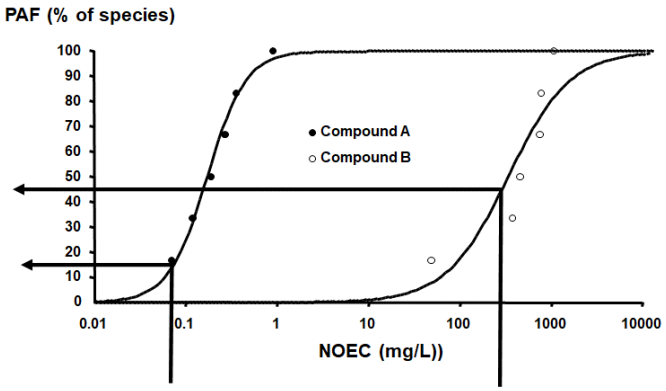


Figure B 4 The Species Sensitivity Distribution model, showing the derivation of the toxic pressure levels caused by ambient exposures to compounds A and B, respectively, which differ in hazard (A far more toxic than B). The mixture toxic pressure is calculated for compounds with different modes of action as msPAF=1-((1-0.15)(1-0.45))=0.53 (53% of the species would be affected above their NOEC due to this ambient mixture).*

Standardised methods to calculate the toxic pressure of individual chemicals and their mixtures are available for the aquatic compartment. Aquatic SSDs have been derived for >12.000 compounds, although the quality of the SSD (and the robustness of the toxic pressure estimates) varies between data-poor and data-rich (tested) chemicals (Posthuma et al., 2019b).

This specific toxic pressure metric (msPAF) has been calibrated to the degree of ecological damage in exposed surface water systems, and it has been shown that an increase in toxic pressure implies an increased probability that the good ecological status cannot be maintained or reached. In other words, mixture toxic pressure (msPAF) relates to ecological impacts, i.e. a decline in biodiversity. This provides an interpretation framework that closely relates to the interpretations of the previous steps. In short, situations for which the PAF-NOEC and msPAF-NOEC<0.05 are considered sufficiently protected, and values >0.05 indicate increasing toxic pressure.

In short, the steps for the aquatic compartment are:

1. Follow the instructions of the Calculation Tool to calculate the msPAF;
2. Collate and interpret all the results (go to Section 9.1). Following the assessment, the user can decide whether the current assessment has provided sufficient information to answer the societal concern or whether more information is desired or needed.

The steps for other compartments, such as soil and sediment, are:

1. Collect ecotoxicity data (NOECs) for the contaminant(s) of interest;
2. Generate SSDs
   - Use, among others, the EPA Species Sensitivity Distribution Generator;
3. Derive PAF-values per compound from measured concentrations (values between 0-1);
4. Derive multi-substance PAF (msPAF) values for measured concentrations of a mixture of multiple chemicals in a sample (values between 0-1)
   - $msPAF = 1 - ((1-PAF_1)(1-PAF_2)\ldots(1-PAF_n))$;
5. Collate and interpret all the results (go to Section 9.1), with PAF and msPAF-NOEC<0.05 as criteria (as in the description of the assessment for aquatic ecosystems). Following the assessment, the user can decide whether the current assessment has provided sufficient information to address the societal concern or whether more information is desired or needed.

Notes

- Above steps (1-4) can be repeated with EC50 as ecotoxicity data, whereby situations with msPAF-NOEC>0.05 are insufficiently protected (presence of toxic pressure) and increasing values of msPAF-NOEC or msPAF-EC50 imply increasing toxic pressure.
- Great effort is being made to improve methods to assess the effects of chemical mixtures on the environment. This means that it is likely that current calculation tools will be improved, new tools will arise, and new and improved ecotoxicity information will be generated. The user of this Guidance should be aware of these developments, and new tools may be used to calculate msPAFs.
- Generating robust SSDs may be difficult as the quality and number of ecotoxicity data may differ per compound and environmental compartment. It is recommended to be aware that SSDs derived from less than 5 or 6 data points are not robust, and can yield biased insights into toxic pressure levels, because the curve can be – by accident – very shallow or steep (see Figure B 4).

Note that the PAF or msPAF<0.05 outcome may be perceived as generally protective for ecosystems, but not may be protective for the vulnerable or protected species of interest. As ecotoxicity tests are often performed with standard test organisms, there are in fact just no data available to characterise the sensitivity of the species of interest to the chemicals of interest.

6.7 Method 4: Virtual Bioassays

Whereas Method 2 provides a conclusion in terms of *sufficient protection of the environment according to regulatory standards*[10] (thus: a legally valid interpretation on toxic pressure levels), the data underlying that method also allows for a more refined assessment.
This could be done by replacing the EQS/PNECs of the previous step by the no-effect level metrics for separate taxa or taxonomical groups. This provides insight into the likelihood that the specific taxonomical groups are affected by the pollution. For example, one could imagine that the RCR values for insects will be higher in case of pollution with insecticides than the RCR values for species groups that are typically unaffected by insecticides (e.g. plants).

In this newly developed method, the RCR approach is replaced by a taxonomic group specific Risk Quotient (RQ) to yield for the aquatic compartment:

- RQ_{algae} = measured concentration$_i$ / $NOEC_{i,\ algae}$
- $RQ_{invertebrates}$ = measured concentration$_i$ /$NOEC_{i,invertebrates}$
- RQ_{fish} = measured concentration$_i$ /$NOEC_{i,\ fish}$.

For the soil environment fewer NOEC values tend to be available, but for specific pollutants it may be possible to derive similar RQs, such as:

- $RQ_{earthworm}$ = measured concentration$_i$ / $NOEC_{i,\ earthworm}$
- $RQ_{springtail}$ = measured concentration$_i$ / $NOEC_{i,\ springtail}$
- RQ_{mite} = measured concentration$_i$ / $NOEC_{i,\ mite}$
- $RQ_{nematode}$ = measured concentration$_i$ / $NOEC_{i,\ nematode}$
- RQ_{plant} = measured concentration$_i$ / $NOEC_{i,\ plant}$

For sediments it is possible to derive RQs, such as:

- $RQ_{chironomid}$ = measured concentration$_i$ / $NOEC_{i,\ chironomid}$
- $RQ_{lumbriculus}$ = measured concentration$_i$ / $NOEC_{i,\ lumbriculus}$

The NOECs are to be taken from tests representing chronic or semi-chronic effects.[11] One can choose to use the lowest value available for each trophic level or, in case a specific species is of interest, use data for that specific species.[12] Ecotoxicity data can be collected from various public sources, such as REACH registration dossiers, market authorisation of products containing the substance, ecotoxicity database (e.g. US-EPA Ecotoxicology database; Pesticide Properties Database; Atlas bestrijdingsmiddelen (Dutch only)) or scientific literature.

The RQs derived in Method 3 are a refinement of Method 1 (with the typical conclusion: *"there are chemicals of man-made origin"*, or not) and Method 2 (*"there is yes/no sufficient regulatory protection"*, or not) as the outcomes allow specifying the possible implications of the toxic pressure:

[10] The analysis in Method 2 (Risk Characterisation Ratio) also allows assessors to determine whether there is sufficient protection of humans, as environmental standards are also intended to be protective for human health. However this is beyond the scope of this Guidance document.
[11] The technical guidance for deriving environmental quality standards by the European Commission provides insights into the interpretation of toxicity tests performed according to established guidelines.
[12] As ecotoxicity studies are often conducted with standard test species, there is limited availability in tests with other species.

- Values RQ<1 imply that it is unlikely that the typical life history traits, such as growth and reproduction of a species or species group, would be affected under chronic exposure. The set of RQ values all with RQ<1 is interpreted as a *"minimal toxic pressure for all three species groups, unlikely to pose a threat (as the NOEC is not exceeded)".*
- Values of RQ>1 suggest that the species group(s) for which this is found *"would be affected at a level beyond their no-effect level if they would be exposed to the sample of interest".*
- The set of RQ data for the different species groups provides a further specified insight when chemicals are found (Method 1), when their concentrations suggest a non-specific interpretation of insufficient regulatory protection (Method 2), by specifying which species group(s) might be affected. Moreover, higher values of RQ imply a higher impact magnitude.
- This step offers an *initial ecological interpretation of the type and magnitude of harm, related to the toxic pressure*. The set of RQ values provides a 'fingerprint' of the toxic pressure in terms of (groups of) species potentially affected.

In short, the steps are:

1. Collect NOEC values for the species groups of interest;
2. Compare environmental concentrations to NOECs to derive RQs
   - An RQ can be derived for each 'contaminant-trophic level' combination (aquatic compartment) or each 'contaminant-species combination' in each individual sample;
3. Collate and interpret all the results (go to Section 9.1). Following the assessment, the user can decide whether the current assessment has provided sufficient information to address the societal concern or whether more information is desired or needed.

Important considerations and notes Method 4

Considerations

1. *Do I use acute or chronic data to calculate RQs?*
   The RQ values can be calculated using NOECs, yielding conclusions on chronic effects at the no-effect level. However, the calculations can equally well be based on acute effects, for example RQ=concentration/EC50 per species group. In that case, the assessment yields an insight on the magnitude of effects of the acute exposure, if the species would be exposed to the sample. An RQ(EC50, daphnids)>1 implies that the daphnids would be affected by more than 50% when exposed to the sample, signaling substantial acute toxic pressure.
2. *As a bridge to 'Effect-Based methods' (described in Section 7), this method can be referred to as a 'virtual bioassay'.*
   This is because the assessor takes *field concentration data* whilst mimicking a bioassay with each species group *on the basis of literature test endpoint data*, to conclude whether that 'virtual bioassay' signals harm. It is a virtual bioassay, as it helps judging the toxicity of field-collected materials with biological test data, be it that those are taken from a database. A true bioassay exposes a sentinel species in a field collected sample. This implies that the selection of a true bioassay can be based on the information collected in this step: a bioassay with insects may be executed if the RQ(insects) in this step is high.

Notes

- Chemical pollution may be high for one or more compounds, showing up as, for example, >10-fold exceedance of the NOEC-level in this step. In such cases, it is warranted to also collect EC50 data, and calculate risk quotients with these. Outcomes RQ-EC50>1 in such assessments imply that exposure to the organism group in the environmental sample would induce substantial effects (>50%).
- Depending on the assessment, it may be that not all trophic levels or species are assessed. In addition, toxicity tests are often performed with standard test species, and the results may not represent the sensitivity of all species present in the environment. Results should therefore be interpreted with caution.
- The assessor is responsible for the quality of the used NOECs, meaning that values should be checked for their reliability.
- If the concerns have been voiced on specific (sensitive) species groups, it may be feasible to collect specific ecotoxicity data for species related to the species group of interest. This provides an opportunity to further specify the 'virtual bioassay-approach', with emphasis on the species group of interest.

7 Phase IV – Effect-Based Methods

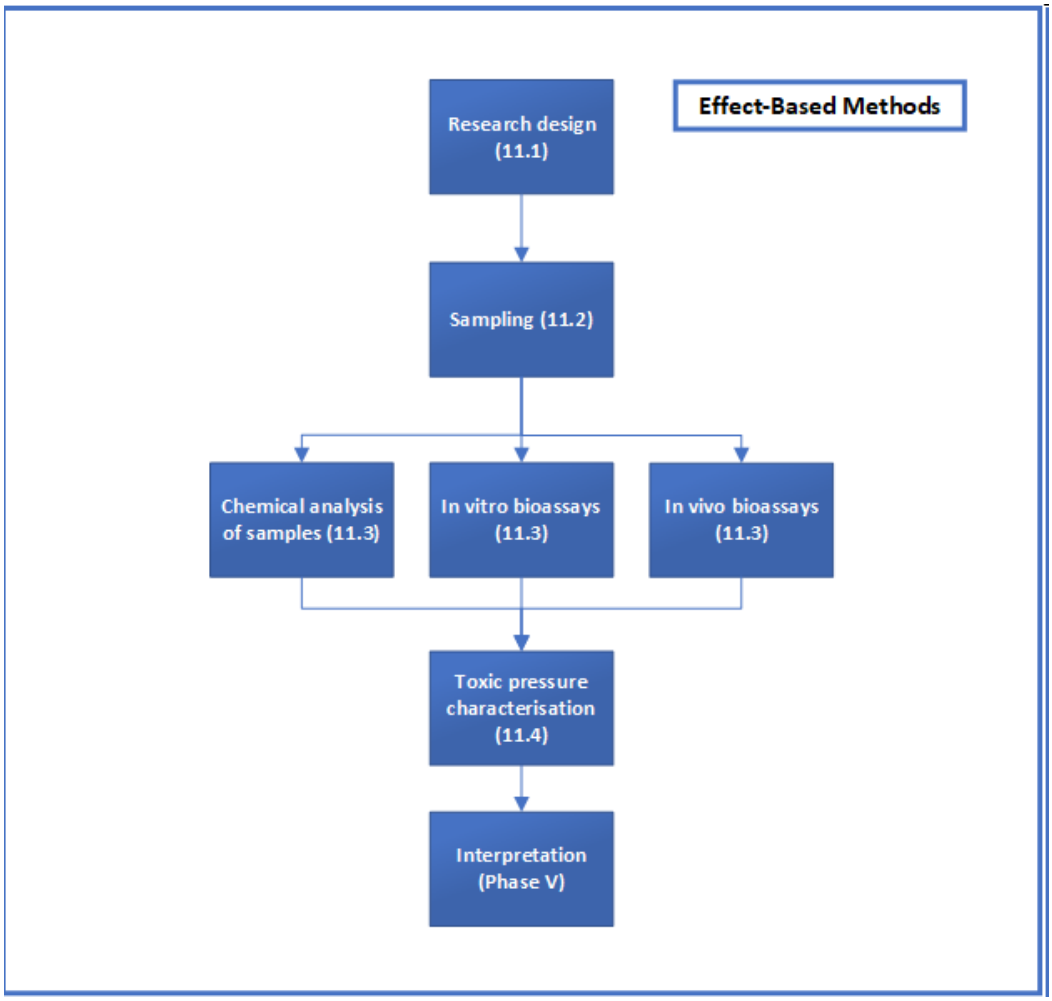


Figure B 5 Steps to determine toxic pressure with Effect-Based Methods. Note that the user may select the (type of) bioassays appropriate to answer to the societal concern. Note that in vivo bioassays may in part be executed at the study site (in situ bioassays).

The Effect-Based Methods described here (Figure B 5) can be optionally applied and may provide further biologically relevant evidence for the occurrence of toxic pressure in vulnerable areas.

Effect-Based Methods are based on two types of information:

- The response of sentinel organisms when exposed to a environmental sample;
- Information that helps distinguish whether that response exceeds the response that is expected under no-pollution conditions.

If responses exceed the response under no-pollution conditions, this is interpreted as possible effect of chemical pollution.

That is, if a bioassay is specific for the mode of action that is shared by

a group of compounds and responds quantitatively to increased exposure levels, and if it shows a reaction in a bioassay executed in field-sampled material, and if the result is not a consequence of confounding factors (other physicochemical characteristics of the sample), then the bioassay result can be used to trace increased toxic pressure of mixtures.

7.1 Research Design

There are various aspects that are important for the research design of the Effect-Based Methods. These are:

- Target and/or key organisms;
- Available bioassays for the compartment;
- Results of the virtual bioassays (if available, see Section 6.7).

After collating all above information, the bioassays can be selected. All aspects above are described further below.

7.1.1 Target and/or key organisms

The organisms that are the endpoint of concern help decide which bioassays may be of interest. For example, a bioassay with fish may be useful when there are concerns over a population decline in fish, or one with insects may be most warranted if the concern relates to the use of insecticides. Here, a list with organisms of concern (protected, vulnerable, presence, sensitive, trophic level) should be developed. The choice may be based on different types of information, such as the compounds of concern, the endpoints of concern or the information from the virtual bioassays step (see Section 7.1.3).

7.1.2 Available bioassays

In this step, an overview of available bioassays is to be made. Bioassays can be grouped on the basis of different criteria, e.g.:

- Chronic and acute tests;
- *In vivo*, *in vitro* and *in situ* tests;
- Tests for soil, sediment and water;
- Mechanism-based bioassays or species-level bioassays.

The bioassays performed most frequently are some *in vitro* tests and species-level bioassays. However, there are also other types of bioassays that can be performed. An overview of known bioassays is presented below. These have been sorted on the basis of environmental compartments. Note that the list of bioassays is not exhaustive.

Surface water

For surface waters, a base set of bioassays has recently been composed with the purpose to cover a relatively wide array of mode of actions.[13] The base set is partially calibrated to ecological status, which means that there is evidence to state that increased bioassay responses grossly co-vary with increased ecological impacts. However, through a lack of standardisation (so far), the evidence has not yet the shape of a robust observed 'signal-to-field effect' magnitude rule for the interpretation of bioassays.

Still, it is valuable to utilise the available methods, as bioassays react to

[13] Available via list of bioassays for the aquatic compartment (website in Dutch only).

whole-mixture exposures, so that the assessment in that sense can completely cover the unknown set of chemicals that are present.
A list of possible bioassays (not exhaustive) for surface waters are:

- Algal growth inhibition test;
- Daphnia immobilisation test;
- Cytotox calux;
- Microtox basic test (in vivo);
- Ames test;
- Fish larvae acute test;
- Rotifer acute test.

Sediment
A list of possible bioassays (not exhaustive) for sediment[14] are:

- Microtox;
- Ragworm bioassays;
- Amphipod bioassays;
- Lugworm bioassays;
- Phytotoxkit;
- Ostracodtoxkit;

Soil
For contaminated soils, Mesman et al. (2011) present a list of possible bioassays (not exhaustive):

- Tests with elutriates such as the Microtox, Rotoxkit, and PAM algae test;
- Plant tests assessing germination of seeds or growth;
- Nematode tests (survival, growth, reproduction);
- Tests using earthworms or enchytraeids (survival, growth, reproduction);
- Tests using springtails;
- Tests using soil-inhabiting mites.

Numerous protocols and guidelines exist for conducting toxicity tests and bioassays with soil organisms. These are available from the Dutch 'Koninklijk Nederlands Normalisatie Instituut' (NEN), the Organisation for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO).

7.1.3 Virtual bioassays

The information from the virtual bioassays (Method 4 from the Component-Based Methods) can help decide which contaminants may cause effects on which organisms. In this step, it can be determined which bioassays may potentially be successful in demonstrating effects, on the basis of the virtual bioassays. It is also important to consider the modes of action of the contaminants when interpreting the results of the virtual bioassays for selecting real bioassays.

7.1.4 Selection of bioassays

On the basis of the information collected in the previous steps, here the bioassays are selected.
Selection can be based on (non-limitative):

- A specific ecological concern (Section 7.1.1);

[14] One of the parties performing these tests in the Netherlands is Wageningen University and Research (https://www.wur.nl/en/show/marine-and-freshwater-bioassays.htm).

- Available bioassays (Section 7.1.2);
- Outcomes of virtual bioassays (Section 7.1.3);
- Practical considerations (e.g. available resources, urgency).

The user of the Guidance is free to pick the bioassays expected to be relevant. Just as for the Component-Based Methods, a stepwise principle can be employed, that is: simple, cheap, fast and sensitive bioassays would be employed as a first step, and more complex, costly, slower and more precise bioassays would be performed if deemed needed on the basis of the initial results. *In vitro* bioassays, selected for being sensitive and different modes of action, can be selected to form a first step approach, whereby those bioassays may have (in part) been calibrated to ecological impacts magnitudes. If some of these respond (and as they are designed to be specifically sensitive), the more extensive methods can be selected on the basis of observed responses. These methods are thereupon more refined, and they could consist of, for example, daphnid in situ tests, which evaluate behavioural and survival effects in water fleas. Those are considered more 'ecologically relevant', which may be a good characterisation, but they may also respond to different pressures such as acidity or temperature. Before using high-throughput assessments, it is recommended to consider – as for all bioassays – whether and how they relate to field effects of pollution. For example, some results may be contributed to other confounding factors (e.g. temperature and acidity in a daphnia bioassay).

In the absence of specific preliminary information, ideally a bioassay battery would be applied, which would enable tracing multiple chemicals and modes of action that are present, but that are not derived from land use considerations. A stepwise approach has, yet not been widely applied in practice, so the tiering of bioassays for the present Guidance will be a tailor-made option in the research plan of an assessment.

Notes

- A bioassay-approach may consist of testing an environmental sample with a cellular response metric, up to *in situ* tests with, for example, caged daphnids. The former are often more sensitive, the latter are considered more 'ecologically relevant'. The former can be very specifically related to a mode of action of a group of chemicals. The latter may respond not only to chemicals but also to confounding factors, such as low temperature or pH. These matters need be considered when establishing an Effect-Based Method strategy. Such a strategy may be tiered, employing cellular assays first and whole-organism (*in vivo*) tests later. Such a strategy may also employ a so-called 'bioassay battery', a series of Effect-Based Methods that is designed such that multiple chemical groups are covered. A proposal for a standardised set of bioassays, as well as a tiered approach, have recently been designed for the aquatic compartment (see Bioassays in the Ecological Key Factor Toxicity).

7.2 Sampling

A bioassay is performed by exposing biota or a sentinel species to a sample from the environment, or an extract thereof.

Again, it is crucial to design a soil, sediment or water sampling scheme that ascertains that (1) enough material is collected for the bioassays that are planned; and (2) the set of bioassays across the area of concern is sufficiently representative to draw conclusions on toxic pressure patterns in the area. In principle, the sampling scheme for Effect-Based Methods should be based on the same considerations as the sampling scheme for Component-Based Methods. In practice, however, technical considerations on costs and run-times may result in different sampling schemes.

Consider the following when sampling environmental media:

- Collect sufficient material at each sampling location;
- Use appropriate methods to collect samples;
- Collect additional samples for chemical analyses.

Because bioassays are often performed with the same water or soil samples as taken for chemical analysis, the reader is further referred to the Sections 6.2 and 6.3 for information on sampling and chemical analyses and the background information provided in Section C.

7.3 Bioassays and chemical analysis

In this step, the bioassays are conducted, according to the approach selected. Preferably, chemical analyses on the samples are also performed. In this way, the effects can be related to the actual concentrations of contaminants present in the sample, whereby the latter can be used to calculate (proxies for) mixture toxic pressure using the Component-Based Methods.

For methods on how to conduct bioassays, the following resources can be considered:

- 'Sleutelfactor Toxiciteit': Working with bioassays following the Key Factor Toxicity;
- Triad (Mesman et al., 2007, 2011);
- Handbooks (e.g. Biosciences handbook);
- Scientific literature.

7.4 Toxic pressure characterisation

To characterise the toxic pressure, the following steps are to be followed:

Bioassays:

1. Calculate toxic pressure for each sample
    - For surface water: use the available interpretation tool provided by the 'Sleutelfactor Toxiciteit'[15]
    - For soil: use the available interpretation tool provided by the Triad (Mesman et al, 2007, 2011). Note, however, that the associated calculation tools may not all be up-to-date, and care must be taken in advance before using any.

[15] Interpretation tool for aquatic bioassays

If chemical analyses have also been conducted:

1. Where possible, use ecotoxicity data and methods available from the Component-Based Methods exercise (e.g. EQS, PNEC, EC50, NOEC values);
2. Calculate toxic pressure for each sample according to Method 4 from the Component-Based Methods.

After the toxic pressure is characterised, collate and interpret all the results (go to Section 9.1). Following the assessment, the user can decide whether the current assessment has provided sufficient information to address the societal concern or whether more information is desired or needed.

8 Phase IV - Ecological Assessment Methods

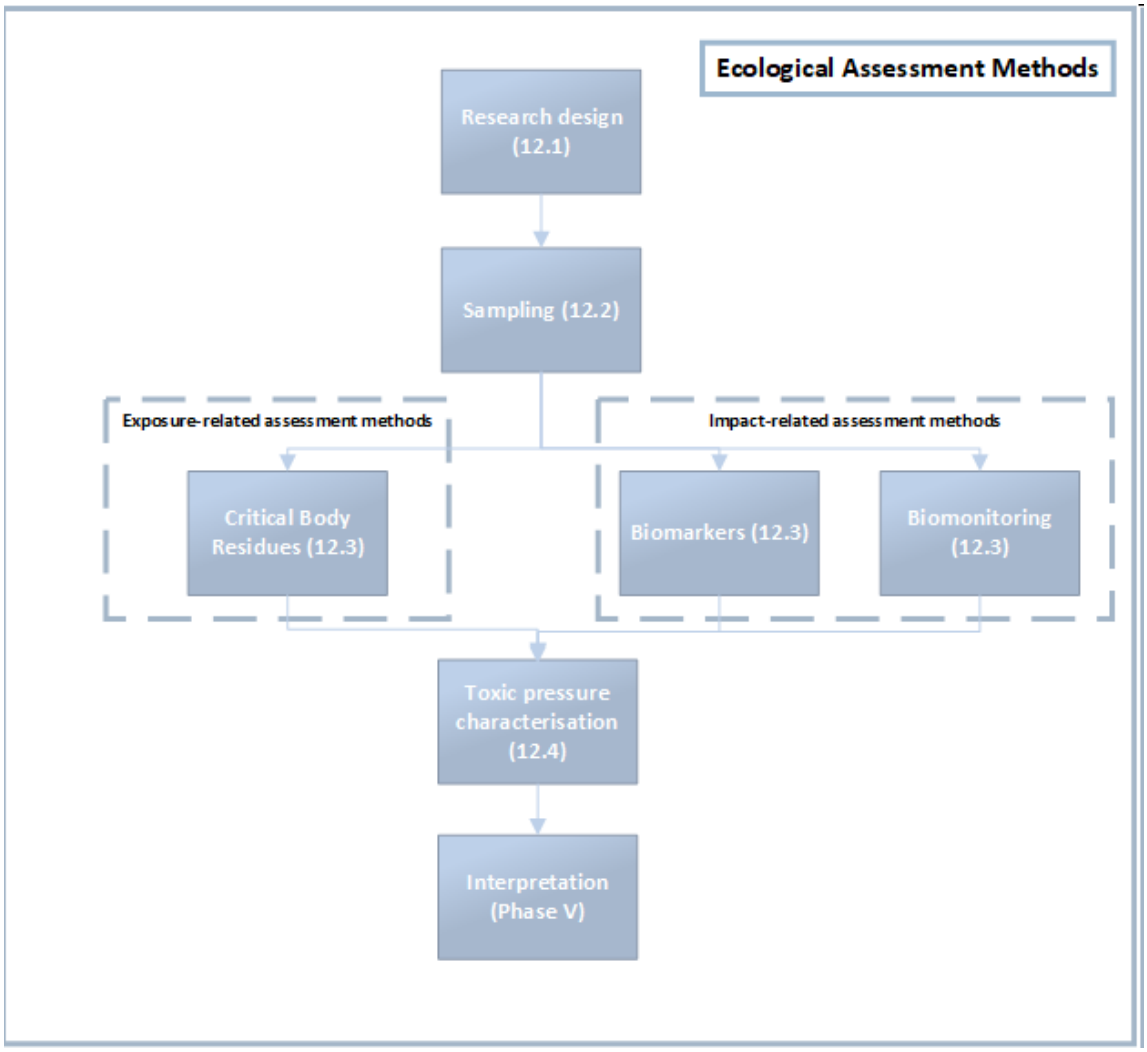


Figure B 6 Steps to determine toxic pressure by using Ecological Assessment Methods. Note that the user may select the (type of) assessment methods appropriate to addressing the societal concern.

Like the Effect-Based Methods (Section 7), the Ecological Assessment Methods are optional (Figure B 6), and they may provide additional, ecologically relevant information to draw conclusions on the occurrence of toxic pressure in vulnerable areas.

There are four ways to evaluate ecological information, viz.:

1. Collect information on body residues of chemicals in wild animals or plants and consider whether those mark the *presence* of increased values compared to reference organisms (like Method 1 from the Component-Based Methods);
2. Body residues in wild animals tested against literature-based critical body residues (like Method 4 of the Component-Based Methods), which marks the presence of *likely effect-initiating* tissue exposure levels, and thus toxic pressure;

3. Biomarkers of exposure or effects, which are physiological markers that exposure or effects occur (with the metric not being a body residue);
4. Biomonitoring → changes in other metrics, such as altered abundance per species, altered species diversity or altered functional parameters.

There are many different methods that can be used to produce an ecological assessment when considering effect-related metrics (methods 3 and 4 specified above). Regarding their focus, one may distinguish between assessments that focus on 1) structural endpoints, and 2) functional endpoints.

Structural endpoints relate to the structure of ecosystems and populations of organisms. Classical examples are inventories of species assemblages and the composition of communities and ecosystems. Functional endpoints measure the result processes that are conducted by organisms and ecosystems. These may include measures such as organic matter breakdown, community respiration and (de)nitrification.

Besides these classical approaches, there are also measures for the health of individual organisms (such as biomarkers and bilateral asymmetry of organisms), studying species' mobility and behaviour, and the assessment of body residues of pollutants in wild organisms. Finally, it is possible at this stage of the assessment to use environmental and ecological modelling approaches, for example, modelling the effect of bioaccumulation of persistent chemicals and their biomagnification in food chains.

Notes

- The operational stage of development of the four types of Ecological Assessment Methods is less far advanced than that for Effect-Based Methods and Component-Based Methods. So far, their utility is not supported by many practical examples of use. Of the available Ecological Assessment Methods (see bullet list above), specificity and interpretability decreases from methods 1→4. Further, exposure-related methods are easier to interpret than effect-related methods. This because the latter are co-determined by other pressures and interpretation is complex due to natural biological variability. Some matters are clearer than others, for example body residues of synthetic chemicals (which theoretically should be zero in pristine unpolluted areas) are easier to interpret than abundance or biodiversity data. For all these reasons, this Guidance only provides some general suggestions for the use of these methods, rather than providing precise methodologies and their interpretations.

8.1 Research design

There are various aspects that are important for the research design of the Ecological Assessment Methods. These are:

- Target and/or key organisms;
- Available ecological assessment methods;
- Results of the bioassays (if available, see Section 7).

After collating all the above information, the assessment methods can be selected. All the above aspects will be further described below.

8.1.1 Target and/or key organisms

The selection of target or key organisms is similar to that of the Effect-Based Methods (see Section 7.1.1) and follows from the considerations made during the problem formulation phase.

8.1.2 Available ecological assessment methods

Exposure-related assessments

Here, the research approach focuses on body residues, regarding presence (first step) and the likelihood of effects (when judged with body residue effect thresholds, as a second step). Body concentrations are measured in field-captured specimens, possibly focusing on specific tissues. Specific compound groups may be known to be stored in fat tissue, for example, so that the research plan can specify whether whole-body residues or tissue residues are measured. The assessor collects critical body residue information from available databases (e.g. the ERED database) or scientific literature, for the step in which measured concentrations are compared to body residue effect thresholds. If the measured concentration exceeds the critical body residue, so that RQ-tissue>1 and/or Σ-RQ-tissue>1, the conclusion is formulated that *"there is evidence that chemicals a, b, c (or: the ambient mixture of a, b and c) is present in organisms' tissues at a level that exceeds the threshold concentration of effects, so that the characterisation shows the presence of toxic pressure".* Like other assessment steps, high exposures could be judged by, for example, tissue residues of EC50-responding specimens, so that the RQ-tissue (EC50)>1 is interpreted as a high likelihood that the toxic pressure results in substantial effects.

Note that data on critical body residue is sparse, and likely to be available for other species than the species of which the tissue concentrations are measured. The interpretation and formulation of the conclusion should reflect the uncertainty that is associated with this.

Impact-related assessments

The employment of ecological methods on impact metrics, such as lowered abundance of species or altered biodiversity, asks for handling the net effect of all pressures that act simultaneously to cause the observed pattern, and distinguishing the role of chemical pollution in that pattern. This involves considerable numbers and types of data on both abiotic (pressure levels) and biotic variables of interest, and often complex multivariate statistical analyses. Furthermore, multiple methods are available for this purpose, each focusing on particular species groups or other specific matters.

Because of the complexity of ecological assessment data regarding the goal to characterise a role of toxic pressure changing an ecosystem, or the abundance of one or more species, and the diversity of the effect metrics that may be used, providing a full overview of all existing possibilities is not feasible. Therefore, in the following sections several examples for surface waters and soil are summarised.

Surface water

The European Union Water Framework Directive (WFD) specifies which elements must be assessed in order to determine the ecological status of surface waters (e.g. rivers and lakes) and describes in a quantitative manner how to conclude that the observed data of the so-called biological quality elements deviates from a reference situation. This reference is a pre-defined water type-specific species assemblage. The approach from the WFD characterises the magnitude of an apparent impact as local deviance from that. If an impact (deviation) is observed that impact may be caused by chemical pollution, any other pressure or pressure combination, or both. The WFD assists in diagnosing the potential role of chemical pollution in an observed effect by evaluating which pressures deviate from those in the undisturbed reference condition. That is, if an assessor checks several pressures and finds those pressures to be present in water type-specific reference conditions, the conclusion may be drawn that those factors are *not* a cause of impacts. If, moreover, the application of Component-Based Methods and/or Effect-Based Methods appears to imply an increased toxic pressure, then the WFD diagnostic approach concludes by stating that there is evidence for effects of chemical pollution.

For the WFD, the quantitative approach to delineate the presence of impacts consider (evaluate the abundance and/or diversity of):

- Phytoplankton;
- Macroalgae;
- Angiosperms (flowering plants);
- Macrophytes;
- Phytobenthos;
- Benthic invertebrates;
- Fish.

Sediment
For sediment, the sediment quality triad can be used as source for available methods (Chapman, 1990).

Soil
Mesman et al. (2011) provide an overview of possible Ecological Assessment Methods to assess the effects of pollutants in soil that can be used in combination with the other methods. The interpretation principles, to judge whether there is an impact and whether that impact may have been caused by toxic pressure, can follow the same reasoning as described for surface waters.

These include inventories in the field or in field samples of:

- Soil micro-organisms (number, biomass, biological diversity, genetic diversity);
- Prior induced community tolerance (PICT) of micro-organisms;
- Vegetation (higher plants), (macro)fungi or lichens;
- Soil nematodes (for example the maturity index by Bongers, 1990);
- Micro-arthropods such as springtails, isopods, oribatid mites, epigeic spiders, etc.;
- Earthworms and enchytraeids (pot worms); and
- Fauna (butterflies, birds, mammals).

In addition, ecosystem functioning can be measured such as:

- Carbon and nitrogen mineralisation (by micro-organisms);
- Soil nitrification (by micro-organisms);
- Soil respiration (by micro-organisms);
- Synthesis by micro-organisms (thymine and/or leucine incorporation); and
- Decomposition (for example the 'litter bag' field method).

Rutgers et al. (2007) provide information on 'references for biological soil quality'. That report may serve as basis to define minimally disturbed reference sites, if those are needed.

8.1.3 Results bioassays and chemical analyses

The results of Component-Based Methods or Effect-Based Methods can be informative for the research design of the Ecological Assessment Methods. For example, if the Component-Based Methods show that insecticides are the most likely group of chemicals to exert toxic pressure, and/or if the Effect-Based Methods show that effects are specifically observed in insects, then this can be interpreted as a signal to focus Ecological Assessment Methods on insect species assemblages (e.g. abundance of specific species or insect biodiversity).

8.1.4 Selection of ecological assessment methods

On the basis of a specific concern, the subsequent research plan, and/or information from other methods conducted, the Ecological Assessment Methods are selected.

8.2 Sampling

While there is a large variety of Ecological Assessment Methods, there is also a wide range of sampling methods. It is beyond the scope of this report to provide an overview of all the possibilities, but the appropriate methods can be found in the standards, protocols for and/or method descriptions of the various Ecological Assessment Methods.

In general, ecological information can be collected either at a site or by collecting samples for analysis in the laboratory. In the latter case, it will only be possible to investigate small organisms that are easily caught and remain in the samples. However, it is of importance to realise that many species are mobile. For these species, organism counts may be more variable. Especially spatial differences and seasonal changes may occur at a location or in different parts of an ecosystem.

Sampling must be performed for endpoints, such as body residue analysis, but is not needed for all endpoints (e.g. when counting mammals). The principles of the sampling design – that the sampling strategy and methods should be adequate – are similar to before (see Sections 6 and 7 for Component-Based Methods and Effect-Based Methods, respectively).
However, the sampling scheme may be (far) denser than for the other methods, if the assessment focuses on a diagnostic assessment in which magnitudes of impacts are studied next to diagnosis of the possible role of chemical mixtures and the other pressures. Such assessments require vast datasets of abiotic (pressure) and biotic (species) field monitoring.

Care should be taken that the number of sampling sites exceeds the number of hypothesised pressures that might alter the species abundance or assemblage composition (i.e. the explanatory variables) several times. A rule of thumb sometimes encountered in this context, is that if *n* pressures might operate in the region of study, then *n! (n-faculty, meaning 1*2*3*4*….*n)* sites should be sampled to obtain a sufficiently dense database for the diagnostic assessment. Furthermore, the sampling scheme should result in a set of data for which it can be shown that covariation between toxic pressure and other pressures is below a critical threshold, because otherwise the statistical interpretation is problematic. That is, in the case of covariance of toxic pressure with other pressures, the assessor cannot distinguish between effects of toxicity and effects of the other pressure(s). This implies that the assessment remains unresolved with respect to evidence for toxic pressure impacts.

8.3 Ecological assessment

In this step, the actual assessment is conducted. The assessments may belong to any of the available methods presented before (body residues, biomarkers and/or biomonitoring).

8.4 Toxic pressure characterisation

To characterise the toxic pressure, the following steps are to be followed:

Body residues:

1. Determine the body residues for each chemical of interest;
2. Compare the concentrations of residues to
   a. Concentrations in reference organisms
   b. Critical body residues (or no-effect thresholds).

Biomarkers:

1. Determine biomarker responses;
2. Determine whether the responses can be linked to toxic pressure
   a. On the basis of toxicity data
   b. By comparing responses to reference organisms.

Biomonitoring:

1. Determine the changed response of interest (e.g. abundance of a species, biodiversity or a functional trait;
2. Determine whether the change(s) can be linked to toxic pressure
   a. On the basis of reference data
   b. By using statistical analyses
   c. By comparing them to reference organisms.

Once the toxic pressure is characterised, collate and interpret all the results (go to Section 9.1).
Following the assessment, the user can decide whether the current assessment has provided sufficient information to address the societal concern or whether more information is desired or needed.

9 Phase V - Analysis and Interpretation

9.1 Collation of results

Here, the results of all methods from Component-Based Methods up to Ecological Assessment Methods are summarised and conclusions can be drawn on the characterisation of the toxic pressure. The summarised results can be entered in Table B 4 to create an overview of all the information, and to decide which further steps can be taken.

Note that, depending on the societal concern and the research questions, information can not only be tabulated (as in Table B 4), but also be presented in various other ways. For example, in case of a spatial concern, the results can be presented graphically, for example by using colour shades.

Table B 4 Characterisation of toxic pressure and recommendations for further steps. Q=Question.

Line of evidence	Method (no.)	Assessment of results	Conclusion	Next step
Component Based Methods	Characterisation of pollution (Method 1)	Q.1) Is the concentration of synthetic compound 'X' equal to/higher than the LOQ? Q.2) Is the concentration of non-synthetic compound 'X' equal to/higher than the natural background concentration?	Yes on Q.1 or Q.2?→ There is evidence for human influence. Note: As yet, there is no information on the presence of a *pressure* caused by the human influence.	Yes on Q.1 or Q.2? → Continue with RCR (Method 2) and msPAF (Method 3) simultaneously. No on Q.1 or Q.2? → Stop the assessment
Component Based Methods	Risk Characterisation Ratio, RCR (Method 2)	Q.3) Does the data show that for compound(s) of interest the RCR≥1 and/or for grouped compounds the RCR≥1 and/or for all compounds Σ-RCR≥1? Q.4) Are there compounds for which no (EQS/PNEC) are available?	Yes on Q.3? → The data shows that the local situation is insufficiently protected according to current regulatory standards. Yes on Q.4? → There is insufficient information from regulatory frameworks to assess the effects of these compounds on the local situation. Judge if the question in the next step can be answered.	Yes on Q.3? → Optionally: continue with virtual bioassays (Method 4). No on Q.3 (and Q5 or Q6)? → Stop the assessment Note: do not forget to perform Method 3, irrespectively of the results of Method 2.
Component Based Methods	Toxic pressure (msPAF) (Method 3)	Q.5) For the aquatic compartment, can the msPAF-NOEC and/or msPAF-EC50 be calculated?	Yes on Q.5? → Use the 'Sleutelfactor Toxiciteit' tool[a] to classify the toxic pressure. If the toxic pressure is	Is there is sufficient protection based on Q5 and/or Q6? → Stop the assessment.

Line of evidence	Method (no.)	Assessment of results	Conclusion	Next step
		Q.6) For the sediment or soil compartment, can the msPAF-NOEC and/or msPAF-EC50 be calculated?	moderate/high/very high, there is insufficient protection. Yes on Q.6? → Use the classification system of the 'Sleutelfactor Toxiciteit' tool[a] to classify the toxic pressure (also for soil). If the toxic pressure is moderate/high/very high, there is insufficient protection.	Is there insufficient protection based on Q.5 and/or Q.6? → optionally continue with virtual bioassays (Method 4), Effect-Based Methods and/or Ecological Assessment Methods
Component Based Methods	Virtual Bioassays (Method 4)	Q.7) Does the data show that for one or more of the compounds of interest and for one or more standard test species $RQ_{chronic,NOEC} \geq 1$? Q.8) Does the data show that for one or more of the compounds of interest and for one or more standard test species $RQ_{acute} \geq 1$? Q.9) Are there compounds for which no or only limited chronic or acute data is available?	Yes on Q.7 or Q.8? → The data suggests that certain species group(s) may be affected after chronic/acute exposure. Yes on Q.9? → There is insufficient information to assess the effects on the local ecosystem (for these compounds). The assessment outcome is uncertain for these compounds.	Stop the Component-Based Assessment. Yes on Q.9? List the compounds that were of concern (problem definition) but for which outcomes are missing (report uncertainties).

Line of evidence	Method (no.)	Assessment of results	Conclusion	Next step
Effect Based Methods	In-vitro Bioassays	Q.10) Have effects been observed in in vitro bioassays?	Yes on Q.10? → The results indicate that organisms might be affected by chemicals present in the sample	Yes on Q.10? → Optionally continue with In vivo bioassays (if not yet performed)
Effect Based Methods	In-vivo Bioassays	Q.11) Have effects been observed in bioassays with water organisms? Q.12) Have effects been observed in bioassays with soil organisms? Q.13) Have effects been observed in bioassays with sediment organisms? Note: the contaminants of concern should always be analysed in the samples (or their extracts) used for the bioassays.	Yes on Q.11? → Use the 'Sleutelfactor Toxiciteit' tool[b] to interpret results Yes on Q.12? → Use the soil TRIAD tool[c] to interpret results Yes on Q.11, Q.12 and/or Q.13? Link effects in bioassays to contaminants based on presence (analyses) or mode of action (e.g. with results Component-Based Methods).	Are there undesirable effects on organisms (e.g. exceedance effect signal values) based on Q.11, Q.12 and/or Q.13? → Optionally continue with In vitro bioassays, Ecological Assessment Methods or Component-Based Methods (if not yet performed).
Ecological Assessment Methods	Exposure-Related Impacts	Q.14) In case of body residues, is there evidence for increased concentrations of (some) chemicals?	Yes on Q.14? → This suggests the presence of human influences (as Method 1 of the Component-Based Methods does).	Yes on Q.14? → Continue with assessing Effect-Related Impacts and answering Q.15. No on Q.14? → Stop assessing body residues.

Line of evidence	Method (no.)	Assessment of results	Conclusion	Next step
Ecological Assessment Methods	Effect-Related Impacts	Q.15) In case of body residues, is there evidence for an increase beyond the critical body residue level? Q.16) In case of biomarkers, biodiversity measurements, functional endpoints or other metrics of effect, is there a change that can be attributed to the presence of chemicals and that is unlikely to be caused by other pressures (compare with results from reference area and/or the Component-Based Methods and Effect-Based Methods)?	Yes on Q.15 → This suggests effects and thus toxic pressure. Yes on Q.16? → This suggests the potential presence of toxic pressure.	Yes on Q.15 and/or Q.16 → Optionally continue with Effect-Based Methods or Component-Based Methods (if not yet performed). No on Q.15 and Q.16 → stop the assessment.

[a] https://www.sleutelfactortoxiciteit.nl/verdieping/werken-met-het-chemiespoor/aan-de-slag-met-de-chemie-rekentool
[b] https://www.sleutelfactortoxiciteit.nl/verdieping/werken-met-het-bioassayspoor/aan-de-slag-met-het-interpreteren-van-bioassayresultaten
[c] https://www.rivm.nl/bibliotheek/rapporten/711701068.pdf

Notes

Here, some general notes are provided that one should bear in mind when interpreting the results of the individual lines of evidence.

Component-Based Methods

- The presence of unintended mixtures nearly always implies that results from multiple methods give better insights into real impacts than separate methods, simply because it is usually the case that multiple chemicals cause aggregated impacts, whilst it is rarely (if ever) the case that the toxicity is determined by a single (the most toxic) compound.
- Component-Based Methods provide a good insight into the calculated mixture toxic pressure, and the relative role of different chemicals contributing to it, but one should bear in mind that these methods nearly always underestimate the true toxic pressure due to compounds that are present but not measured, or not measured sufficiently accurately (if the Limit of Detection is higher than the protective standard).
- Risk Characterisation Ratios (Method 2) may not be easily interpreted. That is, higher Σ-RCR do not necessarily indicate higher toxic pressure on an ecosystem. This is caused by the fact that protective environmental quality standards (EQS) are based on the most sensitive endpoint for each compound. This can be human health, ecosystem harm via direct effects or ecosystem effects via indirect exposure routes. In addition, EQSs (and PNECs) are regulatory concepts that are derived from (eco)toxicity data by applying a compound-specific safety factor (which is applied to ascertain sufficient protection under a degree of uncertainty). This means that compound-specific RCRs can represent different types of endpoints and different levels of uncertainty, and that their sum therefore does not necessarily entail a clear scientific interpretation. Note that Σ-RCR<1 does not necessarily have a clear scientific interpretation either, but that it does have a clear regulatory interpretation: the situation is sufficiently protected against the adverse effects of chemical pollution for the most sensitive endpoint (as it is for the less-sensitive ones).

Effect-Based Methods

- It should be considered that bioassays may respond to other environmental factors.
- As yet, the results of a set of bioassays is not often congruent with the results of a set of chemical assessments. There are no large-scale assessments of bioassay results together with chemical measurements, enabling the calibration of the latter to the former and to ecological impacts in the field.

Ecological Assessment Methods

- Ecological assessments are often complicated to interpret, especially when focusing on the effects of pollutants. Therefore, this Guidance only provides some general suggestions for the use of these methods, rather than providing precise methodologies and their interpretations.

- Often, a lot of data is needed to assess toxic pressure using Ecological Assessment Methods. For example, the collection of multi-year (bio)monitoring data for large areas required intensive data analyses of thousands of observations to enable delineating clear statistical patterns and they need to be checked according to alternative hypotheses to make sure that the observed ecological patterns relate to ecotoxicological changes.

9.2 Interpretation results

In case information is available from multiple lines of evidence, the results must be assessed in combination.

In the interpretation phase, the assessor combines the various types of information and describes the uncertainties that are typical for each method and for the ones that were formulated in the assessment. As a general expectation, the various types of methods (chemical, bioassay, ecological assessment) (so far) rarely yield identical magnitudes and types of information on toxic pressure presence and magnitude. That is simply a consequence of the various strengths and weaknesses of each of the methods, and of the fact that methods are rarely employed to their fullest extent in practice. That is, if a PNEC-based set of RQs and sum-RQs is determined for a series of samples, and if those are also subjected to bioassays, these two lines of evidence solely co-vary in their results if (a) all relevant chemicals are sufficiently well measured chemically, and (b) the bioassays that are used relate to the locally present modes of action in a sufficiently sensitive manner.

Thus, assessors should not (yet) expect uniform outcomes of any combination of the three lines of evidence. Rather, they should focus their interpretation on clear conclusions within each of the lines of evidence separately, followed by deriving across-methods aggregate conclusions.

An option to assist in that is to collate the results of the different methods in a tabular form, such as schematically summarised in Table B5, or in the format of Figures (such as illustrated in the aquatic case study in Section D, for example Figure D 9. The example table must be set up in such a way that it collates all types of information for a specific assessment, that is: for the three different method types (1st column) and – with schematised entries to illustrate the approach – for the associated methods (2nd column), on the details (such as compound being judged, or the bioassay being used) and the associated metrics and values in the next columns, eventually followed by the final evaluation (schematised as grey shades). The schematised table results suggest for the three methods, (1) that at least one measure compound exceeds its protective standards, (2) that the toxic pressure is increased (on the basis of both chronic and acute data), and that effects of exposure to toxic mixtures probably decrease in the order algae>insects>fish. Note that the information from the various lines of evidence is not fully congruent (see also Table B 6).

Table B 5 Schematic overview table, in which the various lines of evidence are combined, to derive conclusions on the characterisation of toxic pressure information for each line of evidence, and each table line separately, but also on the overall pattern from the combined lines of evidence.

Method type	**Method details**				**Toxic pressure evidence**
	Method	**Compound**	**Metric**	**Value**	
Component-based methods	#1	A	>LOQ	0	
		B	>LOQ	0.05	
	#2	A	RCR-EQS-A	0	
		B	RCR-EQS-B	22.8	
		A+B	sumRCR-EQS	22.8	
	#3	A+B	msPAF-NOEC	0.86	
		A+B	msPAF-EC50	0.22	
	#4	A	RCR-Daphnia-A	0	
		A	RCR-Fish-A	0	
		A	RCR-Algae-A	0	
		B	RCR-Daphnia-B	35	
		B	RCR-Fish-B	12	
		B	RCR-Algae-B	81	
		A+B	RCR-Daphnia-A+B	35	
		A+B	RCR-Fish-A+B	12	
		A+B	RCR-Algae-A+B	81	
	Bioassay	Specific			
Effect-based methods	Base	Test A	Response (Algae)	0	
	Base	Test B	Response (Invertebrates)	0.9	
	Refined	Test C	Response (Algae)	0	

Method type	**Method details**				**Toxic pressure evidence**
	Method	**Compound**	**Metric**	**Value**	
	Refined	Test D	Response	0.5	
	Endpoint				
Ecological methods	Exposure	Tissue residue	A (presence/absence)		
		Body residue	B (presence/absence)		
	Effects	Biomarker	C		
		Biomarker	D		
	Abundance	Species	1 (Algae)		
		Species	2 (Invertebrates)		

In the schematic example, the degree of grey shades summarises the presence (not white) and characterisation of the degree (shade) of toxic pressure to the ecosystem.

In this step, the user can also start checking whether research questions can be answered. Here, both the interpretation step and information from steps 9.3 and 9.4, can be used for this cause. In some cases, no sufficient data may be available. In that case more research may be necessary, for example by revising the conceptual model (step 9.5) and performing additional measurements/analyses. Important aspects to consider when answering the research question are for example:

- Is sufficient information available to address the research questions?
- Are the results sufficiently specific for the concerns (e.g. regarding area/species/endpoint)?
- Are results unambiguous or multi-interpretable?
- Have deviations in the research plan influenced the results?

Notes

- A Dutch national sampling programme executed by De Baat et al. (2019a) suggested that data from Component-Based Methods, Effect-Based Methods and Ecological Assessment Methods do relate and that combined data signals differences in toxic pressure of ambient mixtures across land uses and land use intensities. However, the data set is limited, and hints at the fact that the concept is broadly confirmed by the data but that there is no room for a refined conclusion yet.
- When information is available for every line of evidence, a specific interpretation approach is to follow the Dutch soil TRIAD. For the Dutch soil TRIAD Mesman et al. (2011) recommend calculating a 'TRIAD effect value' (TE), as a numerical summary value of the applied methods. As to the value threshold for the TE itself, there is no fixed value above which combined effects from the three different lines of evidence is unacceptable. Instead, it is recommended for the soil TRIAD that relevant stakeholders set this threshold value in a joint meeting before a soil TRIAD study is conducted. Chapman (1996) proposed an evaluation scheme for the results of TRIAD research that was also reproduced by Mesman et al. (2007, 2011) for the Dutch soil TRIAD in order to decide if one should stop or continue the research. This Weight of Evidence scheme, provided in Table 4, can also be used to draw conclusions about toxic pressure in sensitive areas if all three lines of evidence are fully conducted during a study.

Table B 6 Possible conclusions based on multiple lines of evidence in a complete TRIAD study.

Contamination (chemistry)	Toxicity (effects)	Alteration (ecology)	Possible Conclusions
+	+	+	Strong evidence for pollution-induced degradation
-	-	-	Strong evidence against pollution-induced degradation
+	-	-	Contaminant(s) are not bioavailable
-	+	-	Unmeasured contaminant(s) or condition(s) have the potential to cause degradation
-	-	+	Alteration is not due to toxic contamination
+	+	-	Toxic contaminants are bioavailable but in situ effects are not demonstrable.
-	+	+	Unmeasured toxic contaminants are causing degradation.
+	-	+	Contaminants are not bioavailable, alteration is not due to toxic chemicals.

+= clear response, -= no response. Reproduced from Chapman (1996).

9.3 Dominant substance group(s)

The set of results collected in the previous section is most often composed of information from underlying steps, such as: the sum-RQ is always traceable to the underlying Σ-RQ of, for example, the group of insecticides.

This allows one to derive chemicals or chemical group(s) that dominate for a site. That is, the tabular summary of Table B5 can be analysed for the specific toxic pressure of separate compound groups (in the case of Component-Based Methods), so that the table reveals which compound group yields the darkest grey tone, signalling the compound group that apparently exerts the relatively highest toxic pressure.

9.4 Dominant chemical(s)

As for substance groups, it is possible to deduce chemical(s) that play an important role for the toxic pressure in an area. On the basis of the chemicals, the source of the contaminants can potentially be traced. For example, when pesticides cause (a big part) of the toxic pressure, nearby agricultural companies or lands may be the source. It could be that the contaminants are very specific to a certain type of process or use. For example, a specific metal could be emitted by a metal processer that solely works with the metal in the area.

9.5 Update conceptual model

Finally, the conceptual model, with the hypothesised source-pathway-receptor causal chains, is updated according to the gained results. Suggested changes could include, but are not limited to:

- Chemicals that were expected but prove to be absent are erased (and this is motivated). Ditto for pathways;
- Chemicals that are present, and/or the concentrations of which exceed regulatory standards, and/or that are likely to affect specific species groups, and/or that exert a certain toxic pressure – alone or as mixture – can be plotted through use of colours and symbols;
- The breadth of arrows that depicts exposure pathways may be adapted to the results obtained (broader for larger exposures, narrower for smaller exposures).

Affected species groups and/or toxic pressure are indicated.
Next to the clear findings, where assessment data yields a clear conclusion, the uncertainties of the assessment should be listed in the final summary conclusions. This pertains to substances that are hypothesised to be present, but are not measured or not detected due to a LOD that is higher than the exposure level causing toxic pressure, or any other aspect expected but missing in the output.

9.6 Mitigation measures

Selection and implementation of mitigation measures are not part of this Guidance, as those are dependent on the regulatory and local context. For example, the EU-Water Framework Directive implies that measures must be considered to improve water quality if it is found that any RQ-EQS>1 occurs, but measures taken are to be prioritised according to the principles outlined in the WFD.
When the level of toxic pressure is considered undesired, actions can be taken to help avoid or reduce adverse effects from chemical pollutants. Effectivity of mitigation measures depends on the availability and quality of information. Information that helps determine whether mitigation measures can be useful are:

- Which contaminants or substance groups significantly add to the toxic pressure in an area?
- Which (nearby) sources potentially emit these contaminants and how likely is it that the emissions result from these sources?
- Which measures can be taken to reduce or avoid emissions to the area of concern?
- Are measures available to reduce the toxic pressure of contaminants currently present?
- What are the benefits and expenses of available measures, e.g. regarding effectivity, costs, implementation time and general acceptance?
- Which stakeholders can be involved in addressing toxic pressure?

10 Recommendations

The resulting Guidance is a first method to characterise toxic pressure on ecosystems in vulnerable areas. Further development is warranted to improve the functionality of the Guidance. Below, some recommendations for that are listed:

Practical/operational

- Collect experiences, data and interpretations that result from the use of this Guidance in practice, and to collate and evaluate experiences and conclusions. An option could be to start a Community of Practice (CoP) for this.
- Develop a practical tool that guides users through all the phases of the Guidance, and that helps assess aspects such as the availability and interpretation of data. Such a tool enhances the usability of the Guidance.
- Develop (new) practical tools for frequently used methods, so that their use is supported by intuitively and practically easy-to-use and easy-to-interpret tools, especially for the newly developed 'virtual bioassays' approach.
- While using the methods, keep an eye on 'the rules of engagement', which may often relate to cost vs. benefit. It is reasonable to assume that the 'costs' of characterising toxic pressure should be (far) lower than the costs of measures that might be considered to reduce toxic pressure in a specific case.

Content/methods

- The calculation methods described by Mesman et al. (2011) to express results from msPAF estimates, bioassays and/or ecological methods on a scale from 0 to 1, were not found to work properly. It is advised for the assessment of these parameters in vulnerable (and other) areas that the worksheets for these calculations are updated.
- Extend methods to other environmental compartments, such as groundwater, sediment, biota etc.

Innovation/development

- Technological innovations may result into new methods and tools that can be used to characterise toxic pressure, e.g. genetic methods. Considering further development of such methods is recommended. These are often characterised by high-throughput approaches, which allows for the cost-effective analysis of many samples.
- Optionally consider the use of estimates from fate models instead of using measured concentrations. Guidance on the use of fate models could be implemented in an improved extended version of this Guidance.
- The proposed use of 'virtual bioassays' in this report is still new and must be further developed.
- Combine available ecological data for vulnerable areas with available data on chemical pollution, and subsequently employ

the methods delineated in the present report, to obtain insights into the current characterisation of toxic pressure in such areas.
- At present, the methods described in this report are generally applicable, i.e., they are suitable but not specific to the assessment of toxic pressure on ecosystems, or particular species, in vulnerable areas. It could be worthwhile to assess what makes such areas more vulnerable than other areas in terms of both the intrinsic sensitivity of the species and ecosystem and in terms of threatened and protected species that are present. This information can be used to extend the method in this report, notably the evaluation and interpretation of the results.

RIVM recommends testing how well the approach and guideline work in practice and developing them further, for example to determine toxic pressure in groundwater and organisms as well.

Section C – Background information on concepts, definitions and approaches

This section of the report provides a non-exhaustive inventory of the aspects that are relevant for the characterisation of toxic pressure in vulnerable areas. It looks at the concepts that are relevant, their definitions, and at an inventory of scientific approaches that can be used to characterise toxic pressure in vulnerable areas. The various sub-sections address the various parts of the inventory. Section C is meant to serve as background information for those who use the Guidance presented in Section B and who want to know more. The Section subsequently contains information on concepts and definitions, sampling, the array of methods to characterise toxic pressure and information on the eventual interpretation of obtained data and the listing of remaining uncertainties.

11 Concepts and definitions

In the context of the present study, two key concepts and definitions require specific attention, i.e.:

- Toxic pressure;
- Vulnerable areas.

Societal, regulatory, scientific and practical aspects of these concepts and their definitions are enumerated in this section.

11.1 Toxic pressure

11.1.1 General

The toxic pressure of a compound, a compound group or a complete ambient mixture is defined as the pressure on ecosystems (or parts thereof, such as 'a species exposed and impacted via the food chain') that is induced by exposure to toxic chemicals and their mixtures, and that implies that ecological impacts may occur (Klepper and Van de Meent, 1997). Toxic pressure is thus a characteristic of the environment that exerts pressure on biota due to exposure, which can result in direct toxic effects of exposure in exposed species, in indirect toxic effects via secondary poisoning or in indirect ecological effects, such as effects on a prey species that is less predated if a predator is affected by toxic exposure.

An increased toxic pressure has been shown to co-vary with impacts on species assemblages in (especially) aquatic ecosystems. Studies have shown that increased toxic pressure co-varies inversely with biodiversity, i.e. leads to a decrease of the latter (e.g. Posthuma & De Zwart, 2006). The studies that have since been performed have confirmed the association between toxic pressure – especially the metric of multi-substance Potentially Affected Fraction (see below for its definition) – and ecological impact metrics, so that the most recent study could conclude that increased mixture toxic pressure implies increased limitations to maintain or restore a good ecological status (Posthuma et al., 2020).

11.1.2 Toxic pressure: scientific aspects

The term 'toxic pressure' is of relative recent origin and was first used in an RIVM report by Klepper & Van De Meent (1997). These authors calculated the so-called Potentially Affected Fraction of species (Y-values in Figure C 1) from concentration data (X-values), to express the 'pressure' exerted by the observed ambient concentration on a species assemblage. The first mention of that approach – without use of the term 'toxic pressure' – traces back to Van Straalen & Denneman (1989). Those authors proposed a method (see Figure C 1) that has played an important role in developing the global basis for deriving protective environmental quality standards for the environment as well as the characterisation of toxic pressure levels from ambient exposure levels. The method is based on the observation that available ecotoxicity test data (for single compounds tested on multiple individual species and expressed on a log-scale) simply appeared to resemble a normal (bell-

shaped) distribution (on a log-scale). This resulted in the name of the associated sigmoid model: the Species Sensitivity Distribution (SSD, Posthuma et al., 2002).

The initial way of characterising the toxic pressure by a chemical pollutant in the environment thus boils down to the derivation of Y (PAF) from X (ambient concentration) with a compound-specific SSD. This is exactly what is needed for the assessment of the toxic pressure by chemical pollution in the environment, the subject of the present report.

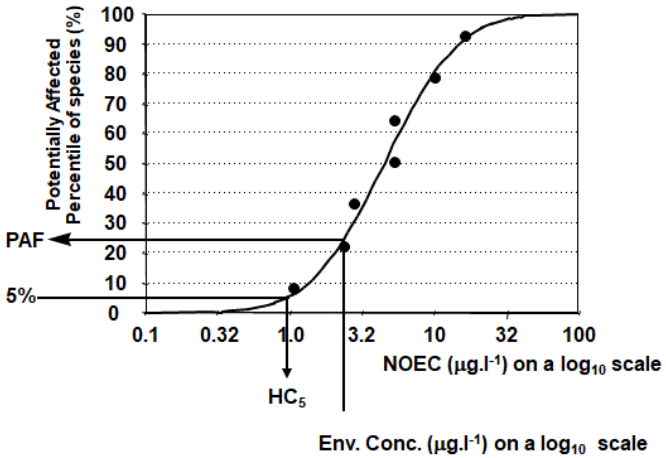


Figure C 1 Dual utility of a Species Sensitivity Distribution (SSD) to derive environmental quality standards from a selected (maximum, protective) Y-value (Y→X), yielding a critical concentration (HC5, Hazardous Concentration for 5% of the species), and to derive a toxic pressure from an ambient concentration (X→Y), with the latter expressed as Potentially Affected Fraction of species. Hereby, the derivation of protective standards is to be based on an SSD-NOEC, whereas toxic pressure assessments can be based on SSD-NOEC as well as SSD-EC50. Figure adapted from Posthuma et al. (2002).

A key aspect of toxic pressure calculation from monitoring data is the characterisation of the pressure on the basis of the bioavailable fractions of the compounds that are present. Those fractions can locally be lower than the total concentration of compounds due to sorption of the compounds, e.g. to clay or organic material. That renders a part of the compounds unavailable for uptake, which lowers the fraction of species potentially affected. Commonly, this is accounted for by using empirical formulae, via which the available concentration is derived from the total concentration and compartment characteristics (e.g. Solomon et al. (2008) for such formulae).

A key expansion of the method consisted of adding realism, given that environmental exposures always consist of unintended mixtures. Therefore, De Zwart and Posthuma (2005) expanded the method of the per-chemical toxic pressure assessment towards mixtures. This results in the characterisation of the per-sample multi-substance Potentially Affected Fraction of species (msPAF), which – given available ecotoxicity data – is commonly expressed as msPAF-NOEC (fraction of species experiencing at least 'nuisance') and msPAF-EC50 (fraction of species in which the effects of exposure results in effects of 50% or more.

A further key expansion of the method considered that there are more than 350,000 compounds produced and utilised (Wang et al., 2020), so that collation and analysis of the global ecotoxicity test data yielded SSDs for (currently) more than 12,000 compounds for the aquatic environment (Posthuma et al., 2019b), with methods pending further development for data-poor or data-lacking chemicals (Hoondert et al., 2019).

In summary, the term 'toxic pressure' originates from a methodology in which concentrations of chemicals in the environment are re-calculated in a metric that represents the fraction of species that is likely affected, in a four-step method:

1. Collate the identities and concentrations of chemicals in the environment;
2. Calculate the fraction of each chemical that contributes to the exposure of species in the environment (bioavailable fraction);
3. Calculate (with the SSD) the toxic pressure per chemicals;
4. Calculate (with mixture modelling) the net toxic pressure exerted by multiple compounds; this can be performed for specified compound groups (e.g. insecticides) or for the total ambient mixture.

The principles of toxic pressure calculation have been adopted widely. As per May 2023, the key paper on the mixture toxic pressure characterisation method (De Zwart & Posthuma, 2005) was cited 397 times (approx. 22 cites/year since 2005), and the one on >12,000 SSDs (Posthuma et al., 2019b) was cited 145 times (approx. 36 cites/year since 2019). Mixture toxic pressure results are reported for countries and areas around the globe.

11.1.3 Additional remarks

Despite the specific historical developments, the contemporary meaning of 'toxic pressure' is not limited to the specific case in which an SSD is used to characterise the potentially affected fraction of species. Given that risk assessment methodologies are often tiered, especially for authorisation purposes (to enable simple, conservative methods in initial stages, see Figure C 2), there is often an array of simplified up to complex and refined methods by which information on toxic pressure can be derived. In such cases, the lower-tier attempts to characterise toxic pressure can be considered to yield 'proxies' of the toxic pressure.

For example, if the environmental concentration of a chemical exceeds its protective regulatory quality standard, that can be interpreted as proxy information that toxic pressure might be present at a level that is considered insufficiently protective in the regulatory domain. The available types of methods for characterisation of toxic pressure are presented in Section 13, classified as Component-Based methods, Effect-Based Methods and Ecological Assessment Methods.

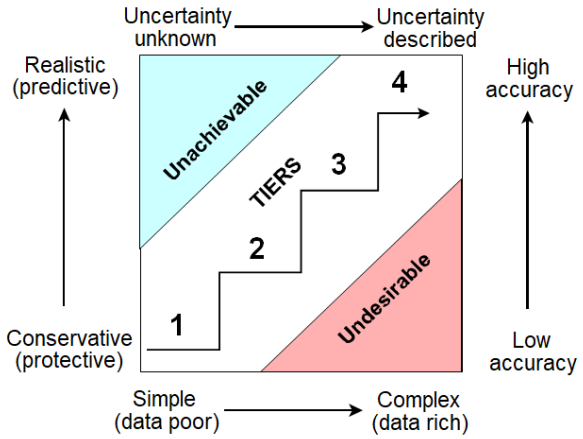


Figure C 2 The principles and characteristics of tiering in risks assessments , which are used in the present report to define proxies of (mixture) toxic pressure. Adapted from Solomon et al. (2008).

Furthermore, when considering the SSD-based toxic pressure characterisation, or any other of the available characterisation methods, it is evident that there are some further specific considerations. First, one of the operational weaknesses of most methods to characterise toxic pressure is that the toxic pressure at a site is nearly always under-estimated when Component-Based Methods are used, if not all chemicals that are present are part of the characterisation, either due to being not monitored, not traceable due to insufficiently sensitive methods or because hazard information (as in the SSDs) is lacking. This occurs frequently, given that monitoring is expensive, and often only focuses on well-known compounds that are frequently measured in constant monitoring schemes. Moreover, toxicity data (and SSDs) are lacking for many compounds.

Second, toxic pressure data needs to be interpreted well, as there are two major types of chemicals that may contribute to toxic pressure:

1. Synthetic chemicals
2. Non-synthetic chemicals

The concentrations and effects of synthetic chemicals are zero in a naturally pristine situation, whilst those of non-synthetic compounds can be naturally enhanced. Example cases are iron-enriched seepage water (e.g. in small rivers in the Dutch Veluwe area), or copper- and zinc-enriched water in the river Geul (that passes a geological surface metal ore deposit). For those cases, the local flora or fauna can be naturally adapted to the conditions, which has even led to metalliferous flora and species such as *Viola lutea* subsp. *calaminaria* (known as the sub-species: zinc violin). For certain compounds, often metals, it may be necessary to correct exposure concentrations for background levels in order to characterise the added anthropogenic contribution to toxic pressure. Methods for that have been developed, for example in Spijker et al. (2011), but a comprehensive overview of all naturally occurring chemicals is not available yet.

Finally, toxic pressure assessment has so far focused mainly on the environment at large, without specific attention for vulnerable or

protected areas. The report of the Dutch RLI has pointed at the potential that toxic pressure might occur due to the presence of unintended mixtures, also within such specific areas (RLI, 2020). However, there is no specific literature or methodology that describes the assessment of toxic pressure for vulnerable areas, or for specifically protected species in such areas. The field of applied ecotoxicology has not developed in that direction, so that methods to specify toxic pressure for these specific situations are lacking, because specific data on sensitivity of, for example, protected species is lacking. That is, the toxic pressure characterisation generated by the methods summarised in the present report provide insights into the pressure exerted by chemicals on a generic basis, which means that impacts on particular species or ecosystems may be larger (if those are more sensitive or vulnerable) or smaller (in case of the opposite).

11.1.4 Operational definition of toxic pressure for the present study

The original definition of toxic pressure relates to a calculated concentration-based value of the Potentially Affected Fraction of species (see Section 11.1.2) and this remains central, also in the Guidance presented here (Section B). However, since the original definition, scholars have developed various ways to quantify toxic pressure, so that the operational definition of toxic pressure for the present study can be expanded. That is, it is proposed to also use the term for studies in which the pressure on species in the environment is quantified by any of the three possible approaches:

1. On the basis of concentrations of chemicals;
2. On the basis of assays with living materials and environmental samples (bioassays);
3. On the basis of ecological observations and attribution of impacts to mixtures of chemicals.

These methods are explained in Section 13.

11.2 Vulnerable and protected areas

11.2.1 General

The work for this report was commissioned to be executed with special emphasis on vulnerable and/or protected areas. However, the inventory of methods to characterise toxic pressure (Section 13) does not specifically encompass methods to characterise toxic pressure in such areas, or for specific species inhabiting those areas. Generically stated, the methods that resulted from the inventory apply to any environmental compartment or area and are not restricted to or specifically designed for such areas. Nonetheless, some aspects of the definition and delineation of vulnerable areas are provided below, considering scientific and regulatory aspects of the term and concept. The term 'vulnerable' is often used interchangeably with terms like 'sensitive' and 'susceptible'.

11.2.2 Scientific context

Scientifically, the term 'vulnerable area' is ambiguous. Wolters & Künzer (2015) cite Füssel & Klein (2006): "*Vulnerability is a term of such broad use as to be almost useless for careful description at the present, except as a rhetorical indicator of areas of greatest concern*." They finally define vulnerability as: "*The degree to which a system, subsystem, or system*

component is likely to experience harm due to exposure to a hazard, either a perturbation or a stress/stressor (after Turner et al. (2003)". There may also be slightly different definitions: "*Vulnerability can be defined as the probability that a feature will be exposed to a stress to which it is sensitive"* (Zacharias & Gregr, 2005). A quick-scan evaluation of the scientific literature did not result in obvious improvements of the definition.

As an alternative, vulnerability has also been defined as the opposite of resilient. That term, in turn, can be characterised by a suite of ambiguous definitions, which reflect opposite characteristics as the above ones for vulnerability.

A brainstorm by the authors of the present report resulted in a suite of other considerations. An area that is considered vulnerable (for example to chemical pollution) may be:

1. An area that combines many functions such as ecological, economic, social, etc.;
2. An area that contains resources, ecosystems, functions, communities and/or species that are relatively highly valued (biodiversity, rare and threatened species) and/or protected (e.g. nature/conservation areas such as Natura 2000);
3. An area that is threatened (by human activities and stressors);
4. An area with high levels of environmental stress such as pollution;
5. An area likely to get easily contaminated (for example the beach in case of an oil spill at sea);
6. An area where effects of contamination are/will be relatively strong, because
    a. the area contains resources, ecosystems, functions, communities and/or species that are relatively vulnerable (to contamination) due to intrinsic sensitivity (high response compared to level of stressor);
    b. of a slow recovery rate after impact;
    c. of slow degradation rates of pollution;
    d. effects occur on key species, which are easily promulgated in the ecosystem;
    e. they are simple ecosystems with only few (key) species and (trophic) relationships (e.g. the Arctic); or
    f. of presence of other stressors (including heat and cold).

11.2.3 Regulatory context

Conservation (protected) areas are commonly defined as designated areas that represent certain ecological and cultural values, and that are managed to preserve these values.

The International Union for the Conservation of Nature (IUCN) defines protected areas as "*clearly defined geographical spaces, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values*".[16]
More specific definitions exist, linked to specific regulatory frameworks of regions around the globe. Well-known examples relevant for specific

[16] https://www.iucn.org/theme/protected-areas/about

environmental compartments in Europe and the Netherlands are designations such as marine protected areas (by OSPAR), 'Natura 2000' (by the European Commission), and 'Natuur Netwerk Nederland' (NNN, by the Dutch government).

The essential characteristic of the areas in the regulatory sense is that the areas can be mapped, and thus be separated from areas without such a status.

11.2.4 Operational definition of vulnerable areas for the present study

Defining whether an area is a vulnerable area can be seen as a step that is made prior to applying the Guidance that the present project aims to provide. The above sections make clear that a variety of regulatory, scientific, societal and practical considerations exist to help delineate a vulnerable area.

The pragmatic approach taken in the present report is that the Guidance should be applicable and relevant for characterising toxic pressure of ambient mixtures in *any* area, as any area can be designated as an area of specific attention or as a vulnerable area. In other words: the Guidance provided in Section B in this document is designed to be applicable to any area, irrespective of the regulatory, scientific, societal or practical definition of the vulnerability status of that area.

11.3 Pollution and toxic pressure in vulnerable areas

Recent research in nature areas has illustrated that the regulatory assignment of an area as a (protected) nature area does not necessarily imply protection against man-made pressures. For example, Hallmann et al. (2017) demonstrated a substantial decline in terrestrial insect biomass in nature areas, without yet being able to delineate the relative role of man-made pressures causing the observed trends. Lemm et al. (2020) demonstrated, for a combined set of all European surface water bodies, that conserving or restoring good ecological status to a substantial extent depends on mixtures of chemicals present. They did not divide water bodies into vulnerable or not-specifically vulnerable categories. Recent research has demonstrated the presence of pesticides in protected areas in the Netherlands, while these compounds are not utilised within the boundaries of these areas (Buijs Agro-Services & Mantingh Environment and Pesticides, 2020).

The presence of these and other foreign chemicals indicates that vulnerable areas may not be free of a man-made chemical pollution pressure.
This is logical as various substances can easily travel by air or water and enter these areas. Research on water quality of Dutch surface waters has shown that the toxic pressure of measured compounds often exceeds the protective environmental quality standards, as reported in Postma et al. (2021), Visser et al. (2023), Natuur & Milieu (2023) and as summarised in Toxic pressure of Dutch surface waters. The results can be summarised as both a map of all studied water bodies, and a map of those water bodies that have a regulatory-assigned protection status (Figure C 3, left and right, respectively).
Evidently, toxic pressure beyond the regulatory protective standards occurs throughout the Netherlands in Dutch surface waters, and – due

to hydrological processes – the regulatory-assigned areas with a specific protection status are in part also characterised by increased toxic pressure.

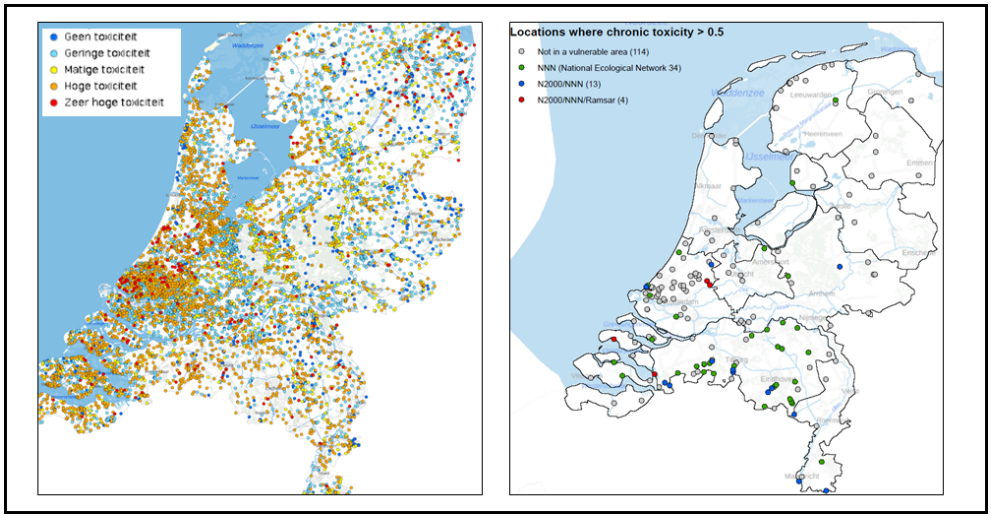


Figure C 3 (Left) Map of the mixture toxic pressure of ambient chemical mixtures for Dutch surface waters (based on monitoring data for 2013-2018, Postma et al., 2021) and (right) an exploratory map of the same data for some selected area types. Note that chemical pollution classification in the left-hand map is based on the classes defined in www.sleutelfactortoxiciteit.nl, and that the right-hand map shows toxic pressures characterised as msPAF-NOEC>0.5 (i.e. ten times higher than the msPAF-NOEC=0.05 criterion), with colours representing different types of regulatory-assigned area classifications.

In summary, there is evidence of man-made impacts of multiple stressors, that can occur in areas of specific attention, where toxic pressure impacts can play a role.

So far, to our knowledge, there is one key study in scientific literature that specifically focused on the assessment of toxic pressure in a vulnerable ecosystem. The study concerns the mixture toxic pressure associated with an array of crops reared along Australian rivers in the vicinity of the Great Barrier Reef (a UNESCO-protected area). Warne et al. (2020) made an extensive study of the crops, plant protection product use and the threats they pose to the Great Barrier Reef, and concluded that the pesticide risks differed across crops and rivers, whereby both could be ranked to identify highest-hazard circumstances to prioritise risk-reduction management.
However, the assessment of the toxic pressure for the Great Barrier Reef was (also) unable to obtain data for reef species, and thus (also) did not specifically highlight that reef species were potentially more or less sensitive to pollution than the species for which ecotoxicity data is available. That is, an assessment of toxic pressure differences across areas can be made on the basis of sensitivity knowledge of 'generic' species (those that have been tested) but not on the basis of sensitivity knowledge of species typical for a designated area.

Furthermore, a European regulation (EC, 2012) aims to protect human health and areas with special protection status from hazardous installations, by keeping sufficient distance between such facilities and

human settlements and the protected areas. This principle was applied in the Netherlands between 2006 and 2010 (https://wetten.overheid.nl/BWBR0020372/2006-10-14). A methodology to derive the 'safe distance' between hazardous installations and protected areas was designed and published, in which the 'safe distance' was derived by evaluating toxic pressure levels that could occur after incidents (Posthuma et al., 2012). That study also indicated that some hazardous installations were close to protected nature areas.

An extensive study of Dutch surface water monitoring data, covering both biological and environmental pressure data, has shown that the aquatic insect fauna is apparently in the process of recovery to a more natural status, which is statistically attributed to a reduced nutrient load and a reduced net toxic pressure of pesticides over time (Hallmann et al., 2021).The study did not discriminate between specific categories of surface waters, and thus did not specify, for example, changes in toxic pressure that would be specific for vulnerable surface water systems. These results provide evidence that management efforts that aim to reduce pressures on ecosystems can result in recovery of biodiversity.

12 Sampling strategies and methods

Now that concepts and definitions have been explained in Section 11, the present report proceeds by describing background information on the various practical steps that are made when characterising toxic pressure in practice. We discern three major steps:

- Sampling (this section);
- Characterisation methods for toxic pressure (Section 13);
- Interpretation of the outcomes of one or multiple methods (Section 14).

12.1 Sampling strategies

All the toxic pressure characterisation methods require sampling of the study area. The sampling evidently precedes the use of the other techniques.

The sampling has various components, namely: the spatial strategy, the identification of what to sample, and optimisation (the potential use of intermediate outcomes to improve the overall efficacy of the method to characterise the toxic pressure). The sampling strategy can be chosen when creating an overview of the hypothesised problem with chemical pollution for an area. As it happens, it matters whether the situation is characterised by a diffuse pollution that may affect the whole area of concern, or whether there is a point source that may have caused a chemical pollution gradient.

Therefore, prior to selecting a sampling strategy, it is suggested that the assessor creates two insight-improving schemes:

- A map of the area, with the hypothesised source-pathway-receptor scheme, which make it *spatially* clear in what ways it is hypothesised that chemical pollution may exert a pressure in an area;
- A conceptual model of the area, with the hypothesised source-pathway-receptor scheme-, which makes it *ecologically* clear in what ways it is hypothesised that chemical pollution may affect certain species (or biodiversity) in an area.

The production of conceptual models and area maps is a standard procedure in ecological risk assessment (e.g. Suter et al., 1993), to make explicit in which ways exposure to chemicals may occur. A conceptual model is helpful to planning the assessment (initially), and to communicating the assessment outcomes (eventually).

12.1.1 Spatial aspects of sampling

There are different types of sampling strategies. Best known are three types of techniques to sample: 1) random, 2) systematic and 3) stratified sampling. They are discussed in more detail below.

Random sampling

In this type, sampling is performed unbiasedly. This means that the probability that a certain sample is taken, is equal to all other potential

samples that could have been taken. Random sampling is considered a fair method of sampling as the results are not influenced by other variables. However, there may also be downfalls. It is possible that random samples may not represent the complete area or population considered. Increasing the number of samples may reduce this uncertainty, but this will also lead to an increased use of resources.

When pollution arises from a point source, random sampling may not be the most representative method as it is likely that most pollutants and subsequent effects will occur in a limited area. For example, when a pipe emits wastewater in a river basin, close to the pipe high concentrations of pollutants are expected to be found and further on, lower concentrations are likely, due to dilution. When sampling locations are chosen randomly, the impact of the point source may not be recorded sufficiently when samples are taken only downstream. In that case, transect sampling (see systematic sampling) may be more appropriate.

For diffuse pollution, there may be several routes to the environment. Via the atmosphere and (ground)water, contaminants may travel long distances. Random sampling may function as a method to show that these contaminants are widespread.

Systematic sampling
In this strategy, samples are taken at a fixed (systematic) interval. This could be useful when a gradient of pollution is expected. In transect sampling, a line is placed along a research area and samples are taken along this line. For three-dimensional purposes, a grid is used. In this way, it is ensured that a gradient is recorded. On the basis of the size of an area and the number of samples to be taken, the sampling locations can be determined. Especially for large areas, systematic sampling is more conventional as the set of rules are fixed while the time spent to sample is lower.

It is also possible to combine systematic with random sampling. In systematic random sampling, the distance between samples is fixed, but other aspects, such as the start position or number of samples over a certain distance, can be chosen randomly. This type of sampling creates a balance between the unbiasedness of random sampling on one hand and the convenience and precision of systematic sampling on the other.

Stratified sampling
In this type of sampling, a study area or population is divided into different subgroups (strata). An example is a nature conservation area with different habitats, such as forest, marshland and/or meadows, that each need to be investigated. The division is generally based on shared characteristics, although the subdivision could also be made for practical reasons. A proportionate number of samples is taken from each stratum. In case differences in variation are expected in the subgroups, the number of samples can be adjusted per subgroup to anticipate on the variation.
The advantage of stratified sampling is that the outcomes provide more precision (consistent results) per stratum than random sampling, and the possibility that samples will be unrepresentative is smaller. It is, however, also possible to combine the strategies. In stratified random

sampling, a subdivision is made prior to the samples being taken randomly from this subdivision. In systematic stratified sampling, samples are taken from each stratum at fixed intervals.

Other sampling strategies
Other strategies include cluster sampling (division of populations or areas in subsets), adaptive sampling (samples chosen on the basis observations during sampling) and targeted sampling (samples based on a specific criterion, such as visual differences). This report will not go into all these, but is important to be aware that more strategies exist that could be suitable for the objective of the study.

12.1.2 Reference areas

One of the key elements is to know whether chemical contamination of a vulnerable area is a natural phenomenon, or if it has increased due to human activity.

Contaminants that are naturally present are known as natural background concentrations. It is well established that various compounds, such as certain metals, can be naturally present in low concentrations in pristine environments. For different environmental compartments and contaminants, natural background concentrations have been established (e.g. within OSPAR, 1992). However, since the industrial revolution, many chemicals have been produced and used, of which a large variety has appeared to be mobile and persistent in the environment. Truly pristine environments may not exist anymore. Contaminants for which no natural background concentrations are available for the type of area investigated, must be compared to a reference area to assess the contribution of additional anthropogenic pollution.

For a reference area, it is of importance that it resembles the area under investigation to a large extent. Differences in both abiotic and biotic factors may influence the contaminant levels found. When areas are comparable, the only difference should ideally be the concentrations of the contaminants under investigation. Preferably, the reference area is close to the contaminated area. This makes it more likely that the area will have similar environmental traits. In addition, it should be ensured that the reference area is not polluted by anthropogenic chemicals. Due to the widespread presence of chemical contamination, it may however be difficult to fulfil these criteria. A reference area should therefore critically be assessed on its suitability before being applied as such.

In nature areas, anthropogenic activity is limited and these areas could therefore serve as suitable reference areas for polluted sites. However, it may be particularly challenging to find a less contaminated reference area when nature areas themselves are the topic of a study.

Data retrieved in a reference area can be used to normalise the results of the area under investigation. If this is done, chemical concentrations measured in the reference area will be subtracted from the chemical concentrations in the study area to assess the contribution of anthropogenic activity. In that case, the toxic pressure is characterised as a result of only this activity. In case the contribution of anthropogenic

activity is not of interest, it is also possible to assess it without a reference area. Toxic pressure will then be based on the complete presence of chemicals, without (opportunities for) correcting for the natural presence.

12.2 What to sample

It is also important to address the question: what to sample. This relates closely to the toxic pressure characterisation method that is employed (as described in Section 13).

Some important considerations are, for the various methods:

- For the Component-Based Methods, it is key to list the chemicals that are hypothesised to potentially contribute to the toxic pressure in an area. These can be derived from the conceptual model, in which the assessor and stakeholders list the chemicals that may be of interest. Further support for the listing of chemicals of potential concern can be derived from a lookup-table, in which information is collated on land uses and associated, typically used and potentially emitted chemicals. For surface water, such a list is provided on the website www.sleutelfactortoxiciteit.nl.[17]
- Some substances may be present only a short time because they are easily degraded or transported. If such substances are suspected to cause toxic pressure in an area, they may be measured using passive sampling, or the (peak) concentrations may be modelled.
- For the Effect-Based Methods, it is key to sample sufficient water, sediment or soil from the compartment(s) of interest to be used for the selected bioassays. Evidently, again, the list of compounds of interest (identified in the previous bullet) is important if the assessor wants to employ specific bioassays, for example when the pollution inventory suggests the presence of insecticides, and the bioassay of preference could therefore be a daphnid in situ assay.
- For the Ecological Assessment Methods, it is key to focus attention to tissue residues of chemicals hypothesised to exert a toxic pressure (again on the basis of the compound list of interest, identified in the first bullet), and to the species groups that are likely to be affected (e.g. insects, because of insecticides being of interest).A good research design for Ecological Assessment Methods may also consider indirect effects of the chemical pollution (e.g. when the pollution affects a sensitive prey species, so that indirectly the predatory species may be affected).

In summary, the question of what to sample concerns the chemicals of interest, the compartment(s) of interest and species groups of interest. The information collected for each of these issues is information that may co-steer the decisions for the other issues. Of key importance is the lookup-table that relates land uses to potentially emitted chemicals, as the number of chemicals in commerce is so high (>350k, Wang et al., 2020) that a motivated selection must be made.

[17] Use the file 'Opzoektabel landgebruik-stoffenlijst'

An assessment plan may start with conducting the Component-Based Methods, followed by verification with methods from the other two lines of evidence. Due to the stepwise nature of practical assessments that result from this, there is ample latitude to consider optimisation of the approaches in what to sample, on the basis of the information gained. For example, if an assessment initially employs component-based methods for four substance groups but finds only one substance group to cause toxic pressure, the optimisation strategy consists of using that information in later assessment steps. Again, insect-related bioassays may be selected as sole type of bioassay in the Effect-Based Methods if the Component-Based Methods only identify insecticides as the cause of toxic pressure.

12.3 Aquatic sampling

Surface water has specific characteristics that need to be considered when determining the appropriate sampling methods for a particular study:

- Water is highly mobile. Surface water may easily move from one area to another, in some cases not naturally but by actively pumping. Via groundwater seepage, substances can enter surface waters.
- Due to drought, water levels decrease, which leads to higher concentration of chemicals. For waters that are exposed to high amounts of wastewater and/or have low fluxes, concentrations can vary to a great extent.
- Suspended particulate matter (SPM) can play an important role in the exposure to contaminants. In waters with low turbulence, these particles may be present at the bottom in the sediment, while, for example during storm and rain events, these particles may be remobilised, and organisms may be exposed to contaminated particulates. SPM will primarily bind organic contaminants with a high adsorption potential (high K_{oc}) but also inorganic contaminants, such as heavy metals.

Active sampling can be performed either manually, by means of a person using a sampling container, or mechanically, for example by a pump. To obtain a sample over a period of time, mechanical sampling is less demanding and it can often be automatised. Compared to passive sampling, active sampling has the advantage that suspended matter can be directly collected, and may contain bound contaminants to which aquatic organisms are exposed.

For passive sampling, on the contrary, no pumps or handling is necessary to collect the sample, only the application of the sampler in the water is needed.
In this technique, contaminants will adhere to the sampling medium by means of diffusion. After a certain period, the sampler can be removed, the contaminants are extracted and the concentrations in the sampling material are determined. On the basis of the concentration and sampling time (and sometimes the flow), information on the concentration can be acquired as each substance has its own diffusion speed (coefficient).

12.4 Terrestrial sampling

To measure the presence of chemicals in terrestrial areas, soil samples can be taken. Soils consist of different layers, with vegetation on top. The number of layers differ between soils, but most soils will contain at least three. These are, from top to bottom, the topsoil, subsoil and the substratum. Some soils may also contain a layer of organic matter on top as a result of decomposition of leaves and other organic matter, while underneath the substratum a layer of bedrock can be found.

When sampling soil, it is important to collect the right layer(s) of soil. This, again, is related to the objective of sampling, but it also depends on the transport towards and fate of chemicals in these soils. Airborne contaminants may end up in the vegetation and topsoil, while these may be transported through the soil by processes as mixing, leaching and groundwater transport. Groundwater can also be sampled in terrestrial areas in case it is expected, on the basis of the chemical/physical properties, that (some of) the contaminants will occur to this medium.

It matters over which depth soil samples are taken. Soil samples for standard investigations of soil pollution, such as described by NEN and/or SIKB, often prescribe fixed depths (see Section 12.5). For soil samples that are also used for bioassays and/or ecological inventories a depth of 20-25 cm is most often applied because soil organisms become much less abundant in deeper layers.

Soil (sub)samples often need to be homogenised. Soils are often sturdy, and more effort is needed to ensure a completely mixed sample than for water. Homogenisation can be done during sampling in the field, but also in the laboratory. Different documents prescribe sample homogenisation (see Section 12.5). Note that homogenisation should be avoided when volatile substances are of interest, as these escape during mixing.

12.5 Sampling standards

For all sampling purposes it is advised to base a sampling strategy on, or adhere to, standards that are already in place. There are different organisations that have published guidance documents on how to take relevant and reliable samples. In some cases, these standards are aimed at sampling certain specific environments or to measure certain contaminants. And in some cases, the use of certain protocols is also laid down in regulatory frameworks.

ISO standards are standards that have been internationally agreed on by experts. The accompanying documents provide guidance on how to perform certain actions.
The ISO 18400 standard for example provides guidance on the sampling of soil. Various standards exist, for example on sampling techniques (part 102), safety (part 103) and recording and reporting (part 107). For water and sediments, the ISO 5667 standards can be issued. Most of the standards are meant to be used in conjunction with each other. Although not mandatory, these documents help sample in a relevant and reliable manner.

Further examples of organisations providing standards are:

- NEN (Stichting Koninklijk Nederlands Normalisatie Instituut, the Netherlands – standards for both soil, water and sediment sampling);
- SIKB (Stichting Infrastructuur Kwaliteitsborging Bodembeheer, the Netherlands – standards for soil sampling);
- US-EPA (U.S. Environmental Protection Agency, United States of America – standards for both soil, water and sediment sampling).

13 Characterisation of toxic pressure

13.1 General

Following the operational definition of toxic pressure, three basic approaches are proposed that can be used to characterise ambient (mixture) toxic pressure levels:

1. Component-Based Methods;
2. Effect-Based Methods;
3. Ecological Assessment Methods.

Any toxic pressure assessment requires field sampling (explained in Section 12) and proper selection of the approaches and methods that are used to characterise the toxic pressure. The three methods can be used separately from each other, in pairs or combined in a so-called 'triad-approach' (Chapman, 1990). In this section, an inventory of all principal methods is provided, including their identification, short description, findings on toxic pressure phenomena in the field, and (dis)advantages. In the Guidance for characterising toxic pressure in a vulnerable area, the set of methods is applied in an operational, stepwise and circular approach (see Figure 1 and Section B).

13.2 Component-Based Methods

Component-Based Methods for the characterisation of toxic pressure are described in, for example, Posthuma et al. (2019c). Apart from the methods to characterise toxic pressure *per se* as PAF or msPAF, there are various proxy metrics, referred to as Method 1, 2 and 3 below.

13.2.1 Method 1 – Characterisation of pollution

Principle

The first method is an evaluation of the *presence* of one or more chemicals in the environmental compartment under consideration. For synthetic chemicals, the concentration without human influence would be zero, and for non-synthetic chemicals the concentration without human influence would be equal to the natural background concentration that is typical for an area. Concentrations higher than zero or higher than the natural background show the presence of chemicals added to the water, sediment or soil by human activity. Those may, or may not, imply the presence of a toxic pressure. Further evaluations (evaluating the concentration against no-effect concentration information) is warranted in such cases.

Approach

The approach is simple and straightforward. Environmental samples are analysed to quantify local concentrations. Local concentrations (found beyond their Limit of Quantification) are interpreted *vis a vis* the expected concentration (zero or natural background).

Example of use

There is no specific example of use of this basic method. Commonly, assessors proceed to more advanced methods such as Method 2 (see Section 13.2.2). This simple method may only be of help to (first)

establish whether, and for which compounds, the concentration data might signal the presence of a man-made concentration increase. If absent, the assessment may stop.

Advantages and disadvantages
Advantage: Simple and straightforward principle
Disadvantage: natural backgrounds are problematic; also, 'presence' does not equal pressure (pressure implies exposure with a level causing impacts; apply Paracelsus' adage ["it is the dose that makes a thing not a poison"]).

Next method
If this assessment step provides information on the presence of chemicals at concentrations higher than zero or the natural background concentration, a further analysis is warranted, to investigate whether the chemicals potentially exert a pressure on the biota.

13.2.2 Method 2 – Risk Quotients or Risk Characterisation Ratios

Principle
In this method, the concentrations that are found are compared to regulatory, protective standards, if those are existent. Note that this method is used throughout the world for environmental quality assessments, for example in policy evaluations.

The most frequently used component-based method is thus to assess whether the concentration of one or more compounds exceeds a protective environmental quality standard, or another standard that is used to signal whether a site would be subject to remediation or other mitigation measures. The website '*risico's van stoffen'*[18] collates contemporary standards for the Netherlands. Additional information can be obtained from other databases, such as the NORMAN Ecotoxicology Database[19], containing standards based on European Guidance documents for deriving such standards.

The principle of this method is always based on deriving a Risk Characterisation Ratio (RCR), defined as the measured concentration divided by the standard. Because the method is based on quotient calculations, the internationally most-often used representation of results is: RQ=concentration/environmental threshold value for a single compound, with RQ<1 indicating sufficient safety for the protection endpoints represented, and RQ>1 the opposite (insufficient protection). In the former case, the toxic pressure of the individual chemical is considered negligible. In both cases, there could be a reason to assess whether impacts of combined chemicals are likely.

According to this quotient-principle, ambient mixtures are characterised by the Hazard Index=ΣRQ that can also be <1 or >1. Note that the ΣRQ calculation could involve data that consists of RQ-human for some compounds, RQ-ecotoxicity(direct) for other compounds and/or RQ ecotoxicity (secondary poisoning) for yet other compounds. It is thus not straightforward to derive a meaningful interpretation of the HI, apart from the (logical) observation that HI<1 again signals a situation of

[18] www.rvs.rivm.nl
[19] https://www.norman-network.com/nds/ecotox/

'sufficient protection' for all three protection endpoints (even if the summed RQs over various compounds would relate to different endpoints).

A key part of the RQ-type calculations is the choice of the denominator: the criterion to judge the exposure concentration. Globally, there are many abbreviations for and ways to calculate a protective standard (which can be used as denominator). For European surface water systems, the standards are called Environmental Quality Standards (EQS). A technical guidance document is available to derive quality standards for water, sediment and biota. This guidance applies to WFD priority substances, priority hazardous substances and river basin specific pollutants (EC, 2018). In the Netherlands, guidance is also available to derive indicative environmental standards for water, sediment, soil, groundwater and air (de Poorter et al., 2015). These indicative standards contain more uncertainty but are less time-consuming than the European derivation method. Indicative standards do not have an official legal or policy status, but values of RQ>1 can still be interpreted as a signal that the presence of chemicals can pose harm. For soil, there are no European EQSs. In the Netherlands, a set of soil standards has been established for soil contamination in order to trigger remediation and/or to assess protection in relation to the use of the soil. Criteria are more stringent for the soil function 'nature' than for the soil function 'industry'. These are covered in the Dutch Soil Quality Decree (VROM/VW, 2017) and the upcoming Environment and Planning Act (in Dutch: 'Omgevingswet') per 1 January 2024. For generic chemical policies, such as under REACH, the protective standard is known as a PNEC (Predicted No Effect Concentration).

In practice, many of these criteria and standards are the lowest value derived from a set of scientific evaluations, i.e. for human health and/or effects on species in ecosystems, both based on either direct or indirect effects. Furthermore, the criteria and standards are often calculated by dividing the scientific no-effect levels by a 'safety factor' to account for uncertainties associated with the (often limitedly) available (eco)toxicity data. This means that 'concentration X < standard for X' implies sufficient safety, whereas 'concentration X > standard for X' implies the opposite, be it with an unclear interpretation (due to the different endpoints that might be affected and the implications of the safety factor).

Approach
The assessor collects data on chemicals present (identity and concentrations), and on environmental quality standards from the database or resources available for that purpose. The assessor then calculates all possible RQs, given the available Standards.
Relevant compounds need be identified by assessing potential sources of the contamination.
For surface water assessments, the project 'Sleutelfactor Toxiciteit' (Key Factor Toxicity) provides a look-up table, which links land uses to probably emitted chemicals, so that a list of potentially relevant compounds can be derived in a pragmatic way from available summary data insights.[20]

[20] See https://www.sleutelfactortoxiciteit.nl/aan-de-slag/de-pressure-van-dpsir

Examples of use
It is common practice to summarise environmental quality data by means of the environmental quality standards. In fact, it is by far the most common approach for regulators. For example, European surface water quality for chemicals is evaluated systematically with this method. Results are mapped in colour maps, so water managers can summarise and communicate assessment results easily for the purpose of decision-making on management priorities and to evaluate effects of measures taken against pollution. An example of an extensive Dutch summary report of monitoring data is provided in factsheets on surface water quality.[21]

Advantages and disadvantages
The advantage of this method is the clear distinction it makes between the label 'sufficiently protected' (RQ<1 and/or HI-mixture<1) and 'insufficiently protected' (RQ or HI-mixture >1), which is a clear regulatory conclusion that is widely accepted as basis for practical decisions and interpretations. When the value >1 occurs, there is a regulatory-valid indication of insufficient protection, warranting either measures or a more refined analysis (depending on the situation).

The disadvantage is the unclear indication of what values>1 imply: are there direct or indirect impacts on ecosystems or indirect effects on human health, or both? And what is the likelihood that these effects occur, i.e. the exact risk? This uncertainty about the meaning of sum-RQ-values higher than one (RQ>1) is caused by two underlying issues: (1) the standards to calculate the RQ for the different compounds can relate to different protection goals, so that the sum-RQ is similar to summing 'apples and oranges', and (2) the standards can be derived with different safety factors, related to differences in quality and amount of scientific data for a compound. The poorer the data, the more conservative the protective standard that is derived. Due to these disadvantages, the exceedance of a protective standard is not directly providing insights into the magnitude of the toxic pressure and the ecological impact magnitude associated with it. For this reason, scholars developed the more refined methods below (Method 3 and Method 4), to specify the meaning of cases where RQ and or HI-mixture >1.

As for all methods, having all compounds that might be relevant for a site in the assessment is key. Non-representative outcomes occur if not all components are accounted for, or if toxicity occurs at a level at which the component's concentration cannot be measured (limit of detection and limit of quantification problem).

Next Method
If this assessment step provides information on the presence of chemicals at concentrations higher than the protective environmental standard, and if this applies to potential effects on ecosystems via direct exposures or secondary poisoning, a further analysis may be warranted to investigate what kind of impacts would be likely. In that method, a 'virtual bioassay' is executed, as refinement of the 'insufficient protection' outcome of the present method. This method can be found in Section 13.2.4.

[21] See https://www.waterkwaliteitsportaal.nl/krw-factsheets

13.2.3 Method 3 - Mixture toxic pressure

Principle

In this approach the evaluation of the toxic pressure is also based on measured or predicted concentration data of chemicals. The principles have been introduced in the Section defining the toxic pressure concept (Section 11.1). This method is described by De Zwart and Posthuma (2005).

Approach

The practical approach, relevant for the Guidance of the present report, consists of collecting concentration data of relevant compounds, collecting the information on the toxicity of the chemicals that is necessary to quantify (mixture) toxic pressures, and deriving the toxic pressure of all compounds by combining the concentration data with the pertinent Species Sensitivity Distributions.

Regarding the outcomes, higher (mixture) toxic pressures imply a higher probability of the combined compound(s) to cause harm to local species exposed to the compound(s). An interpretation method has been developed to assist in summarising and communicating toxic pressure assessment results, based on calibration studies – in which the mixture toxic pressure levels were compared to ecological impacts in the field. The net outcomes of those calibration studies have been summarised in so-called chemical pollution classes, which are shown in Figure C 4. The magnitude of the toxic pressure here relates to a fraction of species exposed beyond a certain level, as summarised in the ‘narrative class boundary’ definitions.

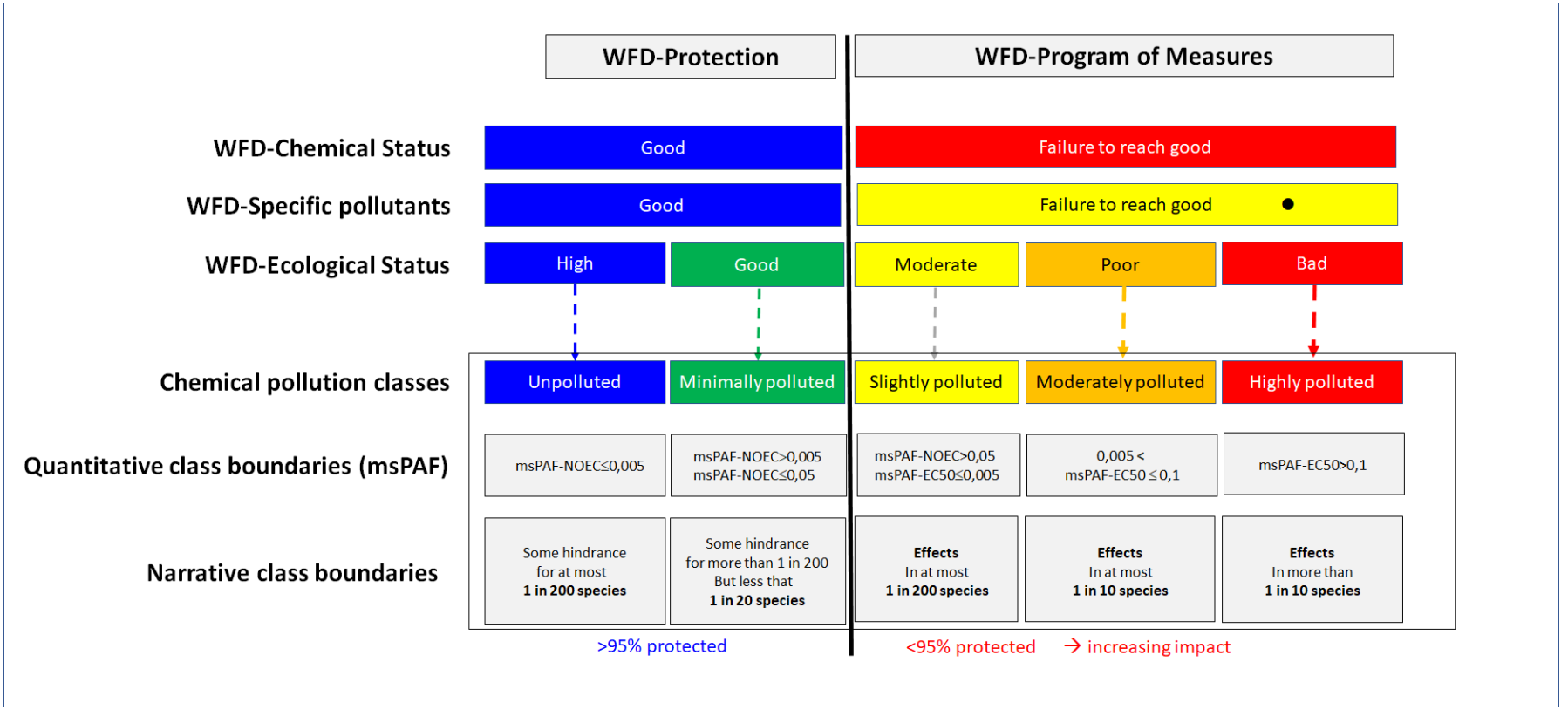


Figure C 4 Scheme that has been proposed to summarise and communicate mixture toxic pressure assessment results (on the basis of results of calibration studies in Dutch surface waters).

Vast datasets on ecotoxicity of substances have been compiled to enable the use of the method. Available ecotoxicity data has been collected, curated and summarised as SSDs for aquatic species by Posthuma et al. (2019b), which allows for calculations of (mixture) toxic pressures for thousands of chemicals. Global data sources also contain soil ecotoxicity data (e.g. https://cfpub.epa.gov/ecotox/), so that the principles and practices can also be applied for terrestrial pollution. For aquatic ecosystems, a website and calculation tool for this approach has been developed (www.sleutelfactortoxiciteit.nl), to support uniform, validated practical assessments with this method. For soil remediation studies such a tool has also been developed for the most common persistent soil pollutants (Mesman et al., 2011; Sanscrit: Risicotoolboxbodem).

Examples of use
The method of toxic pressure assessment is globally used, within highly different contexts. An example of toxic pressure evaluation in relation to a UNESCO-protected area was published for the Great Barrier Reef in relation to pesticide pressures Warne et al. (2020). Extensive European and Dutch studies have recently been published, summarising the spatial and temporal variation in mixture toxic pressure levels across water bodies, and regarding dominant chemical groups (Posthuma et al., 2019b; Postma et al., 2021). These are only two examples of a much wider body of studies.

Advantages and disadvantages
A key advantage of the method is that its outcomes relate to one specific endpoint (ecological impacts), for which it has been established that toxic pressure inversely co-varies with ecological metrics. The best way to formulate the relationship is, that an increased toxic pressure implies an increased limitation of ecosystems (or taxa within ecosystems) to remain in undisturbed, water-body specific reference conditions characterised by the water-body specific biological diversity.

In the interpretation phase, the assessor should be aware of the phenomenon that the method provides a signal of the presence of toxic pressure, but that the biodiversity impact may not be discernible. That is, toxic effects may be masked by other factors and stressors, such as high nutrient loads. In this case, the effects of a toxic exposure on some ecological response metrics are neutralised (not easily demonstrated) due to the effects of excessive food, for example. If excessive nutrients are then removed, the system may not recover, as an effect of toxic pressure persists.

A disadvantage of the method is that it asks for an assumption, namely that the information known from tests with multiple species and summarised as a bell-shaped distribution of species sensitivities indeed represents the distribution of sensitivities across species that occur in the field.

It may even be a critical difference regarding ‘sensitive areas’ – assumingly inhabited by more sensitive and/or vulnerable species – so that this assumption may not hold. Nonetheless, evidence for toxic pressure on a ‘generic’ species assemblage is evidence for toxic

pressure, although the precise impacts on vulnerable species are still not characterised.

13.2.4 Method 4 - Virtual bioassays

Principle

Expanding on Method 2, the assessment proceeds by comparing the exposure concentration data to actual ecotoxicity test data instead of EQS. This data may be taken directly from literature or from the databases compiled for the SSD method (see Section 16.2.3). Again, a risk quotient-approach is applied, but now by calculating (for example) the RQ for the species as:

- RQspecies=concentration/NOECspecies; or
- RQspecies=concentration/EC50species.

Values RQ-NOEC<1 imply that the species would not be affected by the concentrations found, and RQ-NOEC>1 would imply that the species would exhibit some effects, concomitant with the RQ value (higher values imply higher impacts) and the endpoint used in the denominator.

The approaches for regulatory chemical safety assessment (as in REACH) prescribe a minimum dataset of species to be tested. For example, for the aquatic compartment the set of test data comprises algae, daphnids and fish, as a minimum. Therefore, given that ecotoxicity data is likely to be available for these taxa, the virtual bioassay approach can be based on them. A similar approach can be applied for the terrestrial compartment, also focusing on the derivation of RQs for much tested species groups.

In practice, the virtual bioassay approach can be applied to each measured chemical in combination with each measured toxicity endpoint. That is, if the EC50 for an earthworm species is 10 mg/kg soil, and the soil concentration in 20 mg/kg, then RQ-EC50-earthworm=2, and the interpretation would be that substantial impacts are to be expected if the earthworm would be reared in the soil, i.e. there is evidence for substantial toxic pressure (more than 50% of individual earthworms affected).

Upon executing all possible 'virtual bioassays' (by combining measured concentrations with all ecotoxicity data available), the outcomes inform assessors on the taxonomic groups (or species) that are likely to be affected and informs on the degree of impact at the HI values and endpoints of those groups. Given that the assessor uses ecotoxicity test data directly to calculate a RQ, this approach can be coined a 'virtual bioassay', i.e. the assessor is – in fact – answering the question *what would happen if a species with a known sensitivity would be exposed to the water, soil or sediment sample of interest*. A true bioassay (see Section 13.3 on Effect-Based Methods below) consists of truly exposing species to environmental samples taken in the field.

Approach

The approach is like the one used for Standards-based assessments (previous section), with a different step when defining the denominator of the RQ ratio. The assessor now collects data on chemicals present (identity and concentrations), and on ecotoxicity data from a database

for the species that are of interest. The assessor then calculates all possible RQs, given the available data. Results for compound A, B, C… are then summarised, e.g. as RQ-algae, RQ-Invertebrate, up to RQ-fish. In the case of mixtures, RQ values are (again) linearly summed, yielding HI-algae=ΣRQ-algae. Calculations can be performed at the NOEC level (expressing whether species might be affected) or the EC50 level (expressing whether species might be substantially affected).

Outputs are summarised as RQ and/or HI table, whereby values RQ-NOEC >1 suggest the presence of toxic pressure on the species (or taxonomic group) for which this applies, and higher values of RQ-NOEC or RQ-EC50 imply increasing levels of toxic pressure.

Outputs can also be summarised graphically (as illustrated in Section D).

Examples of use
A key example of employing this method is provided by Malaj et al. (2014). These authors collected European surface water contamination data as well as both NOEC and EC50 data for various aquatic taxonomic groups. They were able to summarise the outcomes in the shape of colour maps, which clearly communicate where exposure to certain compounds would lead to impacts on particular taxa. Note that, in contrast to the proposed 'virtual bioassays', the study of Malaj et al. (2014) utilises safety factors that are entered into the analyses to align the method with various regulatory principles (as in Method 2 of the present report). In the proposal on 'virtual bioassays' in the Guidance, however, we propose to derive, using RQs of 'raw data only', whether (standard) test species would be affected at the ambient exposure in an area if exposed in that area.

Advantages and disadvantages
The advantage of this method is that it is based on a simple comparison of concentration data measured in the field with available species-specific test data. Note that data sets of curated aquatic ecotoxicity data – ready for use in this method – are being made available (Swart et al., in prep.). No modelling, and no regulatory assumptions, are employed: if the field concentration exceeds the no-effect concentration, there is a clear signal that the test species would be affected if exposed in that area, i.e. there is toxic pressure. A further advantage is that the outcomes for a set of taxa and chemicals illustrate which taxa are likely to be affected most, and due to which compounds. It is also advantageous that the outcomes of the assessment in this method are not ambiguous, due to potential influences of uncertainty factors or multiple protection endpoints (such as in Method 2). The method can be used for substances with a specific mode of action, such as hormone disruption or bioaccumulation.

A technical advantage is that the outcomes of this method can guide the assessor to the selection of an appropriate (sensitive) bioassay if Effect-Based Methods are applied and to selection of the species group for which monitoring in the field would be warranted with Ecological Assessment Methods.

A disadvantage is, that the results are restricted to the (standard) test species groups for which ecotoxicity data is available, so that extrapolation to the whole species assemblage is not yet done.

13.3 Effect-Based Methods

Effect-Based methods (also known as bioassays) are based on exposing 'living material' to an environmental sample, such that impacts observed in the bioassay(s) can be used to characterise environmental impacts. The first bioassays were performed in the 19th century to assess the activity of medicines in organisms (Van Noordwijk, 1989). Nowadays, bioassays are also used to test the effect of environmental samples, e.g. on cells, tissues, whole organisms or combinations of organisms. The use of bioassays in evaluating surface water pollution is described in Brack et al. (2019).

Principle
There are a few key uses of bioassays. The most practiced use is the classical assessment of environmental quality in the laboratory by exposing a single whole species (*in vivo*) to a single environmental sample transported to the laboratory. But there are also bioassays that are utilised as continuous monitoring tools for water quality assessment and management, such as the mussel monitor (e.g. http://www.mosselmonitor.nl/downloads/brochure.pdf, see also https://www.praktijkcodesdrinkwater.nl/opbrengst/biologische-alarmeringssystemen/). Such continuous biological response-based monitoring is used, for example, to guard the intake of water from the river Meuse for the drinking water production of a few million people[22].

A vast number of bioassays has been developed (EC, 2014; approx. 1300), distinguished in groups such as (cell-line based) *in vitro* assays, *in vivo* assays and *in situ* assays (conducted on site in the environment). A book on the use of bioassays is available for reference (Escher et al., 2021).

The currently available bioassays (Escher et al., 2021) differ in their sensitivity, specificity to compound groups, costs, practices, and co-sensitivity to other pressures. Especially the *in situ* assays (such as a cage test with 10 daphnids) may show that the test individuals are not only responding sensitively to mixture toxic pressure, but also to other non-chemical pressures such as temperature, availability of food and other physical conditions (acidity, salinity, current, etc.).

Approach
The practical approach, relevant for the Guidance of this project's method, consists of collecting environmental samples, and using those to expose the living material, and score their response.

A series of water-related bioassays has been proposed as a subset of bioassays on the website of www.sleutelfactortoxiciteit.nl, for which it was observed that (1) these show divergent responses across Dutch water samples (sensitivity for the study area conditions); (2) represent sensitivity to different chemical groups; and (3) are not correlated

[22] See https://edepot.wur.nl/313404

(which means that two different assays do not respond similarly to the same compound groups).

Soil bioassays with single species such as earthworms and for soil functioning are summarised, among others, by Mesman et al. (2011).

In practice, the execution of a bioassay approach means sampling in the environment, transport of samples to the laboratory, conducting the bioassay, and interpretation of the results. Three principles can be applied for the latter, namely (a) judgement of responses in comparison to an established effect threshold (as with the Component-Based Methods: a standard or criterion), (b) relative to each other or to a control or reference (a sample or set of samples taken from a compartment without the pollutants), and (c) either (a) or (b) but with a calibration to observed field effects (as described in the Section on Ecological Assessment Methods).

Examples of use
Examples of use are covered in Escher et al. (2021) and papers and reports cited there. Recently, the Global Water Resources Coalition executed extensive work on bioassays, and reports of this consortium showed for example that innovative treatment techniques in wastewater treatment plants resulted in lowered bioassay responses (see http://www.globalwaterresearchcoalition.net/reports/, 'water quality' search term). SIMONI is a model in which fifteen bioassays are used to determine the ecological risks of a mixture of chemicals (Van der Oost et al., 2017). By means of passive sampling, extraction of the (un)known chemicals, analytical measurement of these chemicals and the use of these chemicals in bioassays, the environmental risks are characterised and assessed. Bioassay results are compared to threshold values, the results are weighed and combined to indicate the level of environmental risk (low, acceptable, increased, high). Neale et al. (2020) sampled forty-four streams in Germany to assess (indirect) effects of rain events on the presence and effects of chemical pollution. Although only a small fraction of the measured effects could be explained by the chemicals detected, unusually high effects were recorded with the bioassays, which could be related to, in this case, wastewater or overflow of combined sewer systems. An extensive study of Dutch surface waters pollution using bioassays has been executed by De Baat et al. (2019a) and De Baat et al. (2019b).

Advantages and disadvantages
A key advantage of Effect-Based Methods is that the chemical pollution problem, which is immensely complex due to the infinite number of possible ambient mixture compositions, can almost only be comprehensively tackled by using Effect-Based Methods. A well-designed approach contains 'a battery' of bioassays, that represents the full width of modes of action of chemicals of concern for an area, should be employed for that. It is conceivable, that Effect-Based Methods are employed in the format of a battery that consists of sensitive assays and that are used for generic monitoring of environmental quality, that is: monitoring conducted without information on probable emission types. It is thus conceivable, that a specific battery of bioassays is used in conjunction with *a priori* information on modes of action that are likely

to be present. The latter has been proposed in www.sleutelfactortoxiciteit.nl.

A disadvantage of the Effect-Based Methods is that they can only be employed after the pollution event, except for assessments that are used to decide on closing off raw water intake for drinking water production. The latter is a protective effect on the water stored for drinking water production. Another disadvantage is, that various bioassays co-respond to other pressure factors, so that the role of toxic pressure cannot fully be diagnosed with Effect-Based Methods only. Whilst results and geospatial patterns may be informative on a relative, quantitative scale, several also have an Effect-Based Threshold to align Effect-Based Methods with Component-Based Methods principles and practices. However, the derivation of EBTs is complex, and exceedance of the EBT needs not imply clear toxic impacts (like exceedance of EQSs). Finally, the more sensitive, standardised and specific bioassays are *in vitro* bioassays, which employ cell lines, for example. Those are often considered 'of lower ecological relevance'. However, research has demonstrated that those sensitive, specific bioassays do show a systematic covariation with metrics such as ecological status damage (www.sleutelfactortoxiciteit.nl). The disadvantage of unclear ecological relevance can be solved, likely, with calibration studies.

Given the diversity of chemical modes of action, the use of Effect-Based Methods commonly implies the application of bioassays in the form of a test battery. The tests in the battery are selected in such a way that they cover the modes of action of concern, either generically or specifically. It is often argued that such a battery implies high costs, to be comprehensive enough.

13.4 Ecological Assessment Methods

13.4.1 General

Historically, chemical pollution has become a relevant societal concern because of the observation of substantial impacts on wildlife, as summarised in the 1962 book of Rachel Carson (Silent Spring). On hindsight, the observations summarised by Carson represent some clear cases of the presence of toxic pressure at such a level that it caused observable impacts on species, biodiversity and ecosystem services.

Since 1962, scholars developed a wealth of methods that can be part of this section on Ecological Assessment Methods (e.g. Poikane et al., 2020). As the inventory for the present project aimed to serve the practical purposes of being part of a Guidance to characterise toxic pressure (Section B), the overview in this section is limited, and organised in two major sub-sections, viz.: methods based on observations on exposure, and based on observed effects, both in natural systems.

13.4.2 Exposure-related methods

Principle

There are two possible Ecological Assessment Methods, based on exposure analyses, which closely relate to two methods of the Component-Based Methods, that is:

1. Collect information on body residues of chemicals in wild animals or plants and consider whether those mark the *presence* of increased values compared to reference organisms (like Method 1 of the Component-Based Methods);
2. Collect information on body residues of chemicals in wild animals or plants and evaluate those against literature-based critical body residues (like Method 4 of the Component-Based Methods), which marks the presence of *likely effect-initiating* tissue exposure levels, and thus toxic pressure.

As explained in the Section on Component-Based Methods, the assessor establishes with the first method whether man-made chemicals are present, if feasible due to technical limitations such as Limits of Detection, and issues such as the natural background concentration of non-synthetic compounds. If chemicals are found in tissues, the concentrations can be judged against tissue or critical body residue data (e.g. CBR), if available. In the latter case, the RQ-tissue concentration-CBR>1 is a signal of an exposure level inside the organisms' living tissue that is higher than the no-effect level known for that tissue, implying the presence of a toxic pressure in those tissues at a level likely to be causing an effect.

Approach
The practical approach, relevant for the Guidance of this project's method, consists of collecting tissue samples, and quantifying the concentrations of the chemicals that are hypothesised to be a possible cause of toxic pressure.

The assessor evaluates first whether the obtained data provides information on a tissue-exposure level that is caused by human activities, i.e. it is higher than zero for synthetic chemicals, or higher than the tissue concentration found in specimens at the natural background concentration exposure level.

Second, the assessor calculates (again) the Risk Quotient of the observed tissue concentration and the critical tissue concentration of critical body residue level, if available. One of the key sources for such data is a compilation of critical body residues in a systematic database (e.g. McCarthy et al., 2013).

Examples of use
Tissue or body residue assessments serve various purposes in environmental risk assessment around the globe, which is why various scholars and organisations have collated critical body residue data in various databases. There are as yet no publications in which the evaluation of body residues has been used to characterise the presence and magnitude of toxic pressure, in the sense of an evaluation of a likely degree of effects. The main use is the evaluation whether the body residue in field-collected specimens is higher than the critical residue, i.e. the establishment of sufficient or insufficient protection.

Advantages and disadvantages
Comparatively, of the two main approaches (exposure- and effect related assessments), the exposure-related approach yields results that

are more easily interpretable: the study object is clear (one or more concentrations) and the interpretation contexts are clear (presence of contaminants and exceedance of a critical threshold). This is (far) less complex than the effect-related assessments (see below).
Compared to the Component-Based Methods in which environmental concentrations (in the environmental compartments) are measured, the tissue and body residue approaches focus on the ecotoxicologically available (mixtures of) chemicals that have entered the living tissue. This is an advantage, compared to Component-Based Methods for which the available fraction may be unknown. Moreover, the tissue residue represents the temporal and spatial aggregate effects of the net exposure of organisms, and tissue concentrations are often less variable (as aggregate of exposure over space and time) than local concentrations of environmental samples.

However, there are also disadvantages, of which a major one may be the lack of a critical body residue level for many compounds and species. That is, a body residue of any chemical measured in any organism, and within a specific organ or tissue within an organism, may not have its equivalent critical residue level. Moreover, a tissue residue may underestimate the toxic pressure, if the chemical exerts a toxic effect whilst it is biotransformed in the body (and therefore found at relatively low concentration). Tissue residue approaches are therefore less informative for compounds with a high metabolic turnover. As a net conclusion of the advantages and disadvantages, increased body residues (presence of compounds in living tissues) and exceedances of threshold residue concentrations provide relatively clear information on field-relevant exposure levels that may cause harm, especially if the exposure exceeds the critical body residue level. This signals the presence of a toxic pressure on the organism's tissue. If the latter is not exceeded, the interpretation is not necessarily that toxic pressure is absent, for example because of metabolic breakdown.

13.4.3 Impact-related methods

Principle
An impact-related method is based on ecological inventory data for a suite of sampling sites, at which both various pressure metrics – including chemicals and their mixtures – and biotic metrics are measured. Biotic metrics can be of any kind and vary from biomarkers of effects to abundance data for species or integrated biodiversity metrics (such as the Shannon-Winer index) or measurements of ecosystem functioning (e.g. soil respiration). Upon the collection of the monitoring data, the assessor needs to use diagnostic methods to establish which pressures act upon the local species and species assemblages, and – for the present report – whether the toxic pressure of chemicals and their mixtures play a role. The diagnostic methods are often of a statistical nature, and they often involve the use of a concept of un- or minimally disturbed reference sites. The latter are used to define, for example, a 'water type-specific' species assemblage under non- or minimal man-made disturbance, which then acts as 'anchor' to evaluate whether a similar water type is apparently disturbed (diagnosis of the presence of impact) and due to which pressure(s) (attribution of impacts to probable causes).

One of the problems that is encountered here is the historical 'separation' of applied ecology and applied ecotoxicology, which relates to the use of field and laboratory toxicity data in characterising pressure factors, respectively (Schäfer et al., 2023). However, both disciplines have been bridged in diagnostic studies on toxic pressure. Amongst the earliest methods of this kind involving complex mixtures as a chemical pressure is the diagnostic approach named 'effect and probable cause pie diagrams' by De Zwart et al. (2006). More recent and comprehensive studies were published by Lemm et al. (2021) and Posthuma et al. (2019d, 2020). These studies show that mixture toxic pressure is a pressure factor that affects biodiversity in European surface waters.

Approach
(Bio)monitoring data is collected for sampling sites. For the sake of investigating a possible role of exposure to chemicals, the sampling not only needs to characterise the abundance of species or species richness (as response variable), but also a suite of pressure variables (including toxic chemicals), in order to diagnose which pressure(s) cause the impact.

Most often, for investigating toxic pressure, the relevant compounds in the area are selected (as described in the Section on Component-Based Methods). In rare cases, the monitoring can be collated to consist of ecological and Effect-Based Methods data (e.g. De Baat et al., 2019a). Ideally, all likely other relevant pressures are measured (as derived from, for example, an analysis of economic activities and associated pressures), and the species or species groups of interest are identified and monitored. A non-limitative list of (bio)monitoring approaches is presented in Table C 1.

As a rule of thumb, the number of sampling sites should (by far) exceed the number of pressure parameters that could affect the biota. Sometimes, the required number of sampling sites for a potentially successful diagnostic assessment is estimated as *n! (*n faculty, meaning 1*2*3*4*5=120 sampling sites if there are 5 hypothesised pressure factors).

Eventually, upon collecting sufficient monitoring data, the role of toxic chemicals and their mixtures is disentangled from the effects of other pressures by statistical techniques (or by using a combination of chemical, biological and ecological methods). The approach may focus on spatiotemporal patterns in the values of biomarkers of effects, on abundance of species or on aggregated metrics of impacts, such as the Shannon-Wiener index, representing biodiversity. Evidently, the sensitivity of the diagnostic approaches decreases from biomarkers of effects to species abundance data (note that there may be sensitive, neutral and opportunistic species) to aggregate biodiversity metrics. The latter can only reveal a change, if the species abundance data underlying the aggregate metrics has responded – so the latter is always less sensitive than (parts of) the former metric, along the causal chain.

One of the key elements is accounting for natural variation, for example in water types and their associated biota. A small clean river, for example, is populated by other species than a large river or a lake, and north-south gradients and or lowland-highland situations likewise harbour different natural species compositions. Toxic pressure affects those water type-specific natural assemblages in locally different ways, as different species assemblages are affected by toxicity. Hence, the statistical approaches are complex. Another key element is to correctly attribute impacts to the probable cause, here of mixture toxic pressure effects. Statistical methods must be employed to ascertain that the toxic pressure variable is not co-varying, in the available data, with some other pressure, A or B, because in some cases the diagnosis may show a significant impact of toxic pressure, or A, or B, without the assessor being able to diagnose the role of the toxic pressure due to the covariation. In such cases, the calculation of Variance Inflation Factors may show (sufficient) independence of the pressure metrics (O'Brian, 2007). Because of the complexity of the associated statistical methods, we refer to the literature cited above for the statistical approaches that have been proposed and used so far.

Apart from the highly complex (statistical) assessments of effect-oriented methods, there is also an intuitive method. That method has been developed, and is used daily, in water quality assessment and management under the EU-Water Framework Directive. The WFD has designed a water quality classification system, in which all kinds of data on pressures and biotic groups are compared to the water type-specific reference conditions (all are formalised, as standard look-up tables and alike). The raw data of a suite of pressures and biota is summarised in a five-class water quality classification. An intuitive diagnostic approach consists of using the classification system inversely. This inverse use helps the assessor to identify the water bodies where there are ecological impacts (biota deviate from the water type-specific reference) and which pressure factors differ from the non-disturbed status.
This yields a simple list of pressures that may have contributed to the observed biological impact. The method is summarised on the website www.sleutelfactortoxiciteit.nl.

Examples of use
Since the earliest 2006 study that resulted in a landscape-level diagnostic study on impacts of toxic pressure on aquatic ecosystems, an array of studies has been published. Those are summarised in Posthuma et al. (2019b). The *type* of studies can be repeated for vulnerable areas, although the statistical 'rules of thumb' imply that datasets need to be large (many sampling sites) and need to cover all potentially relevant pressures, including all chemicals and their mixtures. All examples of use, focusing on the aquatic compartment, have shown that mixture toxic pressure is a pressure that has impacts on ecosystems in the field. As an example, the EU-wide study on surface waters resulted in the observation that impacts on the ecological status are attributable, on average, for 26% to exposure to chemical mixtures (Lemm et al., 2021).

Advantages and disadvantages
The advantages of the impact-related methods are that the outcomes of such studies are the contemporary reflection of the *Silent Spring* observations of 1962. The contemporary observation could have been that six decades of environmental policies could have solved the chemical pollution issue. However, these types of methods still show evidence for chemically induced effects. Because of the gradients of effects, for example across all Europe's surface waters, these methods may serve as 'anchor' to calibrate all other type of methods (Component-Based Methods or Effect-Based Methods). Such calibrations have been performed, and they are summarised on the website www.sleutelfactortoxiciteit.nl. The calibration studies consistently showed that, and how, impact-related methods can help calibrate results from Component-Based Methods and Effect-Based Methods.
The disadvantages of impact-related methods are that they require large-scale (bio)monitoring data, combined with complex statistical techniques and the designation of reference sites. If it is considered that the Guidance (in Section B) is meant to be operational for an area where there are societal concerns about the potential presence of toxic pressure, the use of impact-related methods is likely beyond reach (due to the disadvantages).

Table C 1 Non-limitative overview of web-links to sets of standardised approaches for biomonitoring, useful as basis for ecological assessment methods.

Source	**Standards (amongst others)**
Standard-subcommission Ecology (NEN)	List of standards NEN
European standard commission CEN/TC 230 'Water analysis'	List of standards CEN/TC230
Internationale standard commission ISO/TC 147 'Water quality'	List of standards ISO/TC 147

14 Interpretation

14.1 Problems and principles

The characterisation of toxic pressure on an area of interest may involve simple outcomes, such as the result of one of the component-based methods, or of a bioassay, but they may also be composite. Composite results may ensue from an assessment that involves multiple lines of evidence (Component-Based Methods, Effect-Based Methods and/or Ecological Assessment Methods), while sometimes multiple methods are used per line of evidence. It is key that the assessor can draw a clear conclusion on the presence and magnitude of the toxic pressure phenomena in an area, as that was the target of the Guidance.

It is suggested to address two issues in the interpretation phase:

1. The interpretation of the outcomes that were obtained;
2. The uncertainties that were recognised when executing any of the steps of the Guidance.

In the next sections, both issues are discussed.

14.2 Outcomes of one or more lines of evidence or methods

Various scholars have developed formal methods to summarise the outcomes of assessments with multiple lines of evidence. In general, those are based on the principle that the outcomes of the various lines of evidence are expressed on a similar scale (e.g. scaled between 0 and 1), upon which the line-scores are combined into a single value. The soil quality triad method employs such an approach (see, for example, Mesman et al., 2011). The outcomes of the various lines of evidence are scaled to a uniform scale, and the deviation that occurs between the lines of evidence is calculated.

In the present report, however, we recognise that the outcomes of the different lines of evidence may vary widely (as also shown in Sections D and E with case study results), and that a single, numerical value is not always the optimal target to summarise information obtained through the various methods. That the results may vary across lines of evidence is a consequence of both the intrinsic limitations of each of the techniques, but also of (still) sub-optimal use of the available methods. Similar characterisation of toxic pressure from component- and effect-based methods are, for example, expected only if one has both measured the concentrations of all (dominant) compounds present and measured responses in bioassays selected for being sensitive to those compounds.

For the present approach, it is therefore proposed to evaluate the information from all methods according to a simple basic concept. That basic concept consists of an initial evaluation of each method separately, with the question *whether there is evidence of the presence of toxic pressure, and if so, what its magnitude is*. This often boils down to the question whether the exposure or a response is higher than a regulatory criterion (or its underlying or related principle), that is:

- If the ambient concentration of a synthetic chemical is higher than zero, there is a human influence, and there may be toxic pressure;
- If the ambient concentration of a non-synthetic chemical is higher than the local natural background, there is a human influence, and there may be toxic pressure;
- If the ambient exposure level exceeds the regulatory protective criterion (accounting for natural background concentrations if needed), there is insufficient protection, and there may be toxic pressure on ecosystems (or on human health);
- If the ambient exposure level in a virtual bioassay exceeds the no-effect level of the tested species, then there is evidence for toxic pressure on that species if it would be exposed in the sampled environment – this is the first clear evidence for toxic pressure at a level that likely affects a local ecosystem (here: a species in that system);
- If the ambient exposure level implies the presence of a toxic pressure (expressed as PAF or msPAF) at a value of PAF or msPAF>0.05, there is evidence for a toxic pressure level that affects a species assemblage;
- If an effect-based method shows a bioassay response beyond an established effect-based threshold, there is evidence for the presence of toxic pressure;
- If an ecological assessment method shows effects (that can be separated from other pressure factors and contributed to toxicants) or the presence of tissue concentrations exceeds the critical body residue concentrations, there is evidence for the presence of toxic pressure.

This summary means that it is feasible to summarise the (sometimes highly different) types of results in a single format (a figure or a table). The aquatic case study (Section D) shows that various graphical and tabulated results summaries can be made to summarise and communicate the results of various lines of evidence.

The collation of results from the various lines of evidence in a table or figure may not always be feasible, as there are some results and metrics for which an 'interpretation anchor' is lacking. The denominator in the RQ approach is such an anchor, as is the effect-based threshold. In such cases, the assessment interpretation must be based on associations, that is, a relationship between the degree of impact and the degree of chemical pollution. Here, the interpretation thus depends on multiple samples, which can be:

- A comparison of a set of samples from a polluted area to a set of samples from reference sites with no pollution and similar characteristics;
- A trend analysis along a gradient of increasing pollution;
- A multiple-pressure statistical investigation of the association between chemical pollution and the abundance of species, or biodiversity, whereby it is ascertained that the chemical pollution level is not co-varying with other pressures.

In these cases, the assessment result can yield conclusions in which the degree of covariation between the pollution and the exposure (body residue levels) or the impacts is described.

Commonly, it is acknowledged that methods that are based on using some anchor point for interpretation provide stronger evidence for the presence and magnitude of toxic pressure than statistical methods. The formulation of the conclusions on the presence and magnitude of toxic pressure should reflect the basis from which the conclusions are drawn and reflect which anchor point was used.

In general, it is also key to summarise (remaining) uncertainties, which is the subject of the next paragraph.

14.3 Reporting uncertainties

Risk assessment is often defined as 'providing scientific support for decision making under uncertainty', and this also holds for characterisation of toxic pressure.

It is advised that the assessors take note of any factor that has been discussed in the making of the assessment plan, including the factors that were not selected, or for which the assessment failed. For example, on the basis of the lookup-table between land use and potentially emitted chemicals, the assessment may have been focused on compounds A, B and C that are typical for the land use. This choice excluded the compounds D, E, F (up till compound 350k, of all compounds in commerce). Therefore, the assessment report should clearly state this fact, why the selected compounds were chosen, and that all other compounds are – in fact – an uncertainty in the Component-Based Methods.

It is not advised to generate an infinite list of uncertainties, because that would imply, for example, that any Component-Based Method would fail by not analysing 350k compounds. That is, in the aquatic case study (Section D), the use of virtual bioassays has resulted in an extensive table of sampling sites, compounds and species for which the no-effect level is exceeded, or not. When such an extensive overview can be made, it is justified to base a conclusion on the presence of toxic pressure, say, on the set of risk quotient values RQ-NOEC>1. If chemicals are lacking in such an overview, it can be reasoned that many other such quotient values *might* exist for chemicals, and that those are unknown (due to lacking data) and thus are not considered – but all that does not invalidate the results of the observed RQ>1-values.

Section D - Aquatic case study

The aquatic case study was undertaken to evaluate the utility of the practical Guidance (Section B) and illustrate its outcomes for data from an aquatic ecosystem. The case study specifically focuses on the use of various Component-Based Methods, to illustrate how those methods can be used to obtain increasingly refined insights, and draw increasingly specific conclusions, on the toxic pressure of chemicals in the aquatic ecosystems of an area.

Because this demonstration case is based on existing data, the choices made are those of the original performers of the work. They have selected the methods, the substances measured and the sampling sites. Because of practical limitations, such as budget and time, the case has also been based on a selection of the available data. For example, not all substances from the dataset were included. When a study is conducted entirely according to the Guidance presented in Section B, various aspects may be different.

15 Introduction

15.1 Content

This report contains the results of a case study that was performed to illustrate the outcomes of the use of the Guidance document (Section B) on characterisation of 'Toxic pressure in vulnerable areas'. The Guidance document is intended to help practically assess whether there is toxic pressure by chemicals in a certain area. The case study illustrates the use and outcomes of the sequential steps in the Guidance for selected compounds, so that it is clear to the user whether the method can be successfully completed. Note that information from the case study assessment activities has been used to improve a draft of the Guidance document, in terms of clarity as well as content. Improvements that could not directly be implemented in the first version of the Guidance were collected and serve as recommendations for future further developments.

The present case study (Section D) focusses on the toxic pressure in the water compartment, for a selected area and problem – here: the Peel. Another case study is available for the soil compartment as well (Section E). The case studies differ with regard to the lines of evidence of the Guidance that are employed. Note that other compartments may also be of interest, e.g. sediment or biota. The case studies are partly based on actual concerns, and partly on fictional ones. The case study has been selected on the basis of scientific, practical and policy criteria.

15.2 Reading guide

The case study report follows the Guidance document, in discerning different phases. In Section 16, the inventory is presented (Phase I of the Guidance). In Section 17, the problem is defined (Phase II of the Guidance). In Section 18, the research strategy, data collection and calculations are given (Phases III and IV of the Guidance). In the last Section (Section 19), the results are analysed and interpreted (Phase V of the Guidance).

16 Inventory

Making an inventory of the situation in the area of interest, of the voiced concerns about a potential presence of toxic pressure, and of optionally available data, is the first phase of the systematic approach to determine toxic pressure. It contains of five steps that need to be followed. These are presented below. Note: the societal concern and other information presented may not represent the actual and complete concern and situation but have been chosen to limit the scope of the case study.

16.1 Societal Concern

The Peel is an area bordering on the provinces of Noord-Brabant and Limburg in the Netherlands. The area is known for its intensive livestock production and agriculture. In the Netherlands, there is pressure on the landscape and on human health due to high levels of nitrogen deposition. The nitrogen deposition threatens biodiversity and may have impact on all types of organisms, such as micro-organisms, plants and aquatic life. One of the known causes of local nitrogen deposition is livestock production. There are ideas to tackle this societal challenge, for example by switching from intensive to extensive livestock production or by changing land use. The Peel has been designated as a NOVEX area, one of sixteen areas that receive priority for altered future landscaping. As the use of land may change, it may also affect the use of chemicals in different sectors and potential emissions of chemicals to the environment.

It is currently unknown what the current toxic pressure is on the environment in the Peel, and especially on the water quality. More broadly, as a context, the Netherlands is known for having the 'worst water quality score' of all European member states, with only low number of waters complying to the criteria for a 'good' status by the Water Framework Directive (PBL, 2020).

16.2 Incentive to determining toxic pressure

It is known that chemical substances are used in the production of livestock and crops. For livestock, these are veterinary medicines (VM). These medicines are partially emitted to the environment, where they may cause a toxic pressure. For several substances, it is known that their concentrations in the environment lead to risks for aquatic organisms (Lahr et al., 2019). For soil and sediment, no risks were found in Lahr et al. (2019). However, it is specified that monitoring data of many VM in the environment is limited. In addition, for some substances, the analytical detection limit exceeds the protective risk limits (PNECs - Predicted No-Effect Concentrations). This means that risks cannot always be evaluated on the basis of regulatory, protective standards, but that toxic pressure may exist, nonetheless.

Plant protection products (PPP) are used on crops. The use of PPP may lead to direct emissions to soil. PPP can also partly end up in surface water as a result of spray drift emissions as well as through surface

runoff, drainage and atmospheric deposition (Kruijne et al., 2020).

The number of exceedances of water quality standards of PPP has been decreasing over the years, but the number of locations in which one or more substances exceed standards is quite stable, while many substances cannot be assessed due to standards that are lower than what is measurable with current analytical tools (Tiktak, 2019; CLO, 2021).

For the Peel, it is known that livestock is held, and crops are cultured (see Section 17 for further information). This implies that VM and PPP are being used. However, it is currently unknown whether these substances are present and whether they result in toxic pressure, which may show up as a decline in nature/biodiversity, or what effects on organisms are to be expected.

By assessing toxic pressure by chemicals in the aquatic environment in the Peel, the current chemical quality of the water systems can be assessed and over the years (given the land use changes) it can be assessed whether chemical pollution decreases and whether the toxic pressure is lowered.

16.3 Collation of existing information

The Guidance suggests starting an assessment by collecting any available information. This was feasible in the present situation. In the 'Brede Screening Maasstroomgebied', various substances in the water bodies of the river basin of the Meuse are measured.[23] Measurements are available for surface water in different areas. It appears that measurements have also been performed in the Peel. For some locations this includes VM and PPP. This data was collected.

Verhagen et al. (2018) relate that in 2016, 10% of measured PPPs and 21% of emerging substances (which include VM such as lidocaine and trimethoprim) exceeded the detection limit of the laboratory. This implies that these compounds are found in the river basin of the Meuse, however presence in the Peel specifically was not assessed. The 2016 data could potentially be used to assess chemical pressure. However, analytical data from 2022 has also become available. As the 2022 data is more recent, it was used to assess toxic pressure in the current study.

No bioassay data for the water compartment in the Peel or ecological data was available yet. On the basis of a case study decision, it was deemed beyond the scope of this case study to collect such data for the purposes of illustrating the Guidance. That is, the aquatic case study focuses on Component-Based Methods, whilst the other case study (Section E) also addresses the other lines of evidence (Effect-Based Methods and Ecological Assessment Methods).

16.4 Research question(s)

On the basis of the above information, there are various research questions that are of interest. We have attempted to answer the following research questions:

[23] See 'Atlas voor een Schone Maas' for more information: https://storymaps.arcgis.com/stories/468a84a07bfa49d694f9229f226b6399

- To what extent were veterinary medicines and plant protection products present in surface water in the Peel area in 2022, and to what extent do they exert a toxic pressure?
- Were there other contaminants present in surface water in the Peel in 2022, and to what extent do these exert toxic pressure?
- Can the toxic pressure be explained by nearby or distant sources and transport of contaminants?

16.5 Go/No Go decision

It is unclear whether there is toxic pressure in the Peel as a result of the presence of VM and PPP in surface waters, caused by chemicals acting individually, or as unintended mixtures. As there is evidence, both from land use data and associated concerns as well as from the available monitoring data that chemicals may be present in the aquatic environment of the Peel, it was decided to continue with the exercise.

17 Problem definition

17.1 Area characteristics

The Guidance suggests proceeding with an assessment by describing the problem to yield a concrete problem definition as a basis for a research plan.

The Peel is an area of which the borders are difficult to define. In the current exercise, we consider the area designated as NOVEX the Peel as the area of interest. In Figure D 1, a spatial outline of the Peel can be found. The Peel is in the South-east of the Netherlands and covers parts of two provinces (Noord-Brabant and Limburg) and 23 municipalities. Part of the area is designated as Natura 2000 area, protected natural areas within a European network. None of the big rivers cross the Peel, however waters such as Zuid-Willemsvaart and Peelkanaal dissect the area from south to north. The area is, thus, of specific interest as a case study for the present Guidance, as the area is characterised (in part) by a sub-region of specific concern regarding nature quality.

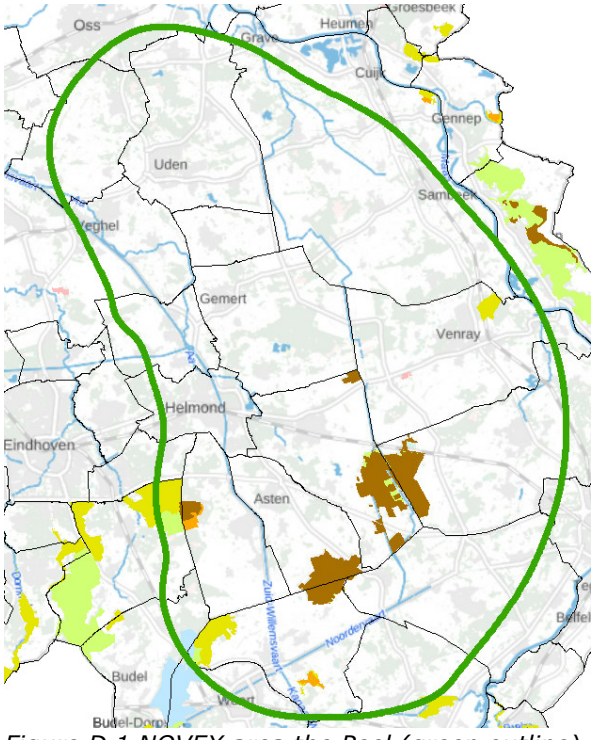


Figure D 1 NOVEX area the Peel (green outline). The Peel covers 23 municipalities , and part of the area is designated as a Nature 2000 area (coloured areas).

17.2 Characteristics of contamination

The research questions focus on VM and PPP. VM are a broad group of substances, with varying physicochemical properties. The toxicity for organisms may depend on the type of substance. For example, antibiotics are likely to be very toxic for micro-organisms, while hormones mainly have effect on more developed organisms, such as

fish. Hence, the toxic pressure assessment focused on a broad array of possible types of exposures and impacts.

In Figure D 2, the density of livestock production is presented. The red colours demonstrate that a lot of livestock can be found in the middle of the area and in the North-west. Around the borders of the area there is less livestock. On the basis of the figure, it is expected that concentrations of contaminants are higher in the middle of the Peel. VMs are generally not volatile, so these substances won't travel far by air. The most logical routes to surface water would be runoff from farms, which would result in local emissions, and emissions as a result of the application of manure on lands. In case of the latter, it depends on to which lands the manure is applied. According to Lahr et al. (2019) application preferably takes place at locations close to the farm, to limit costs. The substances may travel to surface water via drainpipes, surface runoff and groundwater.

Like VM, PPP are a broad group of substances with varying physicochemical properties. The ecotoxicity of a PPP substance will mainly depend on the target organisms. The target could be fungi (e.g. the PPP pyrazole), caterpillars (e.g. the PPP chlorantraniliprole) or weeds (e.g. the PPP glyphosate).

The current production of crops is scattered across the area (Figure D 3). There is no clear relationship between the types of crops produced and the location. A large variety of crops is produced in the Peel. PPP may travel large distances by air, due to spray drifting. Usually, proper equipment and other measures should be in place to limit drifting. In case of surface runoff or drainage, it is expected that emissions are more local.

In most cases, environmental pollution by VM and PPP is diffuse pollution. A point source could be the cleaning of a manure tank, for example, resulting in emissions to the sewage system.
Once contaminants are in the water, they can travel to other locations in groundwater, sewage water and surface water. Some substances may end up in the sediments when these have high affinity with organic matter.

Note: One idea was to gain more information on the area characteristics, for example which type of companies are present in the area. Due to time constraints, the information was not collected.

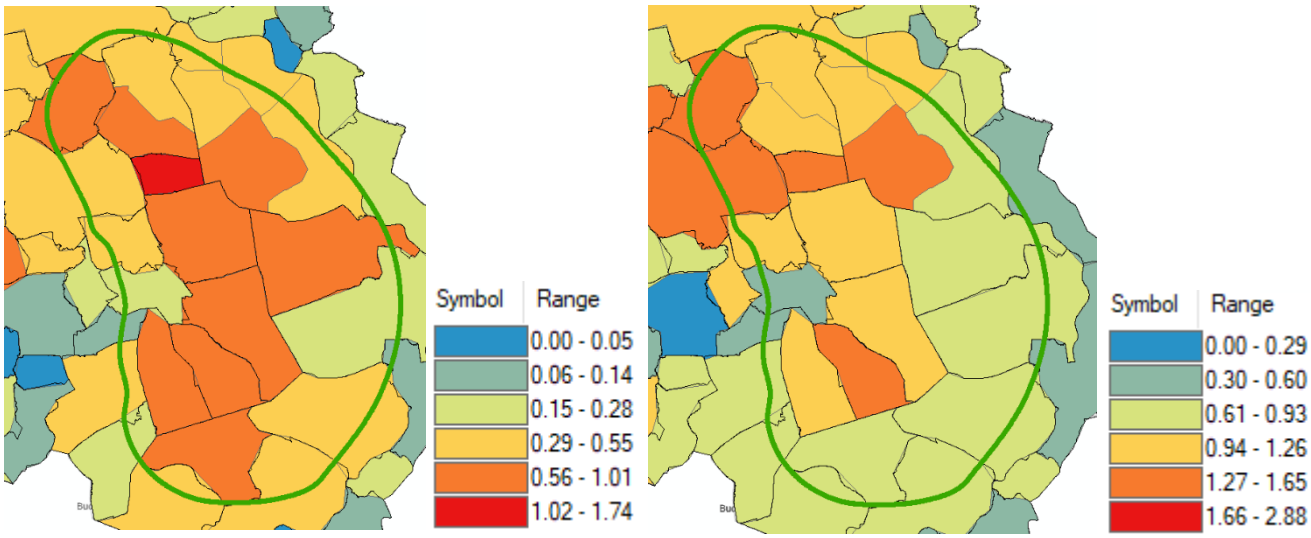


Figure D 2 Geographical overview of number of farms per km2 with (left) barn animals and (right) grazing animals in each municipality in the Peel in 2021. The number of farms increases with colour, from blue to red.

Figure D 3 Different land uses in the Peel in 2021. Colours represent various crops such as grains (yellow), maize (orange), potatoes (brown) and other crops (pink).

17.3 Environmental compartment of concern

On the basis of the concerns, surface water is the compartment of interest. There is no explicit concern regarding specific surface waters in the area. However, on the basis of the emission routes of the

contaminants, it is likely that the highest concentrations, and associated toxic pressure levels, are found in ditches close to farms, and in ditches adjacent to fields used for agriculture and livestock. In larger volumes of waters, concentrations of contaminants might be diluted (and mixed, from various land use sources). It should, however, be noted that the protection goal, and biodiversity in general, may be different for different types of water. There is no specific protection goal in this case, only the general protection of good water quality for all surface water as formulated in the goals of the WFD.

17.4 Endpoint of concern

There is no specific endpoint of concern regarding biodiversity or organisms (e.g. specific organisms or trophic level). Therefore, nature deterioration in general (the effects on all organisms in the water compartment) is chosen as the endpoint.

17.5 Interpretation context

It is chosen to evaluate toxic pressure regarding regulatory protective environmental quality standards or other standards, if available, as well as with virtual bioassays. A second goal is to evaluate the toxic pressure over time, for which this evaluation is being used as a benchmark for future evaluations.

17.6 Constructing a conceptual model

Below, the conceptual model of this case study is presented. Instead of a visual representation, the conceptual model is described in words The conceptual model is as follows:

The research area covers multiple municipalities and can be considered large. The contaminants we focus on are expected to come from diffuse sources, across the entire area. For veterinary medicines, the uses appear to be highest in the centre of the Peel; for pesticides, it is more difficult to differentiate between municipalities. On the basis of the available information, it is not known which specific classes of VM and PPP are used. Some bigger waterways are visible when plotting the area on a map, however it is expected that ditches close to farmlands might be most vulnerable on the basis of the contaminants of interest and dilution. No specific endpoint of concern came forward in this case study. Therefore, nature deterioration, in the broadest sense of the term, is chosen. The current status of chemical pressure in the area is of interest. Therefore, various methods to characterise toxic pressure with Component-Based Methods will be employed.

18 Research strategy and Data collection

18.1 Data collection

Data was already available for the area of concern and the compartment of concern (surface water). Data was retrieved from a project named 'Brede Screening Maasstroomgebied'. In this project, data on the water quality of surface water, groundwater and effluents of wastewater treatment plants (WWTPs) is collected. The water quality of different locations in and around the Meuse river basin in the Netherlands is assessed annually by performing chemical analyses. This concerns a limited number of substances, but every four years, comprehensive analyses are performed. The comprehensive analyses include (veterinary) medicines and plant protection products, albeit not for all locations. Additionally, data was retrieved from 'Waterschap Limburg'. This water board additionally measures contaminants at some of the locations of 'Brede Screening Maasstroomgebied' (at more moments), but also measures contaminants at some other locations. The most recent data available covering VM and PPP is data from 2022; data from that year is used to assess the toxic pressure. In Figure D 4, the different surface water locations, which were sampled in 2022 in or close to the Peel, are presented. In total, data is available for 17 locations (See Table D 1).

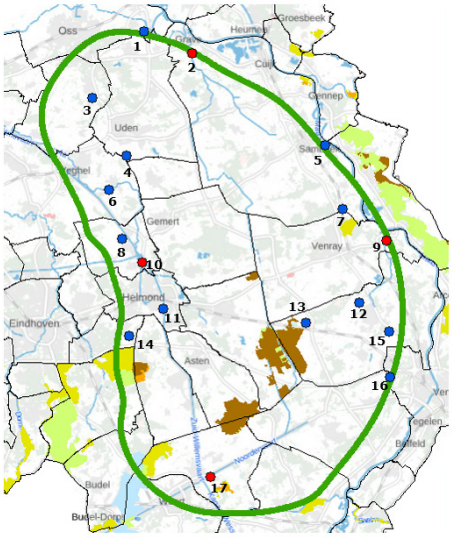


Figure D 4 17 locations of surface water in the Peel or around its border for which data was available in 2022. Blue = one or more plant protections products have been analysed. Red = one or more plant protection products and one or more veterinary medicines have been analysed.

Table D 1 Location characteristics.

Number	Location code	Number	Location code
1	340462	10	140218
2	341427	11	140213
3	140299	12	ODIEP230
4	143273	13	ODRPP500
5	340410	14	900238
6	140219	15	OLAVE200
7	OAFLE900	16	OKRAA600
8	140262	17	ONVAA100
9	OGRMB900		

For 4 out of 17 locations, data on veterinary medicines for livestock is available. Analytic measurements have been performed for 26 veterinary medicines (Table D 2).[24]

Table D 2 List of veterinary medicines for livestock measured in surface water samples. Note that some compounds are also used as medicine in pets (e.g. trimethoprim) and for other uses, such as human pharmaceutical (e.g. paracetamol).

benzocaine	lidocaine*	oxytetracycline	sulfamethoxazol
cloxacilline	lincomycine	paracetamol	sulfaquinoxaline
dexamethason	mebendazol	progesteron	tiamuline
enrofloxacine	monensin	sulfachloorpyridazine	trimethoprim
florfenicol	nafcilline	sulfadiazine	tylosine
flumequine	oxacilline	sulfadimethoxine	
ketoprofen	oxolinezuur	sulfadimidine	

*Is used on horses, which are not commonly considered livestock in the Netherlands, and is mainly used as an ingredient in pharmaceuticals for human use.

The 'Brede Screening Maasstroomgebied' lists 376 substances as plant protection products.

18.2 SWOT analysis

The Guidance document suggests performing a SWOT analysis to select optional lines of evidence. In this case study, the Component-Based Methods were selected as methods of preference, given the vast amount of data of chemical occurrence that is available from ongoing monitoring efforts. Therefore, the SWOT analysis approach was redundant for this study.

18.3 Research Plan

The research plan consists of the following steps:

- Collect all existing chemical measurement data on VM and PPP for the area delineated in Figure D 1;[25]
- Analyse the data in a stepwise fashion following the scheme for Component Based Methods, starting with the characterisation of the pollution and executing subsequent steps on the basis of the results;

[24] Based on current marketing authorisations in the Netherlands (d.d. 15-2-2023)

[25] Note that on the basis of the conceptual model and other information, ideally a specific research plan was set up. On the basis of the area and contaminants of interest it would have been logical to determine toxic pressure as a gradient, sampling nearby and further from sources. This could, for example, be the case for veterinary medicines in surface water close to farms and in drained fields. For pesticides, it could have been interesting to sample over time, in order to link the toxic pressure to time after application.

- The lines of evidence 'Effect-Based Methods' and 'Ecological Assessment Methods' are not applied, as this does not fit the scope and means of the current study.

In the final interpretation phase, the results of all methods are collated, and conclusions are formulated (where feasible) in comparison to regulatory standards, and (in pertinent cases) by interpreting patterns in the outcomes (e.g. virtual bioassays).

18.4 Component-Based methods

18.4.1 Data collection

Information on data collection is presented in Section 18.1.

18.4.2 Sampling and analyses

Sampling plans and chemical analyses were adopted from the existing sampling programmes that were mentioned earlier.

18.4.3 Method 1 - Characterisation of pollution

An evaluation was made regarding the question whether the measurements of chemicals provided insights into the presence of chemicals that only have a man-made origin (and could therefore exert a toxic pressure). This is a first proxy to judge toxic pressure. The results of the assessment are summarised in Table D 3.

Table D 3 Overview of the monitoring effort and number of unique substances measured above the detection limit in the area.

Substance group	**Number of substances**	**Number of substances measured at least once above detection limit**
Total	734	221
Medicine	118, of which 26 veterinary	47, of which 6 veterinary
Pesticides	429	93
Other inorganic substances (metals)	49	48
Other organic substances	138	33

From the monitoring data, it can be concluded that the area has higher concentrations of multiple compounds than expected from the absence of human influences. In total, 221 unique substances were found to exceed the detection limit at least once in 2022. This includes the veterinary medicines paracetamol, ketoprofen, monensin, sulfamethoxazole, trimethoprim, and lidocaine. 93 different pesticides have been found to exceed the detection limit, out of a total of 429 included in the monitoring effort.

Metals and other organic substances have also been found to exceed the detection limit in the available data. Metals have, however, not been compared to natural background concentrations because they are not the focus of this case study, as well as for pragmatic reasons. Their analysis would warrant paying attention to natural background

concentrations. For the present case study, no conclusion is drawn on whether metals are observed in higher concentrations than expected without human influences.

Conclusion

The concentration of multiple synthetic compounds is higher than 0 (see Table D 3). Therefore, there is evidence that chemicals are present due to human influences. The extent to which this potentially causes toxic pressure has not yet been established, as it depends on the exposure level and the total mixture.

As there is clear evidence of anthropogenic pollution on the basis of the observed elevated concentrations of both veterinary medicine and pesticides, the Risk Characterisation Ratio step (Method 2) and Toxic pressure step (Method 3) are performed next.

18.4.4 *Method 2 - Risk Characterisation Ratio*

Concentrations were judged *vis a vis* the regulatory, protective criteria (if available). This is a second proxy to judge toxic pressure. Not all substances measured above the detection limit had JG-MKNs available (Table D 4). Therefore, not all substances could be judged. In total, exposure levels for a substance were observed to exceed their JG-MKN[26] (RCR > 1) 346 times, of which 315 involved metals, 24 were pesticides, 6 other organic substances, and 1 substance used in medicines for human use. Results are summarised in Table D 4Table D 4 and Table D 5.

Table D 4 Overview of number of unique substances measured above the detection limit in the area and number of compounds for which a (Dutch) quality standard for water is available.

Substance group	**Number of substances measured at least once above detection limit**	**Available JG-MKN**	**Number of RCR>1**
Total	221	118	346
Medicine	47, of which 6 veterinary	4, of which 0 veterinary	1
Pesticides	93	41	24
Other inorganic substances (metals)	48	21	315
Other organic substances	33	6	6

We further focus this section on the pesticides, although our research question considered pesticides and veterinary medicines (and thus also excluded the other inorganic and organic substances). For the veterinary medicines, no EQS (JG-MKN) were available. No effort was made to collect PNECs from marketing authorisations of veterinary medicines or other sources due to time constraints. Therefore, their RCRs were not calculated.

[26] JG-MKN=JaarGemiddelde Milieu Kwaliteits Norm, in English: AA-EQS. This criterion is used in the European Water Framework Directive to classify water quality as 'good' (C<criterion) or as 'failure to reach good' (C>criterion). Criteria values were obtained from rvszoeksysteem.rivm.nl.

Table D 5 Locations with individual pesticides with an observed RCR > 1.

Location code	Amount of observations pesticides with RCR > 1	List of pesticides with RCR > 1 (Dutch names)	RCR
140213	2	Chlorantraniliprole	1.69
		2,4-dinitrofenol	1.10
140219	2	2-methyl-4-chloorfenoxyazijnzuur	1.43
		Dimethenamide	1.38
140262	2	Azoxystrobin	2.25
			1.30
143273	1	Metazachloor	1.38
340462	2	**Deltamethrin**	6.45E+3
		Esfenvaleraat	105
900238	3	**Thiacloprid**	5.70E+3
		Azoxystrobin	5.00
		Aclonifen	1.75
OAFLE900	5	**Imidacloprid**	10.8
		Thiamethoxam	6.14
			5.29
			3.43
		Metribuzine	1.08
ODIEP230	1	**Thiacloprid**	3.00
OGRMB900	1	2,4-dinitrofenol	1.20
OLAVE200	1	Spiromesifen	16.0
ONVAA100	4	Hexachloorbutadieen	10.9
			10.9
			7.27
			5.45

The three compounds in bold are investigated further using Method 4 (see Section 18.4.6)

Conclusion

The data shows that the concentrations of chemicals found in part of the Peel samples exhibit RCR- and/or Σ-RCR values >1. This implies that where they occur, the local ecosystem 'fails to reach the good water quality status' at sampling points, and that the water body is insufficiently protected against adverse effects of chemical pollution according to current protective regulatory standards.

In total, 15 individual pesticides with an RCR >1 have been observed at 11 out of 17 locations (see table above). The data shows that the RCR can be as high as 645 for a single compound (deltamethrin at site 340462). As one or more RCRs are >1, it was concluded that it is necessary to further characterise the toxic pressure according to further refined methods (Method 4 – Virtual bioassay).

18.4.5 Method 3 – Toxic pressure characterisation

The toxic pressure of the 17 locations was calculated using the Sleutelfactor Toxiciteit tool (www.sleutelfactortoxiciteit.nl). For this calculation, data on all chemicals (pesticides, metals, other organic

contaminants, etc.) was used.[27] In this instance, it was decided to assess all compounds instead of only the pesticides and veterinary medicines, in order to assess and illustrate the usability of this method. It should be clear that for demonstration purposes, we deviate from the Guidance in Section B. This also means that 104 compounds were assessed by this method instead of the 41 pesticides assessed by Method 2.

Figure D 5 - Figure D 8 show the toxic pressure classifications of the various locations (four per figure) at various points in time. Of the seventeen locations, eleven locations are classified as having moderate or higher toxic pressure for at least one point in time in 2022 (Table D 6Table D 6). Most occurrences with a higher toxic pressure were caused by the metals substance group. It should be noted, however, that the bioavailability of the metals was calculated in the online tool, using default values for the modifying factors. Furthermore, they should be placed within the context of natural background concentrations of these metals, as the local ecosystem could be adapted to these. Nonetheless, the high modelled toxic pressure of metals in the area can be seen as a cause for further research, either into natural concentrations of these models, expected impacts on specific (groups of) organisms or research into effects. At no point in 2022, the maximum toxic pressure by pesticides is higher than 'low' for twelve out of seventeen locations. For these locations, it can be concluded that >95% of the species is likely to be protected from negative impacts by pesticides. For five locations the toxic pressure due to pesticides is moderate or higher at some point in time in 2022. These are locations 140219, 340462, 341427, 900238 and OAFLE900. For these locations, it can be concluded that they are insufficiently protected against toxic pressure of pesticides (Table D 7).

The next steps would be to either address the societal concerns on the basis of this information, or if more information is desired, to continue with the biological or ecological methods. For the purpose of this case study, the biological and ecological methods are not performed as this does not fit in with the means of the current project.

Table D 6 The number of locations with a certain maximum toxic pressure classification during 2022.

Toxic pressure classification	**1-none**	**2-low**	**3-moderate**	**4-high**	**5-very high**
Number of locations	0	6	3	6	2

[27] Note that the Virtual bioassay results in the next Section are incomplete as only for three compounds RQs were calculated. This means that this assessment is of a different order and results may not reflect what is found with the Virtual bioassays. Preferably all contaminants of interest are assessed with both methods.

Table D 7 Conclusions on toxic pressure by pesticides at the five locations with at least a 'moderate' classification of chemical pollution (according to the classes defined in www.sleutelfactortoxiciteit.nl), including the narrative conclusions related to those classes.

Location	**Maximum toxic pressure classification due to pesticides**	**Conclusion**
140219	3-moderate	Negative impacts due to pesticides on a maximum of 1 in 200 species
340462	5-very high	Negative impacts due to pesticides on more than 1 in 10 species
331427	4-high	Negative impacts due to pesticides on 1 in 200 to maximum 1 in 10 species
900238	5-very high	Negative impacts due to pesticides on more than 1 in 10 species
OAFLE900	4-high	Negative impacts due to pesticides on 1 in 200 to maximum 1 in 10 species

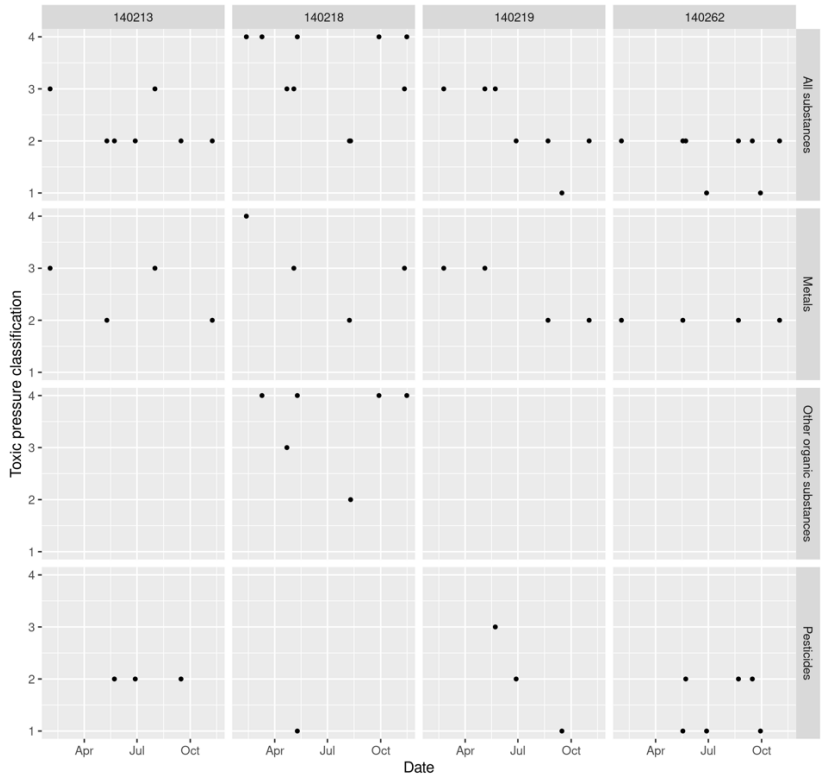


Figure D 5 Toxic pressure classification (according to www.sleutelfactortoxiciteit.nl) during the year 2022 per location and substance group. Classification according to the interpretation scheme (1 = none, 2 = low, 3 = moderate, 4 = high, 5 = very high). Classes 1 and 2 are defined such that the exposure to a chemical or an unintended mixture protects more than 95% of the species against direct effects of the exposure on endpoints such as growth and reproduction (which has been used as criterium to define the regulatory threshold for acceptable risk).

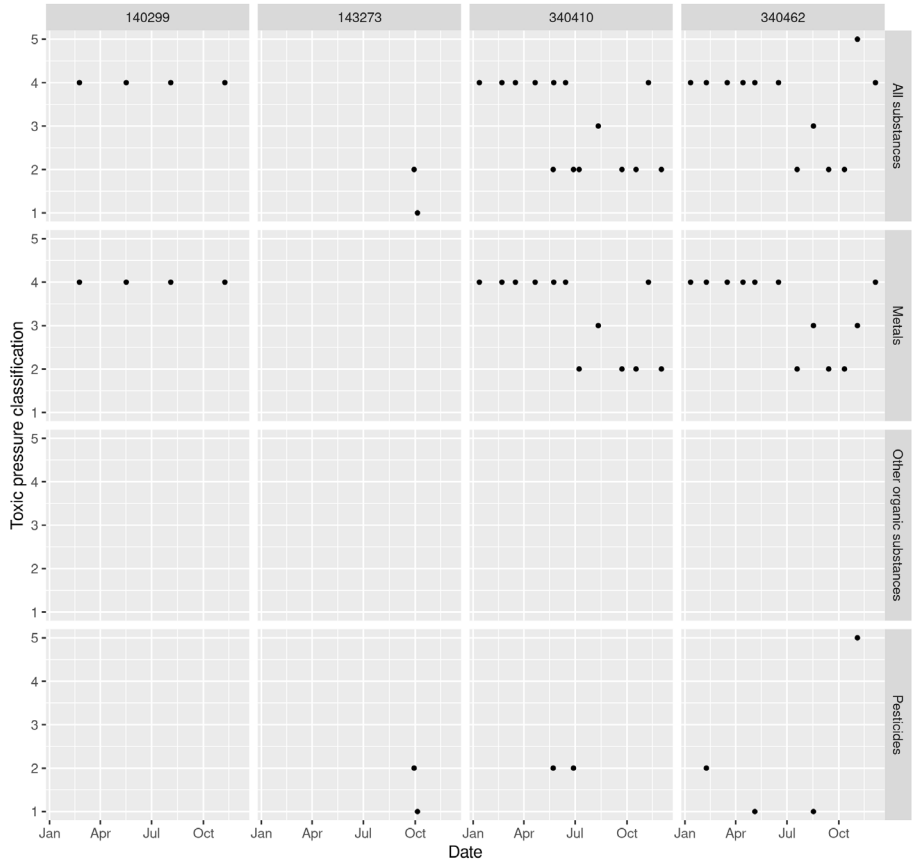


Figure D 6 Toxic pressure classification during the year 2022 per location and substance group. Classification according to the interpretation scheme (1 = none, 2 = low, 3 = moderate, 4 = high, 5 = very high). Further details as in Figure D 5.

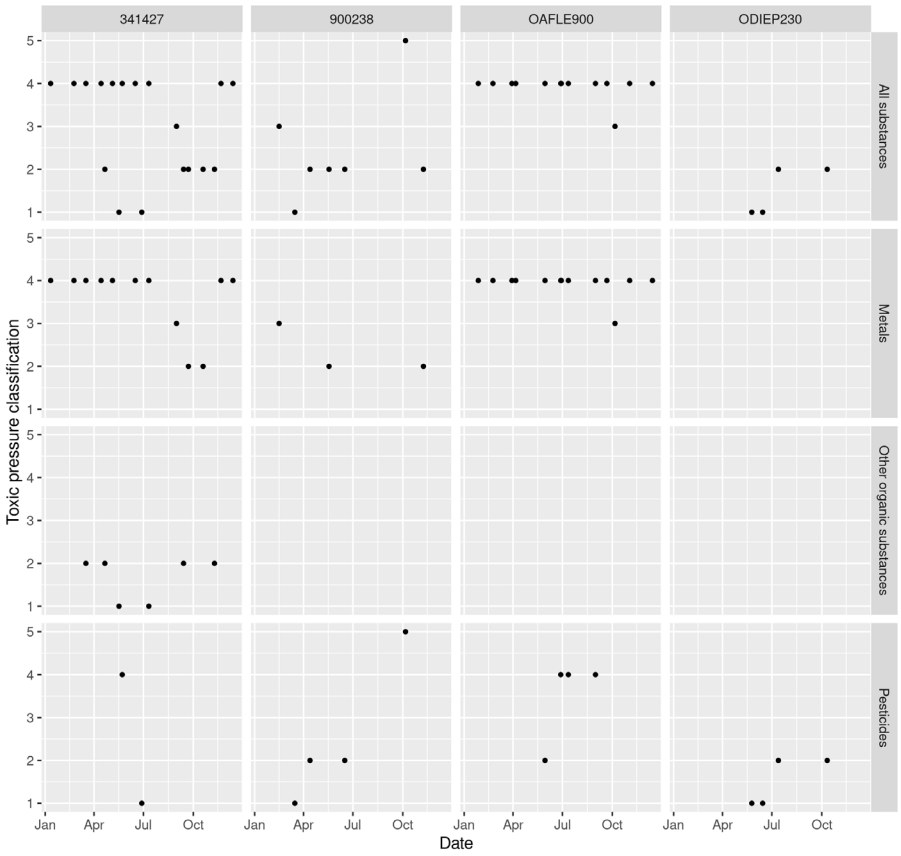


Figure D 7 Toxic pressure classification during the year 2022 per location and substance group. Classification according to the interpretation scheme (1 = none, 2 = low, 3 = moderate, 4 = high, 5 = very high). Further details as in Figure D 5.

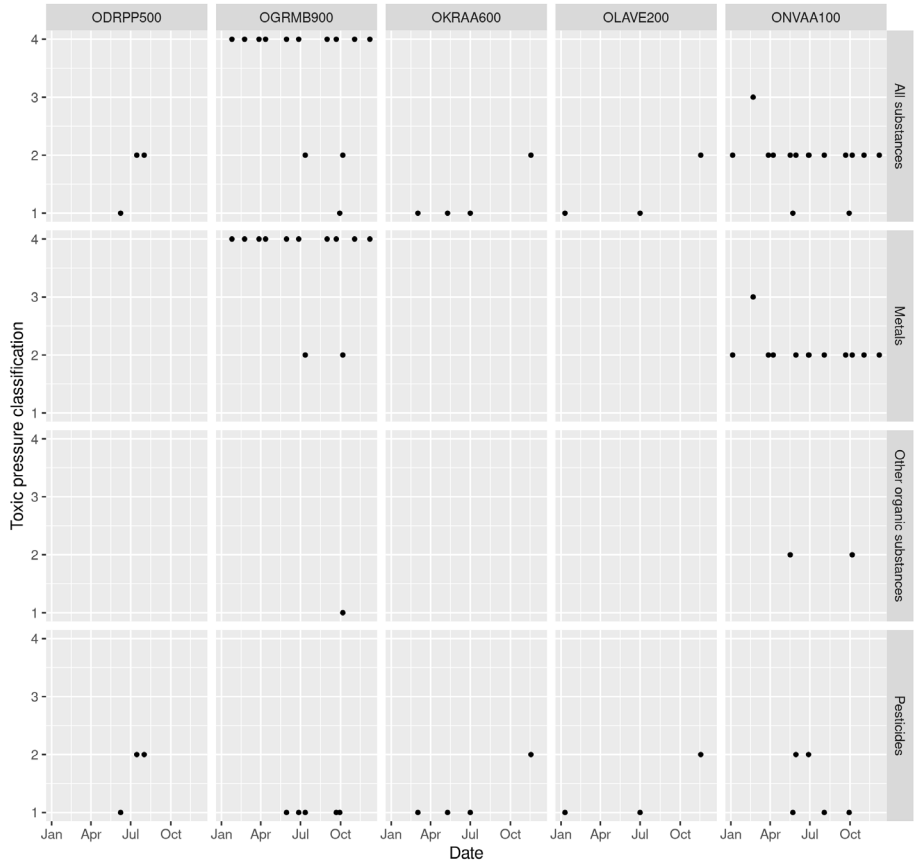


Figure D 8 Toxic pressure classification during the year 2022 per location and substance group. Classification according to the interpretation scheme (1 = none, 2 = low, 3 = moderate, 4 = high, 5 = very high). Further details as in Figure D 5.

18.4.6 Method 4 - Virtual Bioassay

Given the RCR>1 from Method 2, the next step was to execute the virtual bioassay approach. This approach consists (in principle) of comparing all measured data to all available ecotoxicity data. Due to practical limitations, not all possible comparisons were collated and plotted.

As yet, no tool is available that can be used to perform this step of the Guidance for large amounts of data (#sites, #compounds, #species tested). Due to limited case study resources, and the purpose of illustrating the type of outcomes, it was decided that a virtual bioassay was to be performed for only three selected compounds. These compounds are:

1. Trimethoprim (veterinary medicine);
2. Imidacloprid (former pesticide[28]);
3. Thiacloprid (pesticide).

Trimethoprim was chosen as no regulatory protective standard is available for any of the veterinary medicines. The compound has not been assessed in Method 2. It is examined whether any information can be provided on toxic pressure of this compound in the Peel on the basis

[28] Imidacloprid is currently not marketed (allowed) as a pesticide in the Netherlands. It is still marketed as medicine for companion animals.

of the virtual bioassays. Imidacloprid and thiacloprid popped up as compounds for which Method 2 yielded an RCR>1. Both compounds are neonicotinoids and have a similar mode of action. It is assessed whether the results differ between the compounds, and if the results can be combined to assess the combined toxic pressure.

Only measurements of these compounds above the limit of quantification are considered in the assessment. This means that only a small number of measurements are assessed (see Table D 8). Nonetheless, a vast number of virtual bioassay results was obtained (Table D 9), whereby there is evidence for toxic pressure on particular species groups (values in bold).

Table D 8 Number of measurements of imidacloprid, thiacloprid and trimethoprim assessed and total number of measurements.

Compound	**No. of locations above LOQ**	**No of measurements above LOQ (total)**	**Total no. of locations measured**	**Total no. of measurements**
Imidacloprid	2	2	16	98
Thiacloprid	2	2	16	98
Trimethoprim	1	6	4	16

For these compounds, ecotoxicity data for water organisms was collected from the US EPA Ecotox knowledgebase.[29] All NOEC values and EC50 values were collected to assess the acute and chronic effects of these compounds.[30] Following collection, all data was ranked on the basis of the test duration and type of species. Risk quotients were calculated for each combination (measurement of a compound and ecotoxicity endpoint).

For 1 location, virtual bioassay RQs were found >1. This was for Thiacloprid at location 900238. The results are graphically depicted in Figure D 9-Figure D 11. On the basis of both acute and chronic data it can be seen that the presence of the compounds would imply a toxic pressure on the selected species used in the virtual bioassays. The data shows that insects and crustaceans would be affected by the concentrations of this compound, if the tested species would be reared in the Peel surface water sample. This makes sense as the compound is an insecticide (neonicotinoid). For further information on the RQs, see Table D 9.

For all other locations, RQs and Σ-RQs <1 were found. The information is summarised in Table D 9. As thiacloprid and imidacloprid have the same Mode of Action, it would have been possible to add the species-RQs of these compounds and compare the Σ-RQ to the threshold value of 1. However, there are no locations where both compounds were found above the LOQ. Therefore, it was not possible to evaluate the outcome of the virtual bioassay via Σ-RQ evaluation of the unintended mixtures.

[29] https://cfpub.epa.gov/ecotox/search.cfm
[30] Note that LC50 values are missing in the dataset

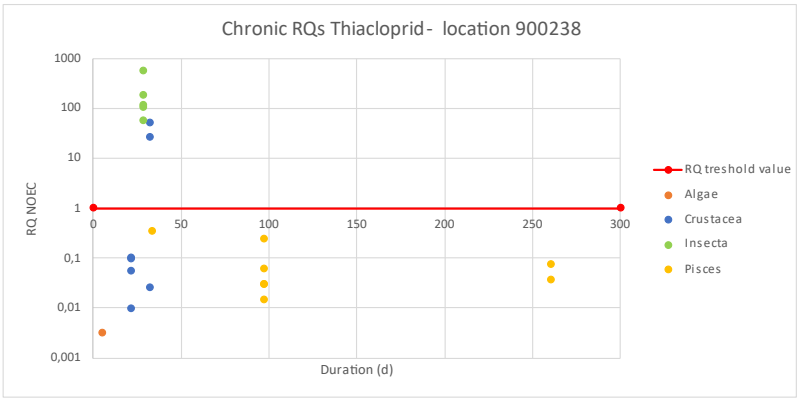


Figure D 9 Overview of all results of virtual bioassays, expressed as Risk Quotients for different species groups (colours) for thiacloprid for location 900238, based on chronic data (NOECs), different species groups (colours) and different exposure durations (X-axis). Y-values >1 imply the presence of an exposure level that would affect the species if reared in the sample.

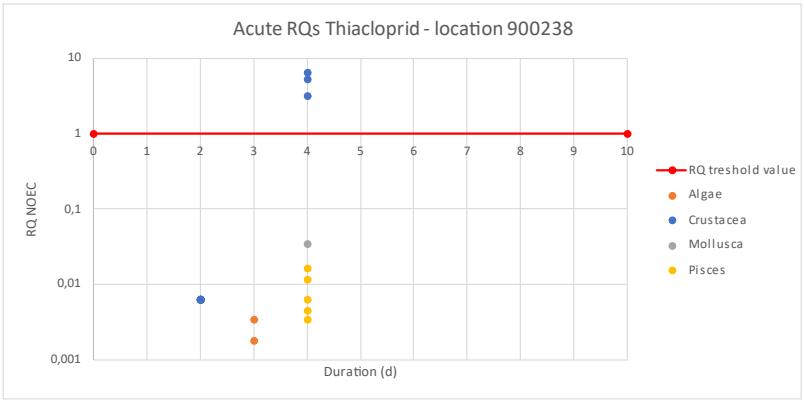


Figure D 10 Overview of all results of virtual bioassays, expressed as Risk Quotients for thiacloprid for location 900238, based on acute data (NOECs). Further details as in Figure D 9.

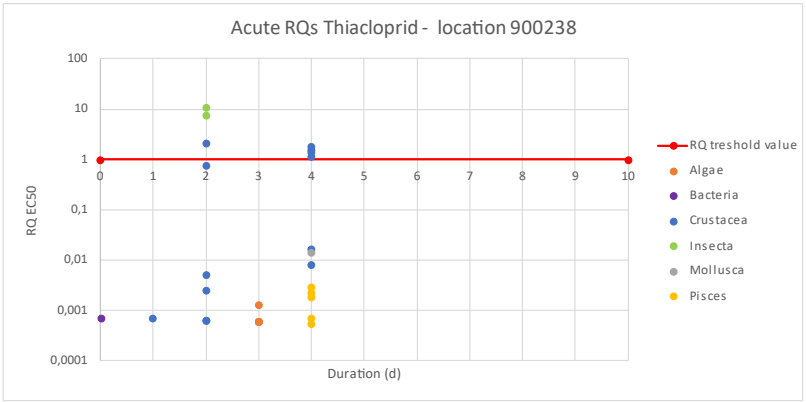


Figure D 11 Overview of all results of virtual bioassays, expressed as Risk Quotients for thiacloprid for location 900238, based on acute data (EC50s). Further details as in Figure D 9.

Table D 9 Average and Maximum Risk Quotients for different combinations of ecotoxicity data, species, and sample-compound combinations.

Compound/location/date	Species	NOEC chronic Avg RQ	NOEC chronic Max RQ	EC50 chronic Avg RQ	EC50 chronic Max RQ	NOEC acute Avg RQ	NOEC acute Max RQ	EC50 acute Avg RQ	EC50 acute Max RQ
Location: 140218	Algae					4.71E-06	4.71E-06	5.31E-06	1.33E-05
Date: 10/Mar	Amphibia					1.20E-06	1.20E-06	1.20E-06	1.20E-06
Compound: Trimethoprim	Crustacea	1.30E-05	2.00E-05					1.10E-06	2.19E-06
Cas no.: 738-70-5	Cyanobacteria	1.63E-05	3.87E-05	2.12E-06	1.09E-05			1.74E-05	1.74E-05
	Macrophyta					1.20E-04	1.20E-04	1.20E-04	1.20E-04
	Pisces	7.64E-04	7.64E-04					1.20E-06	1.20E-06
	Rotifera					3.14E-03	1.20E-02	6.33E-07	6.33E-07
Location: 140218	Algae					3.14E-06	3.14E-06	3.54E-06	8.89E-06
Date: 22/Apr	Amphibia					8.00E-07	8.00E-07	8.00E-07	8.00E-07
Compound: Trimethoprim	Crustacea	8.67E-06	1.33E-05					7.34E-07	1.46E-06
Cas no.: 738-70-5	Cyanobacteria	1.08E-05	2.58E-05	1.41E-06	7.27E-06			1.16E-05	1.16E-05
	Macrophyta					8.00E-05	8.00E-05	8.00E-05	8.00E-05
	Pisces	5.10E-04	5.10E-04					8.00E-07	8.00E-07
	Rotifera					2.10E-03	8.00E-03	4.22E-07	4.22E-07
Location: 140218	Algae					2.35E-06	2.35E-06	2.65E-06	6.67E-06
Date: 10/May	Amphibia					6.00E-07	6.00E-07	6.00E-07	6.00E-07
Compound: Trimethoprim	Crustacea	6.50E-06	1.00E-05					5.51E-07	1.09E-06
Cas no.: 738-70-5	Cyanobacteria	8.13E-06	1.94E-05	1.06E-06	5.45E-06			8.70E-06	8.70E-06
	Macrophyta					6.00E-05	6.00E-05	6.00E-05	6.00E-05
	Pisces	3.82E-04	3.82E-04					6.00E-07	6.00E-07
	Rotifera					1.57E-03	6.00E-03	3.17E-07	3.17E-07
Location: 140218	Algae					3.92E-07	3.92E-07	4.42E-07	1.11E-06
Date: 10/Aug	Amphibia					1.00E-07	1.00E-07	1.00E-07	1.00E-07
Compound: Trimethoprim	Crustacea	1.08E-06	1.67E-06					9.18E-08	1.82E-07
Cas no.: 738-70-5	Cyanobacteria	1.36E-06	3.23E-06	1.77E-07	9.09E-07			1.45E-06	1.45E-06
	Macrophyta					1.00E-05	1.00E-05	1.00E-05	1.00E-05
	Pisces	6.37E-05	6.37E-05					1.00E-07	1.00E-07

Compound/location/date	Species	NOEC chronic Avg RQ	NOEC chronic Max RQ	EC50 chronic Avg RQ	EC50 chronic Max RQ	NOEC acute Avg RQ	NOEC acute Max RQ	EC50 acute Avg RQ	EC50 acute Max RQ
	Rotifera					2.62E-04	1.00E-03	5.28E-08	5.28E-08
Location: 140218	Algae					1.57E-06	1.57E-06	1.77E-06	4.44E-06
Date: 28/Sept	Amphibia					4.00E-07	4.00E-07	4.00E-07	4.00E-07
Compound: Trimethoprim	Crustacea	4.33E-06	6.67E-06					3.6.E-07	7.30E-07
Cas no.: 738-70-5	Cyanobacteria	5.42E-06	1.29E-05	7.07E-07	3.64E-06			5.80E-06	5.80E-06
	Macrophyta					4.00E-05	4.00E-05	4.00E-05	4.00E-05
	Pisces	2.55E-04	2.55E-04					4.00E-07	4.00E-07
	Rotifera					1.05E-03	4.00E-03	2.11E-07	2.11E-07
Location: 140218	Algae					2.35E-06	2.35E-06	2.65E-06	6.67E-06
Date: 15/Nov	Amphibia					6.00E-07	6.00E-07	6.00E-07	6.00E-07
Compound: Trimethoprim	Crustacea	6.50E-06	1.00E-05					5.51E-07	1.09E-06
Cas no.: 738-70-5	Cyanobacteria	8.13E-06	1.94E-05	1.06E-06	5.45E-06			8.70E-06	8.70E-06
	Macrophyta					6.00E-05	6.00E-05	6.00E-05	6.00E-05
	Pisces	3.82E-04	3.82E-04					6.00E-07	6.00E-07
	Rotifera					1.57E-03	6.00E-03	3.17E-07	3.17E-07
Location: 140262	Algae					5.00E-07	5.00E-07	2.50E-07	2.50E-07
Date: 22/Aug	Amphibia							4.99E-08	6.10E-08
Cas no.: 138261-41-3	Annelida							4.11E-04	8.06E-04
Compound: Imidacloprid	Crustacea	7.46E-04	3.60E-03	9.48E-04	1.89E-03	2.28E-03	1.43E-02	3.55E-04	5.00E-03
	Insecta	2.46E-03	5.00E-03	1.80E-03	5.49E-03	1.72E-03	4.85E-03	8.82E-04	7.69E-03
	Mollusca							3.45E-08	3.45E-08
	Nematoda							3.16E-06	3.16E-06
	Pisces	2.25E-05	6.28E-05			1.05E-07	2.00E-07	3.04E-08	6.02E-08
Location: OAFLE900	Algae					9.00E-06	9.00E-06	4.50E-06	4.50E-06
Date: 28/June	Amphibia							8.98E-07	1.10E-06
Cas no.: 138261-41-3	Annelida							7.40E-03	1.45E-02
Compound: Imidacloprid	Crustacea	1.34E-02	6.47E-02	1.71E-02	3.40E-02	4.10E-02	2.57E-01	6.39E-03	9.00E-02
	Insecta	4.44E-02	9.00E-02	3.24E-02	9.89E-02	3.10E-02	8.74E-02	1.59E-02	1.38E-01
	Mollusca							6.21E-07	6.21E-07

Compound/location/date	Species	NOEC chronic Avg RQ	NOEC chronic Max RQ	EC50 chronic Avg RQ	EC50 chronic Max RQ	NOEC acute Avg RQ	NOEC acute Max RQ	EC50 acute Avg RQ	EC50 acute Max RQ
	Nematoda							5.70E-05	5.70E-05
	Pisces	4.05E-04	1.13E-03			1.89E-06	3.60E-06	5.48E-07	1.08E-06
Location: 900238	Algae	3.17E-03	3.17E-03	9.41E-04	9.41E-04	2.59E-03	3.39E-03	9.28E-04	1.27E-03
Date: 06/Oct	Bacteria							7.22E-04	7.22E-04
Cas no.: 111988-49-9	Crustacea	**1.11E+01**	**5.18E+01**			**3.67E+00**	**6.33E+00**	7.35E-01	**2.11E+00**
Compound: Thiacloprid	Insecta	**2.04E+02**	**5.70E+02**					**9.11E+00**	**1.08E+01**
	Mollusca					3.35E-02	3.35E-02	1.43E-02	1.43E-02
	Pisces	1.02E-01	3.35E-01			8.33E-03	1.63E-02	1.66E-03	2.89E-03
Location: ODIEP230	Algae	1.67E-06	1.67E-06	4.95E-07	4.95E-07	1.36E-06	1.79E-06	4.88E-07	6.67E-07
Date: 11/Oct	Bacteria							3.80E-07	3.80E-07
Cas no.: 111988-49-9	Crustacea	5.87E-03	2.73E-02			1.93E-03	3.33E-03	3.87E-04	1.11E-03
Compound: Thiacloprid	Insecta	1.07E-01	3.00E-01					4.79E-03	5.69E-03
	Mollusca					1.76E-05	1.76E-05	7.50E-06	7.50E-06
	Pisces	5.38E-05	1.76E-04			4.38E-06	8.57E-06	8.73E-07	1.52E-06

The RQs >1 are presented in bold and imply the presence of toxic pressure for a tested species (represented as taxonomic group). Note that columns with RQs based on NOECs signal a potential to cause harm (beyond the No-effect level) if RQ values in those columns are >1, but that the interpretation of the RQ values in the columns marked with EC50 in the column header imply substantial effects at and beyond RQ-EC50=1. That is, RQ-EC50-values <1 may also indicate toxic pressure, and impacts.

Conclusion

Most virtual bioassay results show RQ and sum-RQ values (much) smaller than 1, for both the RQ-NOEC and the RQ-EC50. When the virtual bioassays are based on chronic NOECs, this implies that the toxic pressure to these species, if reared in the water samples, is lower than the exposure level that causes any of the effects studied in the tests (values of the denominator, commonly defined for endpoints such as growth and reproduction). However, for thiacloprid on one location, the data yields RQ-NOEC>1 for insects and crustaceans, based on both acute and chronic effect data. This implies that these species groups would be affected, given the exposure level, and will respond the more when the RQ increases.

For the other locations in combination with data on thiacloprid, imidacloprid and trimethoprim, the data yields RQ<1 and/or Σ-RQ<1, implying that the taxonomic groups for which data is available are unlikely to show adverse effects for the studied endpoints.

18.5 Effect-Based Methods

No data was collected to apply the effect-based methods to assess toxic pressure. Therefore, the approaches and outcomes of this step are not illustrated in this case study.

18.6 Ecological Assessment Methods

No data was collected to apply the ecological assessment methods to assessing toxic pressure. Therefore, the approaches and outcomes of this step are not illustrated in this case study.

19 Analysis and interpretation

Please note that the analysis and interpretation of the results presented for this case study may not represent the actual situation in the studied area due to limitations in the execution of the case study and practical choices. The information presented should therefore solely be seen as an example for demonstration purposes and testing of the methods.

19.1 Collation of results

The results of the toxic pressure assessment steps are summarised in Table D 10.

Table D 10 Collated results of the case study.

Line of evidence	Method	Result	Conclusion	Next Step
Component Based Methods	1	93 unique pesticides and 6 unique veterinary medicines measured above their detection limit.	There is evidence of human influence on the area due to concentrations of pesticides and veterinary medicine.	Continue with Method 2 and 3.
Component Based Methods	2	24 occurrences of in total 15 individual pesticides with an RCR > 1 have been observed at 11 out of 17 locations. No regulatory thresholds available for the 6 veterinary medicines.	Insufficient data for the assessment of veterinary medicines. The local ecosystem is insufficiently protected against pesticides according to current regulatory standards.	It is decided to continue with Method 4 (as proof of principle).
Component Based Methods	3	This step was performed for all chemicals measured. Toxic pressure is classified as moderate or	The area is not sufficiently protected against mixtures of pesticides.	Stop the assessment.

Line of evidence	Method	Result	Conclusion	Next Step
		higher at 11 of 17 locations, majority due to *metals*. Toxic pressure is classified as moderate or higher due to *pesticides* at 5 of 17 locations.		
Component Based Methods	4	This step was performed for 2 pesticides: Thiacloprid, imidacloprid and 1 veterinary medicine: Trimethoprim. RQs for imidacloprid and trimethoprim were below 1 for all datapoints. Virtual bioassay of thiacloprid suggests effects are likely to show for insects and crustacea.	Effects expected on insects and crustacea. On the basis of limited information, no effects expected due to veterinary medicines.	

19.2 Dominant substance groups

There are cases of anthropogenic influence due to chemicals in the area. Medicines, veterinary medicine, pesticides and other organic compounds are all detected. Metals are also detected and are the cause of toxic pressure above regulatory acceptable levels in most locations. However, metals were not the focus of the case study and background concentrations have not been considered, that is: the toxic pressure of metals may partly be of natural origin (not investigated).

19.3 Role of contaminants

The results warrant further investigation of the question whether the calculated toxic pressure of metals is due to anthropogenic influence or is of natural origin, and to what extent this results in negative impacts on the environment. Apart from metals, pesticides were also a cause for moderate or higher toxic pressure in the area. However, there were different pesticides per location. Other organic compounds (including (veterinary) medicines, as the msPAF tool did not differentiate these) were not a significant cause for toxic pressure, except at one location. For this location (140218, high toxic pressure) it should be further investigated which compounds were the cause of this toxic pressure.

19.4 Mitigation measures

Veterinary medicines were detected in the area, however determining their impact was challenging due to lack of ecotoxicity data. On the basis of the virtual bioassay of one veterinary medicine, no adverse effects are expected on species or taxonomic groups for which data was available. On the basis of the current data, no direct measures would be prioritised. Effect-Based Methods or Ecological Assessment Methods at locations where veterinary medicines have been detected could provide further insight into their impacts.

Pesticides are a cause of moderate to high toxic pressure at multiple locations. However, there is almost no single pesticide that causes toxic pressure at multiple locations. This makes it difficult to implement specific measures. Potentially, the uses of various pesticides are linked to a certain use, such as specific crops and a specific way of using the land. Information from marketing authorisations can be used to identify the crops for which a compound is used. In combination, information from the area characteristics (See Figure D 3) can be used to see where the pesticides are potentially used. Due to time constraints, it has not been possible to examine this in detail.

Other organic substances also cause toxic pressure in the area, primarily at location 140218. It is advised to first adjust the conceptual model and then further investigate these compounds (for example, to assess where they come from, and to conduct the various Component-Based Methods for these) in order to evaluate the need for and prioritisation of mitigation measures. Also, the toxic pressure of metals has been identified as a potential issue. It should be investigated whether their detected concentrations are due to anthropogenic pollution or whether they are of natural origin, and the conceptual model should be adapted accordingly.

Please note that the above mitigation measures are presented for demonstration purposes, and do not represent an actual advice.

Section E - Terrestrial case study

The terrestrial case study was undertaken to evaluate the utility of the practical Guidance (Section B) and to illustrate its outcomes for data from a terrestrial ecosystem. The case study specifically focuses on the use of the various lines of evidence in the Guidance, that is: using both Component-Based Methods, Effect-Based Methods and Ecological Assessment Methods to illustrate how the results from the various lines of evidence and methods can be used to obtain increasingly refined insights into the toxic pressure of an area. Data for the case study was obtained from a published report commissioned by the Province of Noord-Brabant.

Because this demonstration case is based on an existing study with existing data, the choices made are those of the original performers of the work. They have selected the methods, the substances measured and the sampling sites. When a study is conducted entirely according to the Guidance presented in Section B, various aspects may be different.

20 Introduction

20.1 Content

This report contains the results of a case study that was performed to illustrate the outcomes of the use of the Guidance (Section B) on characterisation of 'toxic pressure in vulnerable areas'. That Guidance is intended to help practically assess whether there is toxic pressure by chemicals in a certain area. The case study illustrates the use and outcomes of the sequential steps in the Guidance, so that it is clear to the user whether the method can be successfully completed. Note that information from the case study assessment activities has been used to improve on a draft of the Guidance, in terms of clarity as well as content. Improvements that could not directly be implemented in the first version of the Guidance were collected and serve as recommendation for future further developments.

The present case study (Section E) focuses on the toxic pressure in the terrestrial compartment for a selected area and problem – here: the catchment of the Dommel river. Another case study is available for the aquatic compartment (Section D). The case studies differ in terms of the Guidance methods that have been employed. Note that other compartments may also be of interest, e.g. sediment or biota. The case studies are partly based on actual concerns, partly fictional. The case study has been selected on the basis of scientific, practical and policy criteria.

20.2 Reading guide

The case study report follows the Guidance document, in discerning different phases and steps. In Section 21 the inventory is given (Phase I of the Guidance). In Section 22 the problem is defined (Phase II of the Guidance). In Section 23 the research strategy, data collection and calculations are presented (Phases III and IV of the Guidance). In the last Section (Section 24) the results are analysed and interpreted (Phase V of the Guidance).

21 Inventory

This section describes the inventory made of the situation in the area of interest, of the voiced concerns regarding potential presence of toxic pressure, and on optionally available data as the first phase of the systematic approach to determine toxic pressure. It contains four steps that need to be followed. These are presented below.

21.1 Societal concern

The various branches of the Dommel river in Belgium and the Netherlands are contaminated with metals (zinc and cadmium) as a result of historic non-ferrous metal industrial activities. As a result of flooding, the surrounding floodplain areas are also contaminated with these metals. The Dutch government aims to develop more nature conservation areas in the Dommeldal (the Dommel valley) as part of the 'Natuurnetwerk Nederland' (NNN), the Dutch ecological network of nature areas. However, there are concerns about whether the metal pollution represents an ecological risk. This hampers the development of nature conservation areas in the valley, as it is known that chemical pollution, if sufficiently high, serves as a limiting factor for various species and therefore for nature development.

21.2 Incentive to determine toxic pressure

The concerns are strongly related to chemical pollution. Previous studies have shown that the river water and the surrounding flood areas are contaminated with metals. The impact of the pollution on the ecosystem once these areas are developed into nature conservation areas is, however, unknown.

21.3 Collation of existing information

In 2008 'Actief Bodembeheer De Kempen', a soil quality office created by the Province of Noord-Brabant, commissioned a 'triad' study on the ecological risks of metal contamination in the area, which was conducted by Grontmij, Grontmij-AquaSense and Alterra (WUR). The report 'Ecologische effecten van metaalverontreiniging in het overstromingsgebied van de Dommel' (Ecological Effects of metal pollution in flood plain areas of the Dommel river; Derksen et al., 2008) presents the results of this study in which chemical, bioassay and ecological data was collected in the Dommel area. The study shows that there are clear ecological risks as a result of metal pollution in the area of concern.

21.4 Research question(s)

Given the evidence for ecological risks, how can the results be characterised in terms of toxic pressure of zinc and cadmium contamination in the current or to-be-developed nature conservation areas?

21.5 Go/No go

On the basis of the available information, there is a clear pollution-related concern regarding the ecological status of the Dommel area, so that it is warranted to execute an assessment aimed at characterisation of the toxic pressure. This implies that the next phase of a toxic pressure assessment can be started.

22 Problem definition

22.1 Constructing a conceptual model

On the basis of the information in Sections 22.2 to 21.5, a conceptual model of the area of concern was made, see Figure E 1. The conceptual model comprises the aspects of the sources-pathways-receptors interactions that are considered to dominate the issue of toxic pressure. Through the food chain, metal contamination can reach animals (e.g. badgers, birds) that are not, or to a lesser extent, directly exposed to soil contamination. Thus, metal contamination can result in secondary poisoning.

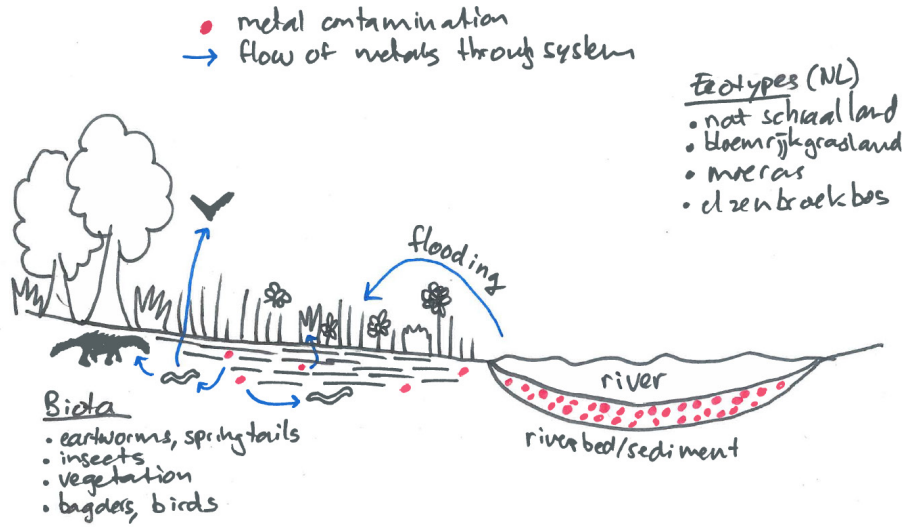


Figure E 1 Schematic overview of metal pollution, the potential flow of contamination and relevant compartments and biota in the Dommel catchment. Red dots indicate metal contamination. Blue arrows indicate the potential flow of metal contamination through the ecosystem. Note that the conceptual model is represented here on purpose, as a sketch with scribbled notes, to illustrate how a conceptual model can be designed in a development process that involves experts and stakeholders.

22.2 Area characteristics

Detailed characteristics of the area of concern are found in Derksen et al. (2008). The list of points below summarises the main area characteristics provided by Derksen et al. (2008) and other sources.

Geographical region
Figure E 2 shows a map of the confluences of the Dommel, Kleine Dommel, Reusel and Beerze rivers. It shows that large areas of the land surrounding the rivers are potentially exposed to contaminated water due to frequent flooding. Detailed maps of different stretches of the river and land use types of the surrounding areas can be found in Derksen et al. (2008).

A more detailed and up-to-date map of the water storage areas of the Dommel confluences can be found on the website of the Dommel Water Board (see https://www.dommel.nl/digitaal-kaartmateriaal).

The maps available on this website show that large stretches along the rivers Dommel, Kleine Dommel, Reusel and Beerze are dedicated flood areas, including the Bossche Broek Natura 2000 area.

Land use and ecological types
Through GIS analysis, Derksen et al. (2008) determined that most (43%) of the flood areas are agricultural lands, followed by multi-functional forest (10.7%) and marshes (6.3%). The various ecological target types (in Dutch: 'natuur(doel)typen') that are found along the river are 'nat schraalland', 'bloemrijkgrasland', 'moeras' and 'elzenbroekbos', which in English correspond to 'wet nutrient-poor grassland', 'flower-rich grassland', 'marshland' and 'alder scrubs', respectively. According to the nature conservation target for the area, these four ecological types should make up 71% of the area in the future.

Environmental (protection) status
Upstream to Den Bosch lies the Bossche Broek nature conservation area. This area has a Natura 2000 classification. Two other Natura 2000 areas (Kampina en Oisterwijkse Vennen, and Kempenland-West) are situated along and around the Reusel and Beerze rivers. These latter two areas are situated upstream of the sampling areas of Derksen et al. (2008).

Soil texture
Information on soil texture, organic matter content, pH and other soil properties of the area of concern are found in Annex 10 of Derksen et al. (2008).

Economic and other relevant activities
The Dommel river flows from its source in Belgium to Den Bosch via Eindhoven. Several other residential areas are situated along (or nearby) the river, including Boxtel and Sint Oedenrode. According to www.bedrijvenopdekaart.nl, several thousands of industrial companies operate in the area, most of which operate in Eindhoven. Details of the specific sectors of these companies are not publicly available through the website and thus are not provided here.

There is a sewage overflow just downstream of Eindhoven. Sampling near this area was avoided.

Other information sources on (a)biotic characteristics of the area
According to Derksen et al. (2008), many other studies and information sources are available. These include:
- Detailed data on water characteristics of the confluence;
- Various reports with data on chemical contamination;
- Flora and fauna inventories;
- Information (maps) on soil types.

Some of these are briefly summarised in the report by Derksen et al. (2008). However, most of these are provided on a CD-ROM.

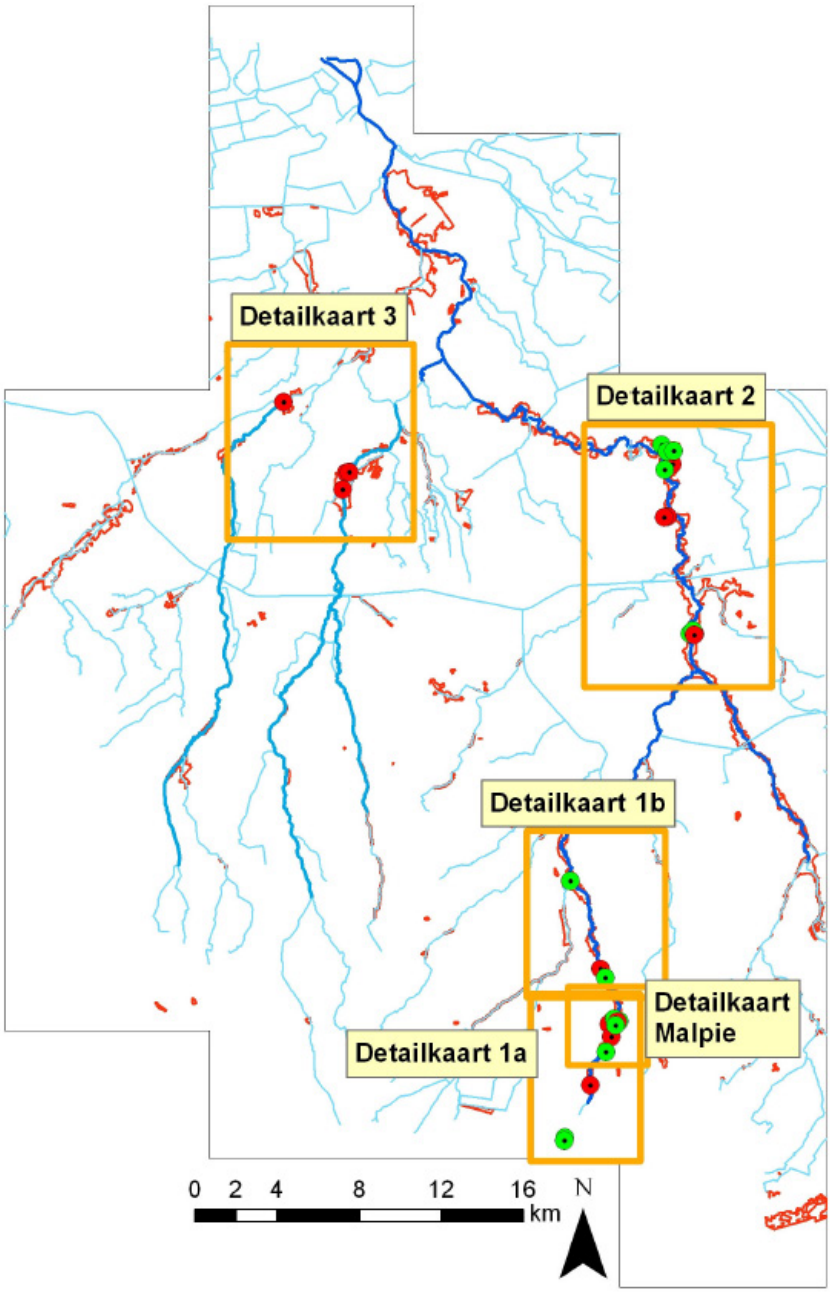


Figure E 2 Map showing the confluences in the catchment of the Dommel and the Kleine Dommel (dark blue) and the Reusel and Beerze (intermediate dark blue). Green and red dots indicate sampling locations. Red lines indicate areas that flood at least once every fifteen years. Detailed maps of sampling location can be found in Annex 9 of Derksen et al. (2008). Source of image: Derksen et al. (2008). Use of this figure in the current report was authorised by the Province of Noord-Brabant.

22.3 Characteristics of contamination

The main contaminants of concern are zinc and cadmium. These have been introduced to the area as a result of historic metal industries in the region. Chemical analysis has shown that the area is also contaminated with naturally present arsenic. Previous studies (Derksen et al., 2008 and references therein) show that at nearly half of the investigated sites, the total metal concentrations exceed Dutch intervention values (critical concentrations used for soil quality characterisation as 'seriously polluted') for one or more of these three metals. Through flooding, the metals bind to clay and organic particles of the contaminated river sediment are deposited onto the land that surrounds the river.

Other sources of contaminations may exist. As mentioned above, most of the flood area is used for agriculture. Through application of pesticides in these areas, (planned) nature conservation areas of concern may be additionally exposed pesticides via run-off, for example. As, to our knowledge, data on pesticide concentrations is not available, pesticide contaminants are not further considered in this review.

22.4 Environmental compartment

The environmental compartments of concern are the soil, soil inhabiting organisms (earthworms, springtails), vegetation (grasses), insects, and (vertebrate) animals that may be exposed through the food chain to soil contamination. Vertebrate model species considered relevant for the area of concern are the badger, godwit and little owl. A justification for a focus on these animal species in the assessments is provided in Derksen et al. (2008), section 7.2.2.

22.5 Endpoint

The relevant endpoints of concern for the area (given the management goal of nature development) are soil quality, survival, growth and reproduction of soil-bound species and bioaccumulation in the food chain.

23 Research strategy and Data collection

23.1 Research Plan

Note: In this case, an extensive 'triad' study has already been conducted (i.e. Derksen et al., 2008). A triad study consists of the three lines of evidence of the Guidance of the present report (Section B) that are done simultaneously, and for which the results are collated in formalised ways. The approaches and results of the triad were re-analysed in order to characterise the toxic pressure according to the Guidance. Thus, the outcomes of this study were used as input for the analysis described in Phase III of the Guidance.

The research plan of Derksen et al. (2008) can be summarised as follows:

Existing data (prior to Derksen et al., 2008) indicates that there is metal contamination in the Dommel area, but the ecological risks in current or planned nature conservation areas are not sufficiently understood. Therefore, a triad study was conducted in which (concurrently) the following studies were performed:

Component-Based Methods
Collection of soil (up to a depth of 20 cm) and measurements of:

- Soil characteristics (texture, pH, organic content, P and N content);
- Total metal concentrations (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn and Tl);
- Available metal concentrations (As, Al, Cd, Cr, Cu, Ni, Pb and Zn) through both mild (0,01M $CaCl_2$) and stronger (0,43 M HNO_3) extraction.

Effect-Based Methods
Measurements of effects in biota using plain soil or soil extracts from the contaminated sites:

- Measurement of bacterial growth;
- Acute toxicity of soil extracts to the water flea *Daphnia magna;*
- Reproductive toxicity studies on a subset of samples the springtail *Folsomia candida;*
- Germination and growth of canola seeds (*Brassica napus*);
- Growth and reproduction toxicity in the earthworm *Lumbricus rubellus.*

Ecological Assessment Methods
Bioaccumulation of metals in:

- The earthworm *Lumbricus rubellus;*
- Insects collected in the grasslands;
- Grass (*Holcus lanatus* or *Glyceria maxima* when the first species was not available);
- Food chain accumulation and effect models (PODYRAS and BERISP).

23.2 Component-Based Methods

23.2.1 Data collection

Details of the data collection strategy and methods can be found in Derksen et al. (2008).

23.2.2 Sampling and analyses

In brief, in total 10 litres of the topsoil (up to 20 cm depth) was sampled using a soil auger. A single (pooled) sample per sample site was collected. Thus, the sample collection was not representative of the different sub-areas within a sample site.

23.2.3 Method 1 – Characterisation of pollution

For the purpose of testing the Guidance, it was assumed (so without formal assessment) that all concentrations for Cd, Zn and As exceeded the background concentration. Note: The assumption was made because the natural background concentration of the metals in the area has not been characterised (due to lack of data) and measured concentrations exceed the (much higher) Intervention Value, see next section. In other cases, it may be necessary to characterise the natural background concentration of non-synthetic compounds in order to correctly characterise the toxic pressure.

23.2.4 Method 2 – Risk Characterisation Ratio

In half of the sampling sites, the concentration of Cd, Zn or As exceeds the available Dutch soil quality standards (here: 'Interventiewaarden', Intervention Values) (see Table 2 of Derksen et al. (2008)). Intervention values (IV) are regulatory standards with which the quality of a soil sample can be classified as 'seriously polluted' if the IV is exceeded.

The sum Risk Characterisation Ratio (RCR) was calculated considering all measured metals. The RCR is the ratio between the measured concentration in the field and the environmental quality standards for a particular metal. In the present study, the RCR-IV was calculated. The sum RCR is the sum of the ratios of all measured metals. The sum RCR-IV was >1 in all samples (Figure E 3). Highest sum RCR-IV values are found in 'moeras' (marshland) and 'vochtig schraalland' (wet nutrient-poor grassland). Given that the regulatory Dutch soil quality classification systems discern seriously polluted soil with the IV, it is implicitly clear that RCRs can be calculated with other soil quality criteria, for example those that discriminate between clean and slightly polluted soil. It is evident that those RCRs are (far) higher than the RCR-IV (and are therefore not shown).

Conclusion: the latter remark implies that the measured concentrations do not represent a case of sufficient regulatory protection according to established regulatory criteria (if so, the sum RCR for sufficient protection should be <1). On the contrary, there is evidence for toxic pressure of the separate metals and their mixtures, as derived from RCR-IV that substantially exceed the Intervention Value.

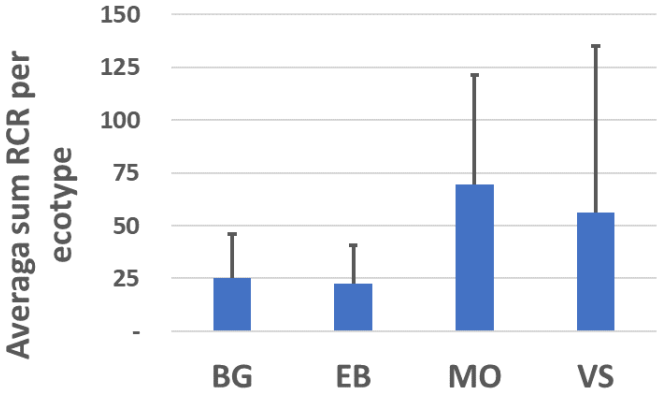


Figure E 3 Sum RCR-Intervention Value based on measured total concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn and Tl in Dommel area per ecotype. BG = 'bloemrijk grasland' (flower-rich grassland), EB = 'Elzenbroekbos' (alder scrubs), VS = 'Vochtig schraalland' (wet nutrient-poor grasslands'), MO = 'moeras' (marshland). IV=Intervention Value, a regulatory concentration standard used to distinguish cases of 'serious soil contamination'.

23.2.5 Method 3 – Toxic pressure characterisation

Derksen et al. (2008) calculated the toxic pressure of combined exposure to total concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn in standardised soil using the msPAF (the multi-substance Potentially Affected Fraction of Species, (De Zwart et al., 2005) as a metric. Details of the approach are described in Mesman et al. (2007). The analysis of the results collated by Derksen et al. (2008) shows that at 9 out of the 30 sampling sites, the msPAF-NOEC was below 0.05 (Figure E 4). This means that at these sites, toxic effects caused by direct exposure of the exposed species may be expected in fewer than 5% of the species, and that these sites are sufficiently protected from effects of metal to soil inhabiting species.

The msPAF values of the remaining sites were above 0.05. Of those, at 12 sites, this value was between 0.05 and 0.5 and at 9 sites, this value was above 0.5. This indicates that biota at these sites are not sufficiently protected from the effects of metal mixtures and that strong effects on biota are expected at some sites. msPAF values in marshes and wet nutrient-poor grasslands were the highest (Figure E 4).

Some of the methods and data to calculate the toxic pressure may have been updated since 2007, therefore some of these values were recalculated with the latest version of the approach described by Mesman et al. (2011). The final scores were nearly identical to those derived by Derksen et al. (2008).

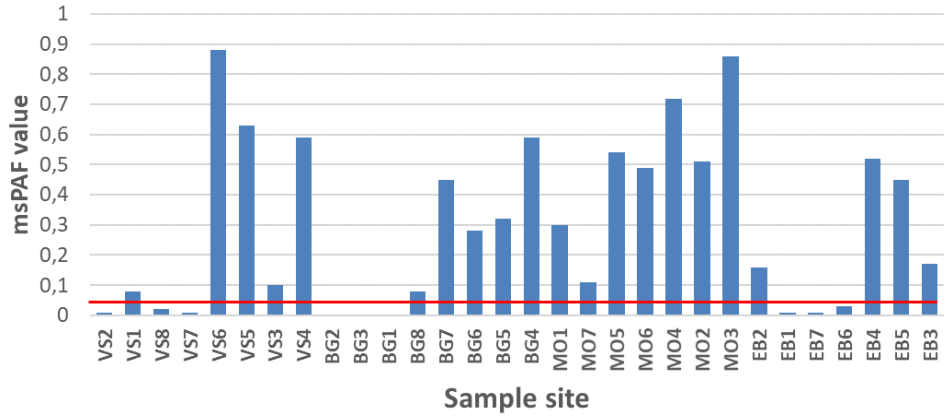


Figure E 4 msPAF-NOEC values based on measured total concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn and Tl in Dommel area per sample site. Abbreviations are (Dutch) names of sub-areas: BG = bloemrijk grasland (flower-rich grasslands), EB = Elzenbroekbos (alder scrubs), VS = Vochtig schraalland (wet nutrient-poor grasslands), MO = moeras (marshland). The red line indicates a msPAF value of 0.05, which is (if representing msPAF-NOEC) considered as threshold value below which the situation is characterised as 'sufficient protection' against direct effects of chemical exposure.

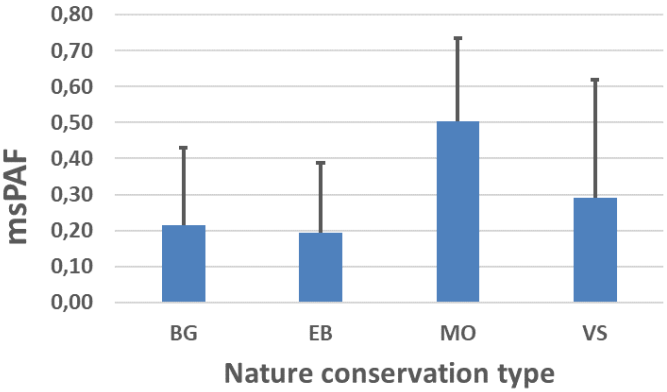


Figure E 5 Average msPAF-NOEC values (± standard deviation) based on measured total concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn and Tl in the Dommel area per ecotype. Abbreviations are (Dutch) names of sub-areas: BG = bloemrijk grasland (flower-rich grasslands), EB = Elzenbroekbos (alder scrubs), MO = moeras (marshlands), VS = Vochtig schraalland (wet nutrient-poor grasslands).

To summarise, at roughly two-thirds of the sites, there is evidence that the environmental concentrations result in a (mixture) toxic pressure level that far exceeds the criterion used to characterise a site as 'sufficiently protected' (commonly: msPAF-NOEC<0.05). This suggests that direct effects on the soil-inhabiting species can be expected. Note that the results of the Component-Based Methods used are in line with each other (see Figure E 3 and Figure E 5).

23.2.6 Method 4 – Virtual Bioassays

No virtual bioassays were conducted because of time limitations for the collection of terrestrial ecotoxicity data, and because the virtual bioassay approach is extensively illustrated in Section D for the aquatic case

study. Given the results of the other Component-Based Methods for the present case study, it is not difficult to envisage that the terrestrial case study would also show a diversity of virtual bioassay case study RQs with values >1.

23.3 Effect-Based Methods

23.3.1 Data collection

Key organisms are earthworms, springtails and plants. These are key species groups in the ecosystem, within the food chain for example, that are suitable for bioassays.

23.3.2 Selection of bioassays

Derksen et al. (2008) conducted an initial screening on the basis of the following assays:

- Measurement of bacterial growth;
- Acute toxicity of soil extracts to the water flea *Daphnia magna.*

A detailed screening of fifteen sites representing different ecotypes and levels of toxic pressures was then conducted using the following bioassays:

- Reproductive toxicity studies on a subset of samples using the springtail *Folsomia candida;*
- Germination and growth of canola seeds (*Brassica napus*);
- Growth and reproduction toxicity in the earthworm *Lumbricus rubellus.*

23.3.3 Sampling and analyses

Details of the sampling and analyses can be found in Derksen et al. (2008) and are further detailed below.

23.3.4 In vitro bioassays

No mechanism based *in vitro* bioassays were conducted.

23.3.5 In vivo bioassays

For bioassays, toxicity can be expressed in relation to effects in a standard reference medium (Mesman et al., 2011) or, if that is not available, in relation to uncontaminated soil from the same area or of the same soil type. In the case of Derksen et al. (2008), an unpolluted reference soil was used.

The results of the single species bioassays are as follows:

- In the springtail bioassay, negative effects on springtail reproduction were recorded at only one site: site 4 (VS4) with wet nutrient-poor grassland. This site has also the highest Zn concentration of all test sites;
- There were clear differences between sites in the hatching and growth of canola seeds. A negative trend between growth and hatching and Zn and Cd concentration was found;
- No statistically significant relation was found between earthworm survival and metal concentrations. An indication for toxic effects on earthworm survival was recorded at the most polluted site. Further, regression analysis showed a significant negative

relation between growth and reproduction and metal pollution (Zn and Cd);
- Together, the bioassays indicate that there are adverse effects at the most polluted sites. However, differences in soil properties (e.g. pH) represent a vastly confounding effect.

Derksen et al. (2008) do not explicitly identify a reference site (rather, regression analyses were used to link exposure to effects). However, unpolluted soils from the Dommel region were used in the bioassay experiments. Using those uncontaminated reference soils, for the present report it was attempted to scale the bioassay effects to a value between 0 and 1 so that different bioassays and methods can be compared. This was conducted according to the method described by Mesman et al. (2011). However, the methods for calculating these scaled values appeared not to fit in with the data described in Derksen et al. (2008) and in some cases, there appeared to be errors in the methods provided by Mesman et al. (2011). Therefore, no final scale values for the bioassay data were calculated.

23.4 Ecological Assessment Methods

23.4.1 Data collection

Derksen et al. (2008) also used Ecological Assessment Methods in the Dommel valley to assess potential toxic pressure. Specifically, they measured body/tissue concentrations of Cd, Zn and As in the following field-collected biota (As only for earthworms and insects):

- earthworms of the species *Lumbricus rubellus;*
- plants;
- insects.

These measurements were conducted at eight sites, four sites with flower-rich grassland and four with wet nutrient-poor grassland.

In addition, Derksen et al. (2008) predicted effects in the food chain through two modelling approaches: PODYRAS and BERISP. In both models, secondary poisoning in the food chain is based on the measured accumulation of metals in earthworms. The BERISP model also takes accumulation via the vegetation route into account. PODYRAS was used to estimate secondary poisoning risks for the black-tailed godwit and badger, whereas BERISP did this for the little owl and badger (via mice).

23.4.2 Selection of methods

See above.

23.4.3 Results

Concentrations in the earthworms and plants were compared to reference values derived from literature. These references values were based on earthworm populations inhabiting unpolluted areas. For plants, tissue concentrations were compared to cattle feed standards.

This resulted in the following results:

Cadmium:

- Regarding the tissue concentrations in earthworms: at two sites, these were within the reference range, at four sites they were <10x the reference range and at two sites they were >10x the reference values;
- Regarding the concentrations in plants: at five sites, concentrations were below the reference range, whereas at three sites concentrations exceeded the reference concentration by <10x;
- No reference values were available for insects.

Zinc:

- Regarding the tissue concentrations in earthworms: at six sites these were within reference range, at two sites these were <10x the reference range;
- Regarding the concentrations in plants: at all eight sites concentrations, they were below the reference range;
- No reference values were available for insects.

Arsenic:

- Regarding the tissue concentrations of earthworms: at four sites these were within the reference range, at three sites these were <10x the reference range and at one site these were >10x the reference values;
- No reference values were available for insects and plants.

For earthworms, tissue concentrations correlated well with soil concentrations, and in particular with the 0.01M $CaCl_2$-extracted fractions. Insect concentrations did, however, not relate to soil concentrations. Based on Cd and Zn concentrations and following existing guidance, the results show that there are no / low risks to cattle grazing in the polluted areas. Only at two of the most polluted sites, there is a slight exceedance of the acceptable tolerable daily dose (up to 1.3x).

On the basis of the cadmium concentrations found in earthworms collected from the field and using the two different models, Derksen et al. (2008) also estimated food chain effects:

- The model calculations with PODYRAS showed that the question whether there is a risk (of kidney damage) in the badger and the godwit strongly depends on the extent to which these animals use the study sites for feeding. In case of 100%, there are increased risks at every sampling site. In case of 10%, in one third of sites, there is a risk for badgers and in half of the sites there is a risk for the godwit;
- The BERISP model showed that at seven and eleven out of thirty sites there were no or low risks to the little owl or the badger, respectively. At all other sites there was an increased or high risk for these two animals.

On the basis of these accumulation and modelling studies, it can be concluded that for some areas in the Dommel valley, there is evidence of toxic pressure in earthworms and intermediate to high risks for effects through bioaccumulation in the food chain and secondary poisoning.

24 Analysis and interpretation

24.1 Collation of results

On the basis of the results discussed above, it can be concluded that:

1. On the basis of Chemical-Based Methods, there is evidence for toxic pressure because concentrations of metals exceed protective- as well as Intervention-related environmental standards in every area, but most in marshland and wet nutrient-poor grassland. The msPAF value in most sampled areas is exceeds 0.05, mostly driven by Cd, Zn and As, resulting in insufficient protection from direct effects of exposure on local species assemblages.
2. On the basis of Effect-Based Methods, there is evidence for toxic pressure because clear associations between metal concentrations in soil and effects on earthworms, canola seeds and springtails are recorded.
3. On the basis of Ecological Assessment Methods, tissue concentrations in earthworms exceed reference values by up to thirty times at some sites and at most sites, earthworm tissue concentrations are high enough to pose intermediate to high risks for secondary poisoning.

Summarising, in many areas of the Dommel valley, there is clear evidence that metal contamination (as a result of historic metal processing industrial activities) results in toxic pressure on species in the ecosystem.

24.2 Dominant substance groups

Only metals were investigated in the current assessment, so no conclusions can be drawn regarding other toxicants. However, among the metals, Cd and Zn were shown to cause most of the toxic pressure in the area.

24.3 Role of contaminants

In addition to metals, the biota in the area of concern may also be exposed to other chemicals such as pesticides that may reach the area through runoff or via the air. In the current assessment, only metals were considered, therefore, risks of other chemicals are unknown. Thus, this represents an uncertainty in the assessment of toxic pressure in the area of concern.

24.4 Mitigation measures

Suggestions for mitigation strategies are provided in Derksen et al. (2008).

References

Bongers, T. (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Brack, W., S.A. Aissa, T. Backhaus, V. Dulio, B.I. Escher, M. Faust, K. Hilscherova, J. Hollender, H. Hollert, C. Müller, J. Munthe, L. Posthuma, T.-B. Seiler, J. Slobodnik, I. Teodorovic, A.J. Tindall, G. de Aragão Umbuzeiro, X. Zhang & R.J.E.S.E. Altenburger (2019). Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. Environmental Sciences Europe 31: 10.

Buijs Agro-Services & Mantingh Environment and Pesticides (2020) Onderzoek naar de aanwezigheid van bestrijdingsmiddelen in vier Natura 2000 gebieden in Drenthe en de mogelijke invloed van de afstand van natuurgebieden tot landbouwgebieden op de belasting met bestrijdingsmiddelen. Report, March 2020, Assen/Bennekom, the Netherlands, 28 pp.

Chapman, P.M. (1986) Sediment quality criteria for Sediment Quality TRIAD – an example. Environmental Toxicology and Chemistry 5: 957-964.

Chapman, P.M. (1990) The Sediment Quality TRIAD approach to determine pollution-induced degradation. Science of the Total Environment 97/98: 815-825.

Chapman, P.M. (1996) Presentation and interpretation of Sediment Quality TRIAD data. Ecotoxicology 5: 327-339.

CLO. Compendium voor de Leefomgeving (2021) Gewasbeschermings-middelen in oppervlaktewater 2013-2019. Web site, visited on February 22, 2023. https://www.clo.nl/indicatoren/nl0547-gewasbeschermingsmiddelen-in-oppervlaktwater.

De Baat, M.L., M.H.S. Kraak, R. van der Oost, P. de Voogt & P.F.M. Verdonschot (2019a) Effect-based nationwide surface water quality assessment to identify ecotoxicological risks. Water Research 159: 434-443.

De Baat, M.L., N. Wieringa, S.T.J. Droge, B.G. van Hall, F. van der Meer & M.H.S. Kraak (2019b). Smarter sediment screening: effect-based quality assessment, chemical profiling, and risk identification. Environmental Science & Technology 53: 14479-14488.

De Gruijter, J., D. Brus, M. Bierkens & M. Knotters (2006) Sampling for natural resource monitoring. Springer-Verlag, Berlin/Heidelberg, 332 pp.

De Poorter, L.R.M., R. van Herwijnen, P.J.C.M. Janssen & C.E. Smit (2015) Handleiding voor de afleiding van indicatieve milieurisicogrenzen. Report 2015-0057, RIVM, Bilthoven, the Netherlands, 96 pp.

De Zwart, D. & L. Posthuma (2005) Complex mixture toxicity for single and multiple species: proposed methodologies. Environmental Toxicology and Chemistry 24: 2665-2676.

De Zwart, D., S.D. Dyer, L. Posthuma & C.P. Hawkins (2006) Predictive models attribute effects on fish assemblages to toxicity and habitat alteration. Ecological Applications 16: 1295-1310.

Derksen, J.G.M., J. Lahr, T. de Kort, T. Tuin, J.F. Postma, T.C. Klok, N.W. van den Brink, H.J. de Lange, S.A.E. Kools, A. van der Hout, J. Harmsen & J.H. Faber (2008) Ecologische effecten van metaalverontreiniging in het overstromingsgebied van de Dommel. Triade onderzoek, ecologische risico's en mogelijkheden voor inrichting en beheer. Grontmij-AquaSense/Alterra, Amsterdam/Wageningen, the Netherlands, 81 pp.

EC (2012). Directive 2012/18/EU of the European Parliament and of the Council of 4 July 2012 on the control of major-accident hazards involving dangerous substances, amending and subsequently repealing Council Directive 96/82/EC. European Commission, Brussels, Belgium.

EC (2014). Technical report on aquatic effect-based monitoring tools. Technical report no. 2014–077, European Commission, Brussels, Belgium, 83 pp.

EC (2018) Technical guidance for deriving environmental quality standards. Guidance Document no. 27, updated version 2018. Document endorsed by EU Water Directors at their meeting in Sofia on 11-12 June 2018. European Commission, Brussels, Belgium, 207 pp.

Escher, B., P. Neale & F. Leusch (2021) Bioanalytical Tools in Water Quality Assessment, 2nd ed. IWA Publishing, London, 462 pp.

Füssel, H. M. & R.J. Klein (2006) Climate change vulnerability assessments: an evolution of conceptual thinking. Climatic change 75: 301-329.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson & H. de Kroon (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE 12: e0185809.

Hallmann, C. A. & E. Jongejans (2021) Long-term trends in aquatic insects in the Netherlands. Report no. 2021-39, STOWA, Amersfoort, the Netherlands, 93 pp.

Hoondert, R.P.J., R. Oldenkamp, D. de Zwart, D. van de Meent & L. Posthuma (2019) QSAR-based estimation of species sensitivity distribution parameters: an exploratory investigation. Environmental Toxicology and Chemistry 38: 2764-2770.

Klepper, O. & D. Van De Meent (1997) Mapping the potentially affected fraction (PAF) of species as an indicator of toxic stress. Report no. 607504001, RIVM, Bilthoven, the Netherlands, 93pp.

Knotters, M., D. Brus & J. de Gruijter (2009) Hoezo representatief? Over de betekenissen van 'representatief' in de KRW-literatuur. Stromingen 15, no.1, 3-8.

Kruijne, R., M. Wenneker, M. Montforts, J. de Weert & A. van Loon (2020) Analyse van de bijdrage van verschillende emissieroutes van gewasbeschermingsmiddelen aan de waterkwaliteit. Report no. 2020-12, STOWA, Amersfoort, the Netherlands, 107 pp.

Lahr, J., C. Moermond, M.H.M.M. Montforts, A. Derksen, N. Bondt, L. Puister-Jansen, T. de Koeijer, P. Hoeksma (2019) Diergeneesmiddelen in het milieu: een synthese van de huidige kennis. Report no. 2019-26, STOWA, Amersfoort, the Netherlands, 117 pp.

Lemm, J. U., M. Venohr, L. Globevnik, K. Stefanidis, Y. Panagopoulos, J. van Gils, L. Posthuma, P. Kristensen, C. K. Feld, J. Mahnkopf, D. Hering & S. Birk (2021) Multiple stressors determine river ecological status at the European scale: towards an integrated understanding of river status deterioration. Global Change Biology 27: 1962-1975.

Malaj, E., P. C. von der Ohe, M. Grote, R. Kühne, C. P. Mondy, P. Usseglio-Polatera, W. Brack & R. B. Schäfer (2014) Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proceedings of the National Academy of Sciences 111: 9549–9554.

McCarty, L. S., J. A. Arnot & D. Mackay (2013) Evaluation of critical body residue data for acute narcosis in aquatic organisms. Environmental Toxicology and Chemistry 32: 2301-2314.

Mesman, M., A.J. Schouten, M. Rutgers & E.M. Dirven-van Breemen (2007) Handreiking TRIADE. Locatiespecifiek ecologisch onderzoek in stap drie van het Saneringscriterium. Report no. 711701068/2007, RIVM, Bilthoven, 53 pp.

Mesman, M., A.J. Schouten & M. Rutgers (2011) Handreiking TRIADE 2011. Locatiespecifiek ecologisch onderzoek in stap drie van het Saneringscriterium. RIVM report no. 607711003/2011, 67 pp.

Mesman M., M. Rutgers, A.J. Schouten, J.J. Bogte & E.M. Dirven-van Breemen (2014) Evaluatie van Triade-onderzoeken op schietterreinen van Defensie. Report no. 2014-0077, RIVM, Bilthoven, the Netherlands, 92 pp.

Mol, G., J. Spijker, P. van Gaans, & P. Römkens, eds. (2012). Geochemische bodematlas van Nederland. Wageningen Academic Publishers, Wageningen, the Netherlands, 275 pp.

Natuur & Milieu (2023) Hoe giftige bestrijdingsmiddelen onze natuur bedreigen. Bestrijdingsmiddelen in en om natuur- en recreatiewater. Report, August 2023, Stichting Natuur & Milieu, Utrecht, the Netherlands, 27 pp.

Neale, P.A., G. Braun, W. Brack, E. Carmona, R. Gunold, M. König, M. Krauss, L. Liebmann, M. Liess, M. Link, R.B. Schafer, R. Schlichting, V.C. Schreiner, T. Schulze, P. Vormeier, O. Weisner & B.I. Escher (2020) Assessing the mixture effects in *in vitro* bioassays of chemicals occurring in small agricultural streams during rain events. Environmental Science and Technology 54: 8280−8290.

O'Brian, R. M. (2007) A caution regarding rules of thumb for variance inflation factors. Quality & Quantity 41: 673-690.

OSPAR (1992) Convention for the protection of the marine environment of the North-east Atlantic. OSPAR Commission, London, UK. https://www.ospar.org/convention

PBL (2020) Nationale Analyse Waterkwaliteit. PBL, The Hague, the Netherlands, 232 pp.

Poikane, S., F. Salas Herrero, M.G. Kelly, A. Borja, S. Birk & W. van de Bund (2020) European aquatic ecological assessment methods: a critical review of their sensitivity to key pressures. Science of The Total Environment 740: 140075.

Posthuma, L. & D. De Zwart (2006) Predicted effects of toxicant mixtures are confirmed by changes in fish species assemblages in Ohio, USA, rivers. Environmental Toxicology and Chemistry 25: 1094-1105.

Posthuma, L., G.W.I. Suter II & T.P. Traas (2002) Species sensitivity distributions in ecotoxicology. CRC-Press, Boca Raton, FL, U.S.A., 616 pp.

Posthuma, L., D. de Zwart, E. Brand, D. van de Meent, H. van Wijnen & H. Den Hollander (2012) Beoordeling risico's gevaarlijke stoffen voor natuurgebieden: grondslagen en randvoorwaarden. Report no. 620550006, RIVM, Bilthoven, 73 pp.

Posthuma, L., D. de Zwart & S. D. Dyer (2019a) Chemical mixtures affect freshwater species assemblages: from problems to solutions. Current Opinion in Environmental Science and Health 11: 78-89.

Posthuma, L., J. van Gils, M.C. Zijp, D. van de Meent & D. de Zwart (2019b) Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12 386 chemicals. Environmental Toxicology and Chemistry 38: 905-917.

Posthuma, L., R. Altenburger, T. Backhaus, A. Kortenkamp, C. Müller, A. Focks, D. de Zwart & W. Brack (2019c) Improved component-based methods for mixture risk assessment are key to characterize complex chemical pollution in surface waters. Environmental Sciences Europe 31: 70.

Posthuma, L., W. Brack, J. van Gils, A. Focks, C. Müller, D. de Zwart & S. Birk (2019d). Mixtures of chemicals are important drivers of impacts on ecological status in European surface waters. Environmental Sciences Europe 31: 71.

Posthuma, L., M.C. Zijp, D. de Zwart, D. van de Meent, L. Globevnik, M. Koprivsek, A. Focks, J. van Gils & S. Birk (2020) Chemical pollution imposes limitations to the ecological status of European surface waters. Scientific Reports 10: 14825.

Postma, J., R. Keijzers, J. Slootweg & L. Posthuma (2021) Toxiciteit van Nederlandse oppervlaktewateren in de periode 2013-2018. Report no. 2021-43, STOWA, Amersfoort, the Netherlands, 77 pp.

Rutgers, M., C. Mulder , A.J. Schouten, J. Bloem, J.J. Bogte, A.M. Breure, L. Brussaard, R.G.M. de Goede, J.H. Faber, G.A.J.M. Jagers op Akkerhuis, H. Keidel, G.W. Korthals, F.W. Smeding, C. ten Berg & N. van Eekeren N. (2007) Soil system profiling in the Netherlands with ten references for biological soil quality. Report no. 607604008/2007, RIVM, Bilthoven, the Netherlands, 96 pp.

Rutgers, M. J., J. Tuinstra, J. Spijker, M. Mesman, A. Wintersen & L. Posthuma (2008) Risico's voor het ecosysteem in stap 2 van het Saneringscriterium. RIVM report no. 711701072/2008, RIVM, Bilthoven, the Netherlands, 75 pp.

Schäfer, R. B., M. Jackson, N. Juvigny-Khenafou, S. E. Osakpolor, L. Posthuma, A. Schneeweiss, J. Spaak & R. Vinebrooke (2023) Chemical mixtures and multiple stressors - same but different? Environmental Toxicology and Chemistry 42: 1915-1936.

Schouten, A. J., J. Bloem, W. Didden, H. Keidel, & M. Rutgers (2003) Bodembiologische Indicator 1999. Ecologische kwaliteit van graslanden op zandgrond bij drie categorieën melkveehouderijbedrijven. Report no. 607604003, RIVM, Bilthoven, the Netherlands, 107pp.

Solomon, K.R., T. Brock, D. de Zwart, S.D. Dyer, L. Posthuma, S. Richards, H. Sanderson, P. Sibley & P. J. Van den Brink, eds. (2008) Extrapolation practice for ecotoxicological effect characterisation of chemicals. CRC-Press, Boca Raton, FL, U.S.A., 408 pp.

RLI (2020). A grip on hazardous substances. Raad voor Leefomgeving en Infrastructuur (Council for the Environment and Infrastructure), The Hague, the Netherlands, 88pp.
https://www.rli.nl/sites/default/files/a_grip_on_hazardous_substances_-_engelse_vertaling.pdf

Spijker, J., G. Mol & L. Posthuma (2011) Regional ecotoxicological hazards associated with anthropogenic enrichment of heavy metals. Environmental Geochemistry and Health 33: 409-426.

Suter, G.W., L.W. Barnthouse, S.M. Bartell, T. Mill, D. Mackay & S. Patterson (1993) Ecological Risk Assessment. Lewis Publishers. Boca Raton, FL, U.S.A., 560 pp.

Swart, E., J. Slootweg & L. Posthuma (in prep.) A database of curated ecotoxicity data, for use in environmental protection, life cycle assessment and environmental quality assessment.

Tiktak, A. (2019) Gewasbeschermingsmiddelen en de realisatie ecologische kwaliteit van oppervlaktewater 2018. Report no. 3878, PBL, The Hague, the Netherlands, 10pp.

Turner, B.L., R.E. Kasperson, P.A. Matson, J.J. McCarthy, R.W. Corell, L. Christensen, N. Eckley, J.X. Kasperson, A. Luers, M.L. Martello, C. Polsky, A. Pulsipher & A. Schiller (2003). A framework for vulnerability analysis in sustainability science. Proceedings of the national academy of sciences 100: 8074-8079.

Van der Oost, R., G. Sileno, M. Suárez-Muñoz, M.T. Nguyen, H. Besselink & A. Brouwer (2017) SIMONI (Smart Integrated Monitoring) as a novel bioanalytical strategy for water quality assessment: Part I–model design and effect-based trigger values. Environmental Toxicology and Chemistry 36: 2385-2399.

Van Klink, R., D.E. Bowler, K.B. Gongalsky, A.B. Swengel, A. Gentile & J.M. Chase (2020) Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. Science 368: 417–420.

Van Noordwijk, J. (1989) Bioassays in whole animals. Journal of Pharmaceutical and Biomedical Analysis 7: 139-145.

Van Straalen, N.M. & C.A.J. Denneman (1989). Ecotoxicological evaluation of soil quality criteria. Ecotoxicology and Environmental Safety 18: 241-251.

Visser, M.D., M. van ’t Zelfde, C. Hallmann & C. Barmentlo (2023) Analyse aanwezigheid, normoverschrijdingen en toxische druk van bestrijdingsmiddelen in geselecteerde meetpunten binnen het Nederlands oppervlaktewater. Institute of Environmental Sciences (CML), Leiden, 50 pp. ISBN: 978-90-5191-208-1.

VROM/VW (2017) Regeling bodemkwaliteit. Regeling van 13 december 2007, nr. DJZ2007124397. Staatscourant 20 december 2007, no. 247, p. 67.

Verhagen, F.T., A. Holsteijn & M. Schipper (2018) Feitenrapport brede screening bestrijdingsmiddelen en opkomende stoffen Maasstroomgebied 2016. Report no. ATBF1729R001D00, RoyalHaskoningDHV Nederland B.V., Nijmegen, the Netherlands, 201 pp.

Wagelmans, M.H.A.B., J.G.M. Derksen, D. Lud, M. Mesman & J.H. Faber (2010) 10 jaar Triade: een evaluatie. Bodem 2, april 2010: 18-20.

Wang, Z., G.W. Walker, D.C.G. Muir & K. Nagatani-Yoshida (2020) Toward a global understanding of chemical pollution: a first comprehensive analysis of national and regional chemical inventories. Environmental Science & Technology 54: 2575–2584.

Warne, M.S.J., C. Neelamraju, J. Strauss, R. Smith, R. Turner & R. Mann (2020) Development of a method for estimating the toxicity of pesticide mixtures and a pesticide risk baseline for the Reef 2050 water quality improvement plan. Department of Environment and Science, Queensland Government, Brisbane, Australia, 233 pp.

Wolters, M.L. & C. Kuenzer (2015) Vulnerability assessments of coastal river deltas-categorization and review. Journal of Coastal Conservation 19: 345-368.

Zacharias, M.A. & E.J. Gregr (2005) Sensitivity and vulnerability in marine environments: an approach to identifying vulnerable marine areas. Conservation Biology 19: 86-97.

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