Key Factor Toxicity

User Guidance

Toxic Pressure calculation tool (version 2.0)

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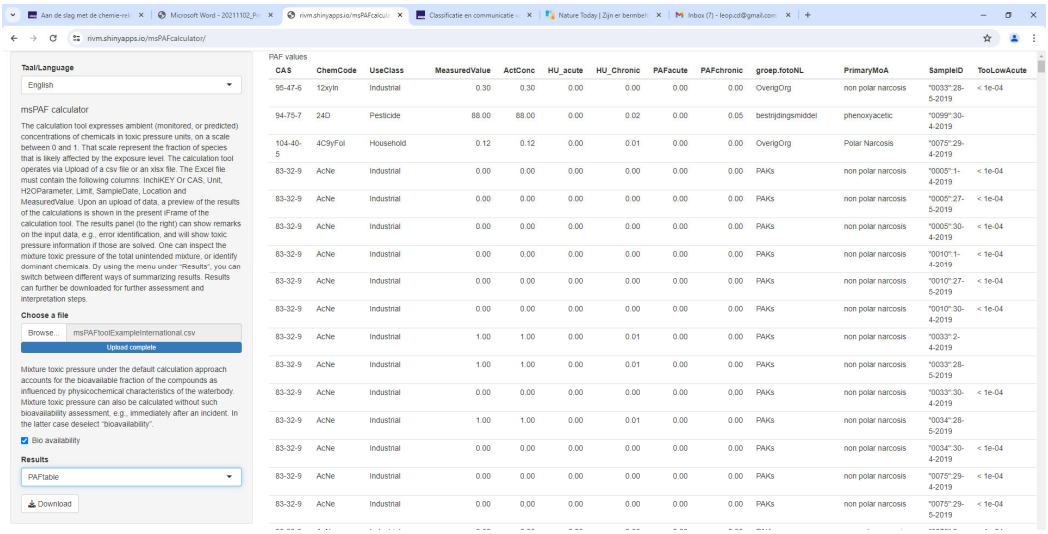
Highlights

1. The Key Factor Toxicity is one of a broader set of Key Factors, which together compose a system designed and employed in the Netherlands to systematically execute a water quality evaluation in a 'water system analysis' in the context of the EU-Water Framework Directive (WFD).
2. The calculation tool allows water quality assessors to quantify the toxic pressure of individual compounds, compound groups and total mixtures, based on bioavailable (default) or total (if so desired) concentrations of individual chemicals.
3. Toxic pressure differences are expressed both scientifically (on a scale between 0 and 1) and practically (in five classes of chemical pollution which relate to the associated damage to the ecological status, defined in alignment with water quality definitions of the EU-WFD).
4. The purpose of toxic pressure assessment and chemical pollution classification is to determine the necessity and priority of measures to achieve the societal targets that motivated the rules in the WFD (good water quality), via protection (if possible) or restoration (if necessary) of good water quality.
5. The Key Factor Toxicity consists of two tracks, referred to as the "Chemistry-" and the "Bioassay"-track. Employing different techniques in water quality assessment, both tracks result in classes that have an interpretation that an increase in the degree of pollution implies an increased limitation to maintain or restore a good ecological status.
6. The calculation tool is available on the internet. (https://www.sleutelfactortoxiciteit.nl/key-factor-toxicity-introduction), and the use of the calculation tool is described in this manual.

Graphical summary



The start screen of the web application, after entering a set of water quality data. The user chooses an input file (input csv or Excel, left), and runs the calculation, with various results (preview, right). Output can be exported for further characterization of chemical pollution.

Release Notes (version 2.0)

Version 2.0 of the SFT2-calculation tool differs from Version 1.0 of the tool, which was published as Dutch-only tool in 2021 on the website www.sleutelfactortoxiciteit.nl. That first version was made in the context of a Dutch stimulation program aimed at improved theoretical and practical approaches under the EU-Water Framework Directive (EU-WFD). Dutch water quality managers have used that first version of the tool for a while, and the present second version was released for various reasons.

The main reason is for the release of Version 2.0 is to allow for Europe-wide use. One of the most important principles of the EU-WFD is that it is based on the hydrological network structure, which implies that water quality management is organized according to the scaling principle of river basins, sub-basins, etc. Priority pollutants are compounds that affect water quality throughout Europe, and are thus a key responsibility starting at the EU-level. River-basin specific pollutants are compounds that affect water quality in a specific river basin, and are thus a key responsibility of river basin authorities, which are often trans-national. Upon recognizing that a Dutch-only version, used by Dutch water quality managers, would neglect the international relevance of the upstream areas of the four major river basins (Rhine, Meuse, Scheldt and Ems) and smaller rivers, it was decided to prepare Version 2.0 as English-language calculation tool. This allows international collaboration in the aforementioned smaller and larger basins affecting Dutch water quality, but also use elsewhere in Europe. For this reason, the calculation tool Version 2.0 has now a choice-menu option, whereby the user can select Taal/Language "English", and whereby calculations and a standard input format in Excel are provided. In the calculations, all internationally common ways for decimal separators and alike have been accounted for. Despite such technical differences, the input and output of the Dutch and English versions of the calculations are the same.

The second reason for the preparation and release of Version 2.0 concerns some small improvements, based on feedback from practice. The most important improvement is a more explicit visualisation (in the results) of outcome values of toxic pressure (PAF) that are considered negligible. If a PAF-value is calculated as 0.01, this means that 1 in 100 species would be affected, but if there are (far) less than 100 species present in reality, such low numbers have no specific ecological interpretation. Moreover, the statistical confidence of toxic pressure estimates in the lower tail of the sigmoidal model that is used are less certain. Both reasons motivated the use of a value below which PAF-values are considered negligible. Further, at specific request of various water quality managers, the Version 2.0 calculation tool allows to deviate from the *default* approach of accounting for water quality parameters that affect the bioavailable fraction of chemicals. By un-checking the "bioavailability" box, Version 2.0 allows to quantify the toxic pressure e.g. just after the incidental release of chemicals.

Kennisimpuls WATERKWALITEIT

Table of Contents

Summary

The toxic pressure of chemicals, subgroups of chemicals or total mixtures can be derived with a web-based calculation tool, the basics and use of which are presented in the present report. Toxic pressure is a metric that quantifies in how far chemical pollution acts as a limiting factor to maintaining or restoring a good ecological status. The toxic pressure metric informs water quality managers and stakeholders on the 'distance to target' to an un- or minimally polluted status, providing nuanced insights into pollution as compared to the Water Framework Directive "one out, all out" classification.

Chemical pollution of surface waters is a frequently occurring phenomenon, Characterization of chemical pollution is usually based on evaluating Risk Quotient (RQ) values that are defined as the (summed) ratios of the concentration- and the protective Environmental Quality Standard(s) (EQSs) of a chemical (mixture). The resulting (sum) RQ- metric is often poorly interpretable due to the diverse natures of the protective standards. That is, such Standards are based on the most sensitive endpoint (human health, ecosystem integrity due to direct effects of exposures, and ecosystem impacts caused by secondary poisoning), combined with a data-quality and -quantity related Safety Factor. Exceedance of an EQS has thus no directly clear meaning in terms of ecological impacts, though (sum)RQ>1 implies chemical pollution at a level above the set safety standards. This effect is also present in the outcome of the "one out, all out" rule of the Water Framework Directive, which classifies water as 'failure to reach good status' if the value of one or more water quality parameters is not in compliance with the value representing good quality. In short, these metrics identify *that* water quality deviates from good. Beyond that, there is the option to characterize *the degree of* pollution with a nuanced and ecologically meaningful metric that quantifies the degree of pollution, related to the degree of damage to biodiversity. The nuanced metric is named 'toxic pressure' or 'mixture toxic pressure', and it is expressed as Potentially Affected Fraction (PAF) or multi-substance PAF (msPAF).

For practical water quality management, it is important to be able to quantify the (mixture) toxic pressure. This is because an increase of this metric implies an increased limitation to keep a good ecological status, or restore that status if a water system is affected. The toxic pressure metric expresses *how much* the ecological status is likely affected by the local pollution. Therefore, the (mixture) toxic pressure metric can assist practical water quality management by ranking water bodies with respect to the degree of impacts caused by chemical pollution, as well as ranking the relative contributions of different chemicals within those water bodies. Both nuanced outcomes help prioritization of management actions, first to priority sites, then to priority chemicals within sites.

To support practical water management, a calculation tool to quantify the local toxic pressure of the unintended mixtures that are present in surface water samples is provided in the present report. The tool based on the theory summarized in the book on Species Sensitivity Distributions in Ecotoxicology and the large-scale use of that for European and Dutch surface water systems. The calculation tool operates in various steps, based on measured or predicted water concentrations of pollutants. The steps are (1) *(default)* calculation of bioavailable- from total concentrations, based on physicochemical characteristics of the water body (the *non-default* approach allows to calculate the toxic pressure without bioavailability correction, such as e.g. after emissions of chemicals from a sudden incident), (2) calculation of the toxic pressure per compound, and (3) calculation of the mixture toxic pressure of multiple chemicals. The calculation tool uses default values for physicochemical characteristics when they are missing, which are currently based on typical Dutch water systems.

The present report is a User Guidance as well as a source of information on the principles and practices of the calculation tool. The guidance describes how to collect concentration data on chemicals, how to prepare those for the analyses, how to execute the calculations, and how to eventually interpret the final results on toxic pressure outcomes.

1 Introduction

The toxic pressure calculation tool is part of a set of methods that has been designed for water quality characterization in a water systems analysis (On the system of Key Factor (in Dutch)). The calculation tool can be used via the link: https://www.sleutelfactortoxiciteit.nl/key-factor-toxicity-introduction.

The calculation tool yields a quantitative metric that represents the toxic pressure of substances and their mixtures based on the bioavailable (*default*) or total (if so desired) concentration(s) of the substance(s) in surface water. The total-concentration calculations can be used, for example, to quantify toxic pressure after an incident, when the bioavailability of suddenly released chemicals is highest. The results can be used to determine the toxic pressure of a mixture of substances within a specific substance group (e.g., insecticides) or of all substances combined.

Insight into the (mixture) toxic pressure in a water system is important, because this parameter is related *to the degree of impairment* of the aquatic ecosystems (and the Ecological Status class as defined in the European Union Water Framework Directive): the higher the value, the greater the impairment, and the distance to good water quality, and the greater the need for corrective measures.

The present report is written as a User Guidance to support the use of the toxic pressure calculation tool and the interpretation of its outcomes. Chapter 2 describes the context of the use of the calculation tool. Chapter 3 describes how to use the calculation tool. Chapter 4 illustrates how the results of the calculation tool are summarized and interpreted.

2 The context of using the calculation tool

2.1 Overview

The calculation tool is meant to be used for water quality protection, assessment and management, to enable water managers to characterize, and rank, water bodies with respect to the degrees and kinds of chemical pollution. The EU-Water Framework Directive stipulates that water quality management can use monitoring data *and model results* to evaluate whether protective or remediation action is needed to safeguard or restore good water quality status, and – if needed – which actions should be prioritized (see WFD-Annex II, Article 1.5). The calculation tool was designed to provide model results that are relevant for practical water quality protection, assessment and management, in line with Annex II.

The calculation tool is meant to support prioritization of programs of measures, by calculating the local toxic pressure of chemicals, chemical groups and/or whole mixtures, for an array of water bodies in a management area. Explicitly, the calculation tool enables considering any unintended mixture that poses harm to aquatic life, provided that the chemicals of which the mixture is composed are accounted for in both the monitoring and the calculations. This is in line with the definition of 'pollutants' in the WFD. The WFD defines 'specific pollutants' as any chemical that poses harm (Article 2.33).

It is, so far, mostly common to consider chemical pollution under the WFD in separate approaches, and on a per-chemical basis, namely:

1. For individual *priority substances*, which are chemicals that have a Europe-wide occurrence and potential to cause harm.
2. For individual *river-basin specific pollutants*, which are chemicals that have a river-basin wide occurrence and potential to cause harm.
3. For individual *substances identified via a watch-list mechanism*, aimed to identifying additional substances of greatest concern.
4. For individual *chemicals monitored or considered for other reasons*

The assessment of (mixture) toxic pressure adds to this per-chemical approach, by enabling the *consideration of mixtures of any composition of chemicals*, whether they are on any of the aforementioned lists or not. This is based on the scientific principle that the net impacts of unintended mixtures on biodiversity and water quality are caused by the effect-contributions of all compounds in the local unintended mixture, irrespective of them being on any of the lists or not.

There are three very important considerations for applying the calculation tool:

1. Consider the appropriate scale:
   Use the calculation tool in a water system analysis, for multiple sites or multiple moments in time, because the comparison of results help in various ways to rank pollution problems, and thereupon to prioritize and focus measures, or evaluate the efficacy of measures taken in the past.
2. Consider the appropriate set of compounds (representativeness):
   Enter the concentrations of the hazardous substances that are present in a water body, and that can be considered as a (potential) 'pollutant' as defined under the WFD[1]; in cause of doubt, monitor the compound, and evaluate whether the toxic pressure is negligible or not.

[1] WFD Article 2, "Definitions", clause 31: "Pollutant means any substance liable to cause pollution, in particular those listed in Annex VIII" and clause 33 "Pollution means the … introduction … of substances … into the air, water or land which may be harmful to human health or the quality of aquatic ecosystems or terrestrial ecosystems directly depending on aquatic ecosystems…".

3. Consider the interpretation sequence:
   The interpretation (most often) begins by identifying and ranking sites from higher to lower toxic pressures, as these results aid in prioritizing measures across water bodies. Only after this, the focus shifts to compounds within these sites that contribute most to the local toxic pressure.

The explanation of these points is given in the following paragraphs.

2.2 Scale: a water system level analysis approach

The calculation tool of the Key Factor Toxicity is used in a water system analysis. This is entirely in line with the basic principle of the Water Framework Directive (WFD), in which the starting point of assessment and management is the hydrological system.

The calculation tool therefore provides insight into the differences in toxic pressure of substances, groups of substances and entire mixtures within the framework of the hydrological system. The calculation tool provides insights into the differences in (mixture) toxic pressure between hydrological units, or during the different periods in a year or other chosen period. The results provide insight into where the toxicity is most restrictive for biodiversity (a comparative question), which is reflected in five classes of degree of chemical contamination. In an ecological sense, no distinction is made between substance groups that are considered specifically in the Water Framework Directive, such as priority substances or river-basin specific substances, Watch List substances (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32022D1307&qid=1658824912292) or any other (list of) substances. After all, it is the local mixtures of the locally occurring compounds (irrespective of their presence on a list) that determine the degree of restriction to biodiversity.

> Note: For the purpose of obtaining specific insights, in the context of specific obligations under the EU-WFD, the user may choose to exclusively import concentration data for certain substance groups, e.g., only Priority Substances that are defined as compounds of Europe-wide concern. This approach allows water managers to gain insight into the toxic pressure of the chemicals that have an impeding effect throughout Europe, and which is also subject to a European level policy control approach. It could also be used for River Basin Specific Pollutants, which are pollutants that have an impeding effect in a certain river basin. The calculation tool can be used to quantify the (mixture) toxic pressure for any chemical or mixture of interest. Water quality managers can choose for any input (measured or predicted concentrations of all or selected compounds) and evaluate any output (toxic pressure per compound, per compound group, or of the total mixture of monitored chemicals; toxic pressure trends over time, etc.).

The results of the calculation tool provide insight into the question of which hydrological units are most heavily affected. For those units the tool also provides insights into which substance groups make a strong local contribution. Both types of results are helpful in identifying the need to consider measures, and in selecting and prioritizing them. Figure 1 shows a typical example of the results of a water system analysis for chemical contamination in the form of a spatial map, with colour codes reflecting the degree to which chemical pollution caused biodiversity impacts.

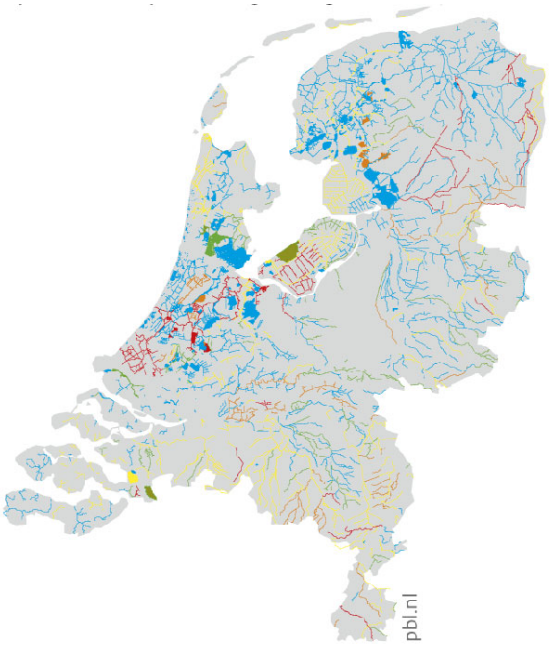


Figure 1. Example of the final results of a water system analysis with the calculation tool for surface waters of the Netherlands. Grey area: land mass. Coloured areas: delineation of the hydrological system (rivers, lakes, etc.). The various colours represent different degrees of impairment of aquatic life caused by chemical pollution, based on calculated values of the toxic pressure of local mixtures. The colour codes in this example map differ from those developed for the SFT2-calculation tool (version 2.0), whereby the five classes were calibrated to the ecological status classes: see further Table 1. Figure source: PBL (2020).

2.3 Representativity: considering all locally occurring substances

The calculation tool quantifies toxic pressure on the basis of concentrations of substances in the water system. These can be measured substances (monitoring) or predicted substance concentrations (modelling), often summarized as PEC and MEC (Predicted and Measured Environmental Concentration, respectively). The user can select to base the calculations on the bioavailable fraction of the chemicals (default) or on the total concentrations (if so desired, e.g., after an incident).

It is important that the mixture toxic pressure is calculated with *all* substances that occur locally or may occur locally. After all, substances that are not measured (MEC) or modelled (PEC) whilst (likely) present cannot contribute to the toxic pressure outcomes from the calculation, whilst they still cause toxic effects. In other words: it is always a good strategy to list the compounds that are emitted from local land uses, and combine those with compounds emitted in upstream water bodies and with compounds on the lists of EU-wide Priority Substances and the pertinent River Basin Specific Pollutants for the study area, and evaluate the concentrations of all those compounds, per chemical, per chemical group or for the total mixtures.

> Note: the calculated toxic pressure of substance groups or mixtures is always an underestimate of the actual toxic pressure. The degree of underestimation is determined by the degree to which ecotoxicologically dominant chemicals are lacking as part of the input data.
>
> Note: Having “missing compounds” is unfortunate, but not a disqualification for prioritizing measures. That is, if for example four substances together produce a strongly increased toxic

pressure (even if 10 other substances may contribute, but have not been measured), then the measures taken against those four substances (on the basis of the insight that is given on their toxic pressure) can already lead to significant improvements in water quality. Nevertheless, it remains important and logical to strive for the calculations with the calculation tool to be representative of the toxic pressure in the aquatic system. This means that the water manager must strive for the best possible assessment of the substances that can occur locally.

There are two remedies that can be used in the assessment to reduce the problem of non-measured substances:

1. Use a lookup table "land use - list of substances" (as presented in the website on the Dutch pages: https://www.sleutelfactortoxiciteit.nl/aan-de-slag/de-pressure-van-dpsir, select "Download de Landgebruik-Stoffenlijst opzoektabel" to download the look-up table that lists potentially emitted chemicals for a wide array of land uses). By looking at local (and upstream) land use, and reading off the associated substances, there is a good chance that all relevant substances will be measured.

   Note: The list of substances that may occur locally consists of the sum of four lists:
   a. The substances commonly found in Europe, and thus also possibly at the specific site under investigation (the Priority Substances)
   b. The substances that are often found in the basins of the major river, in which the specific site is positioned (the River-Basin Specific Pollutants),
   c. The Watch List substances
      and
   d. The locally or regionally relevant substances, which do pose a problem due to the local forms of land use in the region, but are not so on the scale of the basin or Europe, or candidate on the Watch List. The aforementioned lookup table provides suggestions for this part of the compound list.

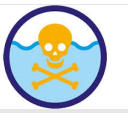
2. Summarize the results for substance groups separately, instead of just the toxic pressure of the total mixtures. This helps to derive an operationally useful interpretation, because – for example – a subgroup of compounds is always consistently measured (such as an array of 16 PAH-compounds), whilst it also provides immediate insight into the potential usefulness of measures that can be envisaged to be taken against the separate groups. This clarifies the results and also provides insight into the potential effectiveness of measures.

To illustrate this we refer to the example of measuring of PAHs and pesticides (plant protection products PPP). For PAHs we always measure all 16 of them, while for PPPs the monitoring of individual pesticides varies over time, for various practical reasons. For the PPPs we know, for example, that substance use varies per season and crop. When all these substances are considered together, the mixture's toxic pressure can fluctuate significantly, depending on the PPP-measurements (as they may or may not cover the substances used in that particular season). The results can vary greatly, and it is difficult to determine whether PAHs also show variability. Separating the measurement of PAHs and PPPs enhances our understanding of the significance of the results, especially if it is known (or clear from the data) that the group of PAHs is measured consistently.

2.4 Interpretation

It is important to use various rules of thumb when interpreting outcomes of toxic pressure calculations:

1. Start at the top:
   Begin by prioritizing remedial action at the highest toxic pressure locations. At those locations, measures make the most sense, as there the impediment of biodiversity is largest.



2. Avoid over-interpretations that have no ecological significance:
   Interpret the 'big picture', which entails analysing the rank-ordering of likely biodiversity impacts as quantified with the toxic pressure metric. Consider that the outcomes can identify, e.g., some major groups of practical interest (high impacts, no impacts, and the intermediate group), whereby management efforts on the former and the latter group are clear (priority for sanitation or protective management).
3. Do not interpret (very) low calculated values of the toxic pressure more precisely than is logically possible. For example, if there are 100 species of insects in an area, interpreting a toxic pressure (PAF or msPAF) <0.01 is not meaningful. After all, the value 0.01 indicates "1 in 100 species would be affected", and a calculated value of 0.001 simply means "very low pressure" and (probably) no substantial impact on biodiversity of aquatic life.

While adhering to general rules of thumb, it's essential to note that exceptions may emerge upon closer examination and because of other types of considerations. Rule of Thumb-1 suggests that addressing the highest pollution class should take precedence when implementing measures. However, this may entail complex or cost-prohibitive solutions for the highest-ranking site. Practical considerations often lead to prioritizing measures that can be more readily and affordably implemented, e.g., not the whole budget to the top-1 site, but the same budget for the 10% of top-ranking sites with less-costly measures and more expected improvement for those sites. It is the overall picture (according to the principle of water system analysis) that leads to fully weighted (science + practice) priority ranking. Thus, the final priority of measures taken also relates to the feasibility and cost of implementation of measures as evaluated by the water quality managers.

2.5 Toxic pressure as classes of chemical pollution

The calculation tool provides output data that give insight into the toxic pressure of individual substances, substance groups or their total mixtures. Because the water system exploration is intended to ultimately be able to deduce in a simple way whether, where and against which compounds specific measures are needed, the output of the calculation tool is summarized in classes. Therefore, five classes of chemical pollution have been derived, corresponding to the five classes of the WFD-Ecological Status. That is, the numerical levels of calculated (mixture) toxic pressure outcomes have been calibrated to Ecological Status class information.

When defining the five classes, the data from two toxic pressure measures (both calculated with the tool) are combined:

- The chronic toxic pressure is calculated as the fraction of species exposed above the *no-effect* level (NOEC) under chronic exposure; the exposed species here experience *a beginning of nuisance*, with an onset of effects on characteristics such as growth and reproduction (a toxic pressure increase does not immediately mean species loss).
- Acute toxic pressure is calculated as the fraction of species exposed above EC50 levels, for which empirical studies have shown that a rising msPAF-EC50 value is associated with an increasing species loss, and a stronger impediment to Ecological Status.

The concept of classification by classes of chemical pollution is illustrated in Figure 2. This figure shows that the ecological state (Y) can have all kinds of values (the points), due to multiple stress. The sigmoid line of all these observations is the so-called 'quantile regression', the line that describes the 'outer envelope' of the observations as a function of the potential pressure variable 'X', and that lines means the following:

- if there are no observations 'top right', this means that the factor on the X-axis at elevated values of X is a limiting factor for Y (Cade and Noon, 2003).

Since that effect has indeed been observed, it has become clear that the toxic pressure of mixtures was thus indeed found to be limiting to the Ecological Status (Posthuma et al., 2020; Postma et al., 2021).

Based on the principles of classifying Chemical and Ecological Status of the EU-WFD as well as the observed calibration of toxic pressure levels with degree of ecological impact, the following chemical pollution classes are discerned (**Error! Reference source not found.**).

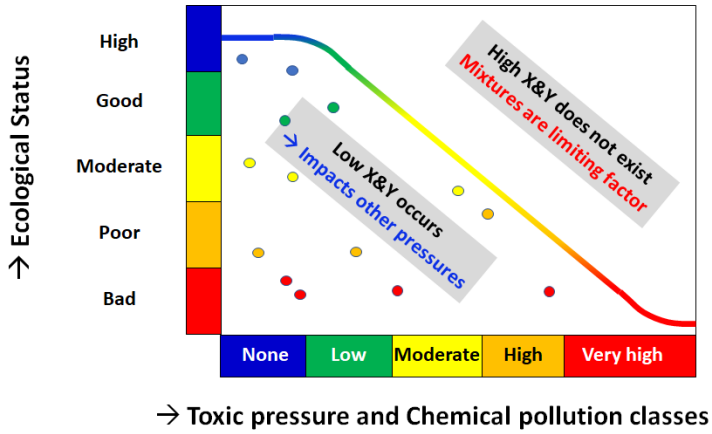


Figure 2. The concept of the classification of chemical pollution into five classes (X), including calibration to the Ecological Status classes (Y). The higher the degree of pollution, the more strongly the Ecological Status is impeded. That the observations (dots) are not on the sigmoidal curve is caused by other pressures (e.g., an "unpolluted" site can have a 'red dot' due to another pressure, as shown in the lower-left corner).

- The class **None**, coloured 'blue', is characterized by the absence of human influences (or those are minimal or negligible), and thus has no or negligible increased toxic pressure (very low msPAF-NOEC).
- The class **Low**, coloured 'green', is characterized by a certain degree of exposure to a substance, substance group or mixture, but that exposure does not yet have a significant effect on the Ecological Status. The upper limit of the green class is defined by the long-time honoured principle known as the '95% level of protection'. That is the concentration at which 95% of species would have been exposed chronically below their no-effect level (NOEC). For each substance separately, this exposure level would be equal to the HC5 (Hazardous Concentration for 5% of the species, also known as the 95%-protection level) concentration, and for a mixture it is equal to msPAF-NOEC<5%.
- The classes coloured yellow, orange and red, referring to **Moderate-, High- and Very high** samples, are characterized by an increasing degree of species loss, and impediment to the Ecological Status. The class boundaries have been derived from a number of field studies in which msPAF was calibrated to Ecological Status information.

The class boundaries, the alignment between the evaluation of chemical pollutants with the WFD-Environmental Quality Standards and the evaluation of impacts in the form of Ecological Status classes, and the use contexts of "protective action" or "recovery action" are summarized inTable 1 and include the terms used for chemical pollution classes and the msPAF-boundaries between them.

Note that the system to classify chemical pollution with respect to the degree of harm to aquatic life uniquely *liaises the Chemical- and Ecological Status information* into a single, internally logical system. In the EU-WFD, the evaluation of chemicals and of ecological impacts is

founded on two separate assessment principles, originating from applied ecotoxicology (and laboratory tests) and applied ecology (and field observations) respectively.

	WFD-Protection		WFD-Recovery Measures		
Chemical condition	Sufficient		Insufficient		
Specific contaminating substances	Sufficient		Insufficient		
Ecological condition	Very Good	Good	Moderate	Inadequate	Bad
Chemical pollution classes	None	Low	Moderate	High	Very High
Threshold values of Toxic Pressure	msPAF-NOEC≤0,005	msPAF-NOEC>0,005 msPAF-NOEC≤0,05	msPAF-NOEC>0,05 msPAF-EC50≤0,005	0,005 < msPAF-EC50 ≤ 0,1	msPAF-EC50>0,1
In words	Onset of nuisance in up to **1 in 200 species**	Onset of nuisance in more than 1 in 200 but up to **1 in 20 species (>95% protected!)**	**Effects** in a maximum of **1 in 200 species**	**Effects** in a maximum of **1 in 10 species**	**Effects** in more than **1 in 10 species**

Table 1. Chemical pollution classification based on toxic pressure assessment. Class limits for Ecological Status and chemical contaminants are calibrated to each other on the basis of field impact studies, uniquely relating chemical pollution impacts and chemical pollution classes to the WFD-principles that are used to define Chemical- as well as Ecological Status.

3 Technical manual

3.1 General

The calculation tool is used in a water system analysis, and aims to provide insight into the extent to which substances, substance groups and mixtures affect aquatic life. At the start of using the tool there is always input of a large number of data, processing it, and (at the end) interpreting the results in relation to the water system.
Results are presented in a such a way that they support the formulation of programs of measures. This can have any relevant format, but as an example earlier shown in Figure 1, outcomes can represent a water system analysis of chemical pollution for the whole of the Netherlands (or a region, or a Waterboard).

> The toxic pressure is shown in various classes (colours). By expanding the map regarding types of data shown, by increasing granularity (more local scale), and showing e.g. land use types (and associated chemicals in use) and point source coordinates (and emitted chemicals) in relation to the mixture-toxic pressure patterns and hydrological flow information (e.g., flow direction), such maps allow to deduce probably causes and consequences of chemical pollution. Such a map, which combines land-use and hydrological data, emission insights, and toxic pressure information, would firstly assist in identifying areas with the highest toxic pressures, and next the dominant chemicals within areas. Both would aid prioritizing and implementing mitigation measures.

The present User Guidance now proceeds with the steps that are technically made to use the calculation tool, to enable water quality professionals to make their own analyses.

3.2 Step 1: Collect water quality data

The input data consists of a number of types of data:

1. Sample data (such as xy-coordinates, time and day of sampling, etc.)
2. Data on physico-chemical properties of water sample which are important to calculate the bioavailable fraction of the chemical contaminants (such as the pH for metals, and the organic matter for organic contaminants)
3. Concentration data of chemical contaminants

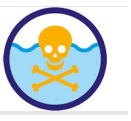
The data is stored per line in Excel, where each line is ultimately the concentration of one substance, with the corresponding physicochemical and location-time data.

3.3 Step 2: Format the data for input in the calculation tool

3.3.1 The Excel file

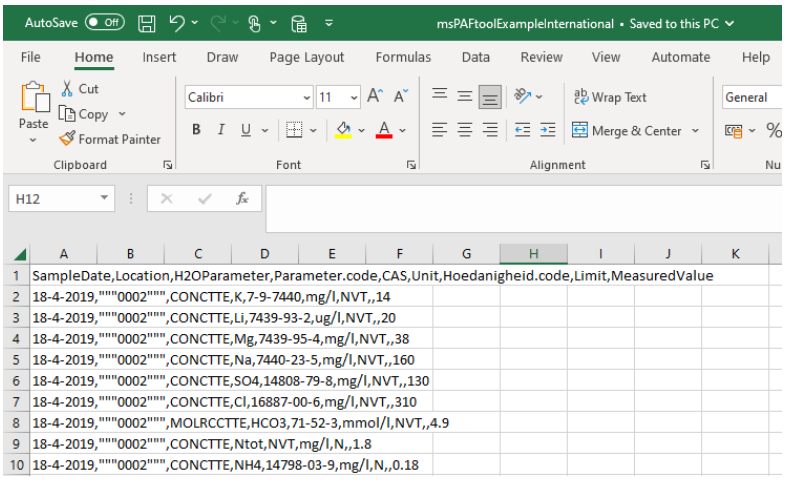
The input file is identical to the IHW[2]-format that was designed for Dutch water quality managers to act as standard input format to send Dutch water quality data to the EU-WISE database. The WISE-database contains data from the River Basin Management Plans reported by EU Members States, Iceland, Norway and the United Kingdom according to article 13 of the Water Framework Directive (WFD). The database includes information about surface water bodies (number and size, water body category, ecological status or potential, chemical status, significant pressures and impacts, and exemptions).
The collected data for the calculation tool (result of the previous step) must be copied in the pre-defined format, and have a .csv file format (see also the included sample file on the website

[2] IHW=Informatie Huis Water, the Dutch organisation responsible for collecting, curating, storing and reporting water quality data, for (amongst others) transfer to the EU-WISE database. References: www.ikw.nl and the WISE European data hub.



(page:https://www.sleutelfactortoxiciteit.nl/key-factor-toxicity-introduction). The maximum size for the input file is 5 MB. This is done to keep the calculation time within reasonable limits for online use.

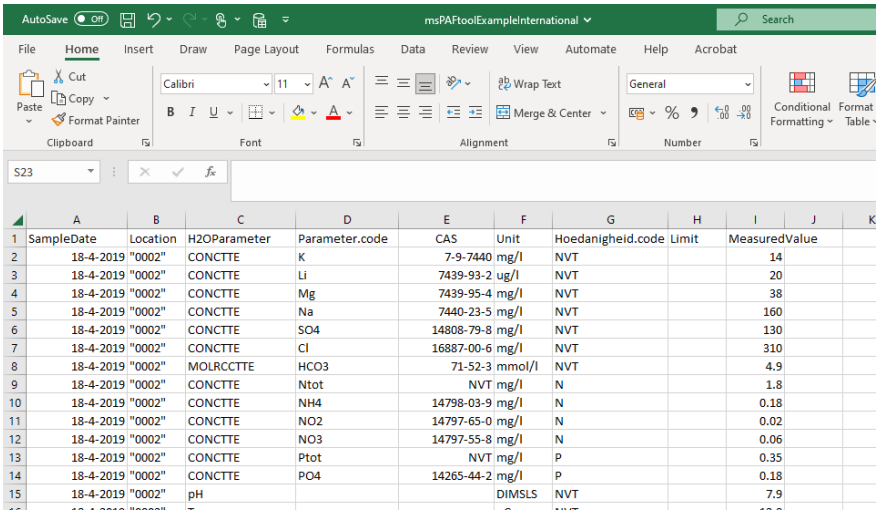
An example of the .csv-file (the example *default-formatted* file) is shown in Figure 3



	A	B	C	D	E	F	G	H	I	J	K
1	SampleDate,Location,H2OParameter,Parameter.code,CAS,Unit,Hoedanigheid.code,Limit,MeasuredValue										
2	18-4-2019,"""0002""",CONCTTE,K,7-9-7440,mg/l,NVT,,14										
3	18-4-2019,"""0002""",CONCTTE,Li,7439-93-2,ug/l,NVT,,20										
4	18-4-2019,"""0002""",CONCTTE,Mg,7439-95-4,mg/l,NVT,,38										
5	18-4-2019,"""0002""",CONCTTE,Na,7440-23-5,mg/l,NVT,,160										
6	18-4-2019,"""0002""",CONCTTE,SO4,14808-79-8,mg/l,NVT,,130										
7	18-4-2019,"""0002""",CONCTTE,Cl,16887-00-6,mg/l,NVT,,310										
8	18-4-2019,"""0002""",MOLRCCTTE,HCO3,71-52-3,mmol/l,NVT,,4.9										
9	18-4-2019,"""0002""",CONCTTE,Ntot,NVT,mg/l,N,,1.8										
10	18-4-2019,"""0002""",CONCTTE,NH4,14798-03-9,mg/l,N,,0.18										

Figure 3. Snippet-picture of the comma-delimited input file (required input format).

An example of the same file if stored as Excel, with separate columns (via the Excel function "Data→Text to columns"), is shown in Figure 4:



	A	B	C	D	E	F	G	H	I	J	K
1	SampleDate	Location	H2OParameter	Parameter.code	CAS	Unit	Hoedanigheid.code	Limit	MeasuredValue		
2	18-4-2019	"0002"	CONCTTE	K	7-9-7440	mg/l	NVT		14		
3	18-4-2019	"0002"	CONCTTE	Li	7439-93-2	ug/l	NVT		20		
4	18-4-2019	"0002"	CONCTTE	Mg	7439-95-4	mg/l	NVT		38		
5	18-4-2019	"0002"	CONCTTE	Na	7440-23-5	mg/l	NVT		160		
6	18-4-2019	"0002"	CONCTTE	SO4	14808-79-8	mg/l	NVT		130		
7	18-4-2019	"0002"	CONCTTE	Cl	16887-00-6	mg/l	NVT		310		
8	18-4-2019	"0002"	MOLRCCTTE	HCO3	71-52-3	mmol/l	NVT		4.9		
9	18-4-2019	"0002"	CONCTTE	Ntot	NVT	mg/l	N		1.8		
10	18-4-2019	"0002"	CONCTTE	NH4	14798-03-9	mg/l	N		0.18		
11	18-4-2019	"0002"	CONCTTE	NO2	14797-65-0	mg/l	N		0.02		
12	18-4-2019	"0002"	CONCTTE	NO3	14797-55-8	mg/l	N		0.06		
13	18-4-2019	"0002"	CONCTTE	Ptot	NVT	mg/l	P		0.35		
14	18-4-2019	"0002"	CONCTTE	PO4	14265-44-2	mg/l	P		0.18		
15	18-4-2019	"0002"	pH			DIMSLS	NVT		7.9		

Figure 4. Snippet of the input file, after the transformation of the comma-delimited format (used in the tool) to the Excel-format with separate columns.

SampleDate	Information on sampling data (DD-MM-YYYY)
Location	Information on sampling location (free field; unique site coding)
H2OParameter	Standardized code for Dutch water quality parameters (in Dutch: Aquocode)
Parameter.code	Common code for identity of parameter
CAS	CAS-number of the chemical parameter

Unit	Dimension in which the parameter value is expressed. Possible values are DIMSLS (dimensionless), °C, ng/l, ug/l, mg/l, mmol/l. For dimensionless parameters the field may also be empty.
Hoedanigheid.code	Code summarizing the 'hoedanigheid' (Dutch for sample pre-treatment or another characteristic of the sample).
Limit	Rows with a "<" or a ">" are not included in the calculations.
MeasuredValue	The value in this column must be a number.

3.3.2 Dealing with concentration limit symbols

The quality of water systems is often assessed by monitoring certain substances that could potentially be present in the system. This may occur, for example, because these substances have been detected in the past, and measures have been taken to reduce them. However, if these measures prove to be effective, these substances can still be part of the measurement program, but are reported as "less than" the detection limit because they have dropped below measurable levels. In these cases, the calculation tool cannot determine local toxic pressure, and the same applies to concentrations "greater than" the detection limit.

In certain lines of research or management assessments, half of the detection limit is used. *This is never done* for the calculation of the toxic pressure of a substance. Experience with a large number of field samples has shown that the toxic pressure of a mixture of, for example, 10 or 20 substances can be increased in this way, to quite high values (especially for msPAF-NOEC). In that case, these would be *results that are not based on any measured concentration, but that still lead to the interpretation that there is evidence for chemical pollution – which is not supported by the available data*.

Therefore, in the calculation tool, the numbers "<" or ">" are not included in the bulk calculations (many data lines with measured substances) and are filtered out by the tool. What the user of the tool could do to explore the numerical relevance of skipping these data is to enter e.g. the detection limit itself, and then calculate the PAF value from it. It then becomes possible to convert concentrations smaller than a detection limit into a toxic pressure that is reported as "lower than a specified value", allowing for an expanding a trend analysis over time, if that started with measured values which were later reported as "<"-values. The toxic pressure of five consecutive periods with measured values can lead to a proven decrease in toxic pressure of (for example) PAF-NOEC 15% to 12% to 7% to 4% to <3%, the latter number indicating that the toxic pressure is less than 3% (whilst the 3%-value represents the PAF resulting from detection-limit assumptions).

3.3.3 Dealing with low PAF values: lower limit interpretation

Toxic pressure quantification is based on Species Sensitivity Distribution modelling, as illustrated in Figure 5. When toxic pressures are calculated (Y) based on monitoring data on concentrations (X), it is key to recognize that the number of available ecotoxicity data for various chemicals can be relatively low – so that the sigmoidal shape can *statistically* be determined (by statistically fitting the log-normal model to the few available data), but that this may result two particular forms of potential SSD-bias: first, a bias caused by an accidentally flat sigmoidal SSD, which – due to that – results in high PAF-values in lower-tail exposure situations (unrealistic over-estimation of low-exposure toxic pressure) or second: a bias caused by an accidentally very steep SSD, which – due to that – results in extremely sharp toxic pressure increases at minimal increases in ambient concentrations. Specific attention has been put on characterizing toxic pressure at low concentrations, here defined as concentrations that are (far) lower than the lowest ecotoxicity data point. This implies extrapolations, in the left-hand tail of the distributions – and such extrapolations warrant care.

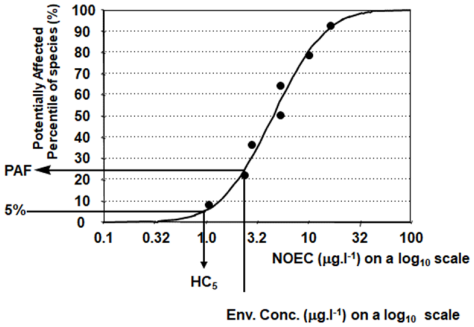


Figure 5. The Species Sensitivity Distribution of a chemical is the sigmoid-shaped statistical distribution of a set of ecotoxicity test data for a chemical, tested on different species. The arrows of Y→X and X→Y show the two uses of SSDs, for deriving Environmental Quality Standards and Potentially Affected Fractions, respectively.

As can be intuitively derived from Figure 5, the best estimates of toxic pressure interpolations would be based on SSDs for which the ambient concentrations fall within the range of the available ecotoxicity data, especially in the range of the slope-part of the sigmoid. Such exposure levels imply interpolation, when deriving PAF, with an SSD that is insensitive (in shape and position) for adding or removing some test data. However, the measured concentration is often (much) lower than the lowest measured ecotoxicity value. This positions the calculations in the 'lower tail' of the sensitivity distribution. That this may pose interpretation problems is illustrated below.

For data-poor compounds, the SSD may have a 'shallow slope', which means a lower tail that is positioned relatively high on the Y-axis (see Figure 6). Regarding toxic pressure results, experience with large data sets and 'artificially' removing and adding data points to SSDs, have shown that the left-tail effect for data-poor substances may yield (very) high toxic pressures, which 'disappear' if additional ecotoxicity data are added (and the slope is normalized by that).

To avoid considering uncertain toxic pressure numbers, except when they are clearly low, the calculation tool only reports on- and calculates mixture toxic pressure with outcomes when "PAF value >0.01" holds. Lower PAF-values are discarded. At the 0.01-PAF level, the outcome means that 1 in 100 species would be affected by the substance or mixture.

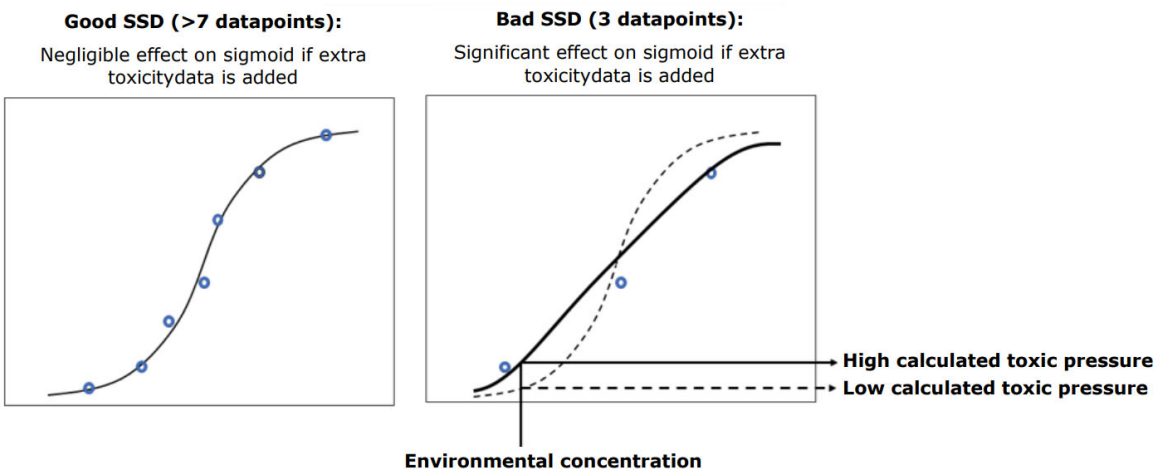


Figure 6. Schematic illustration of a robust SSD and a data-poor and non-robust SSD with a shallow slope. Left: good SSDs are derived from a relatively large number of toxicity data from different taxa, and are robust: they hardly change their location and shape (steepness) when an extra data point is added. The probability of having a shallow slope, and estimated high PAF-values at low X, is low. Right: shallow-slope SSDs can be the result for compounds with relatively few ecotoxicity tests data. In the example, only three toxicity tests were available. The lognormal (sigmoid) model is estimated as the solid line, and leads to a high toxic pressure at a low concentration. However, if one extra data point is added, the line would, for example, become equal to the dotted line, and the toxic pressure that would be calculated *at the same ambient concentration* decreases sharply.

Note: this interpretation rule helps to seeking for a plausible 'biological' interpretation for the situation in which the calculation tool calculates that msPAF-EC50 > msPAF-NOEC. This is not ecologically possible, because species are always first hindered at sub-lethal levels (msPAF-NOEC) and only then are affected seriously and disappear (msPAF-EC50). In incidental cases, however, the 'lower tails' of the NOEC and EC50 models may be extrapolated such that the numerical outcome of msPAF-EC50>msPAF-NOEC due to the slope-effect. If it is found that msPAF-EC50>msPAF-NOEC in incidental cases, despite the aforementioned 0.01-cut-off rule, the user is recommended to inspect this potential slope-bias effect.

3.3.4 Dealing with qualities of metrics

The quality of a measurement in the set of monitoring data can be encoded as a data line for a parameter with the code 'nf' (meaning: "after filtration" [nf in Dutch is 'na filtratie']) and also as a data line (for the same place-time sampling point/moment) as parameter without filtration ('zonder filtratie'). It In such cases, it is important to avoid that double measurement data of the same parameter are entered for a substance. The reporting of both modalities (concentrations measured with and without filtering as sample pre-treatment) of a parameter has occurred regularly in older Dutch data sets. This could cause, for example, metals to be entered twice (with and without filtration). Where possible, only data 'after filtration', encoded with 'nf', is used.

3.3.5 Dealing with extreme values

A measurement series can always contain extreme (high) values. When using the tool, this can be checked by evaluating the range and distribution of the data series for each measured parameter. Usually, there is a normal distribution of observations, with logical differences between measurements such as

pH, zinc, or PAKs (often measured with no known special emissions of the substance) and, for example, pesticides (which can exhibit high concentrations during the growing season at the time of application). Extreme values can also arise due to incidents. In the context of water system analysis, it is a good practice to evaluate the characteristics of the input data set (all parameters), to search for possible extreme values and evaluate whether there is a logical (water-system, or sampling-regime related) reason for the unusual value. If there is a clear error, the value can be removed. If no error can be detected, the value can be retained, and a comment can be added regarding the impact of the extreme value(s) in the final interpretation. The tool does not issue warnings for extreme values and includes them in the calculations.

3.3.6 Taking into account substances that occur naturally

The Water Framework Directive distinguishes between synthetic and non-synthetic substances. The synthetic compounds are all man-made, so the natural exposure concentration and the toxic pressure of these compounds should be negligible, respectively. Substances that can occur naturally form a separate category. This consists of substances such as ammonium, sulphate, chloride and all metals. The toxic pressure of these substances can be calculated. However, the assessor must always thoroughly check whether the local water body concentration is a consequence of a natural background concentration, or whether there is evidence for an increase in concentrations.

For example, some groundwater-affected water systems are characterized by high iron and manganese concentrations, while in brackish waters sulphate and boron concentrations are often naturally elevated. The naturally elevated concentrations of these substances can lead to toxic effects on an 'average' community of species. This is what represents the 'toxic pressure': the degree of threat to the 'average' species assemblage (as embodied in the tested set of species, on which the SSD is based). If the natural background concentration is elevated, then the local communities are adapted to it, and there is no '(man-made)toxicity against which measures must be taken'. The species composition that occurs in those areas is characteristic of those areas (think of the zinc pansy – a typical metal-vegetation species – that occurs on old zinc deposits). However, the need arises to take measures if human action has led to increased concentrations of these substances.

The calculation tool does not yet make it possible to take into account the natural background concentrations of the naturally occurring substances in its calculation routines. The water quality assessment must be expanded here, by the assessor and based on other information (on natural background levels), with an extra interpretation step. The extra step would consist of an explicit evaluation of the extent to which the measured concentration has been increased by human action.

3.3.7 Taking sum measurements into account

Measured concentrations of substances can in practice (due to analytical equipment, or due to reasons embedded in specific policies) be summarized as summation terms, such as the sum of 16 PAHs or the sum of four 'drins.' Since SSD modelling always operates on a per-substance basis, the concentrations are to be loaded into the calculation tool per separate substance. Given that some water managers report both the sum-value and the separate compounds, the user of the calculation tool should take care that a substance is never counted twice.

3.3.8 Taking into account the quality of the SSD models

The toxic pressure of a substance is ultimately derived from the sigmoid relationship between environmental concentration (X) and the Potentially Affected Fraction species (Y), the *Species Sensitivity Distribution* (SSD, Figure 5). In a fully data-driven analyses, Posthuma et al. (2019) described SSD for more than 12,000 compounds, but recognized substantial differences in input data numbers, and (thus) SSD robustness. The robustness can be characterized by statistical methods, such

as bootstrapping. Note that (as explained above too) non-robustness has two influential but undesired outcomes:

1. The 'lifting of the left tail', causing high *calculated* toxic pressures at very low ambient exposures, whilst the SSD shape in reality is not as flat as shown (illustration in Figure 3B).
2. The steepness-effect, causing *high increase of toxic pressure over a low increase of ambient concentration*, which occurs if the SSD is accidentally very steep (sometimes even 'near vertical') when based on a haphazard collection of nearly identical sensitivity data.

The calculation tool currently operates for chemicals from the two highest SSD robustness categories (A and B) that have been defined. The number of substances for which SSDs have been derived, and classified, and the ones currently used by the calculation tool, are shown in Table 2.

Table 2. The number of substances that the calculation tool calculates with, and the number of substances for which the SSD is of insufficient robustness (type C) or missing (type D), or where there are substances with a natural occurrence, such as Al, Fe, Mn, Si, F, sulphate, nitrate and natural toxins such as microcystin).

Group	Substances (n)	Quality of the SSDs	Included in the calculation tool
A	215	High	Yes
B	486	Sufficient	Yes
C	534	Moderate to poor	No
D	367	No toxicity information	No

3.3.9 Taking variable exposure into account

An aquatic community is often exposed to a long-term (and often time-varying) load from diffuse sources, combined with peak loads such as those caused by the use of pesticides and those that may occur after heavy rainfall (sewer overflows). In a water system analysis, the assessor should therefore expect and handle a complex pattern of exposure that varies significantly in both space and time.

The calculation of the toxic pressure of the cumulative exposure is carried out by a pragmatic, ecological approach. For example, if a map is to be made of the differences in toxic pressure in an area, then all differences in exposure patterns must be incorporated into the intended insight, by summarizing measurements over time.

> Suppose that two substances, by their application, lead to a toxic pressure of 50% and 30%. If they are both used equally, this leads to a mixture toxic pressure of msPAF=1-(1-0.5)*(1-0.3)=0.65 (the mixture leads to a toxic pressure of 65%).
>
> If the substances are applied one after the other, but there is still no ecological recovery, the latter phenomenon leads to an identical value for the calculated toxic pressure, even when there has been no simultaneous exposure to both substances.

In other words, the summary of toxic pressure in a water system analysis requires a good understanding of the dynamics of the exposure to chemicals, but surely also of the way the available exposure data are handled.

3.4 Step 3: Check decimal points in Excel

The English version of the calculation tool operates with the Excel operations done according to UK standards. This relates to matters such as decimal separators, etc..

The columns in the .csv input file are separated by a comma. The decimal separator must be a period (“.”).

In Excel the decimal separator can be adjusted as follows: [File > Options > Advanced > Decimal separator] (see below). Don't forget to check and change the thousand-separators to a comma (,), and alter the separator if needed (Figure 7).

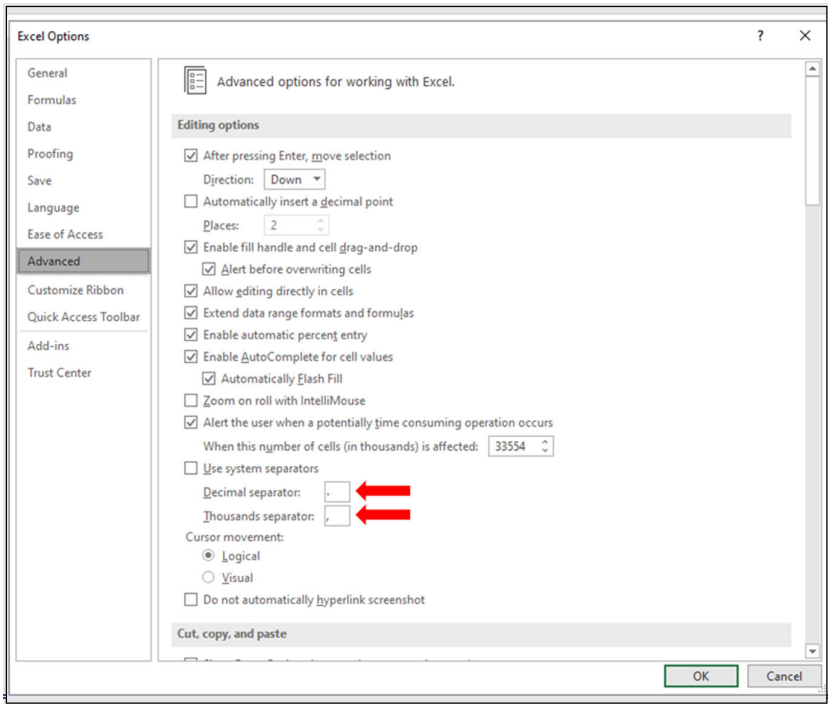


Figure 7. Screen to alter the decimal separator, if needed for proper calculations based on your data.

3.5 Step 4: Launch the tool

The calculation tool is available as iFrame (and embedded tool) on the English-language web page of the website of the Sleutelfactor Toxiciteit (https://www.sleutelfactortoxiciteit.nl/key-factor-toxicity-introduction).

That webpage presents the calculation tool as iFrame, as shown in Figure 8.

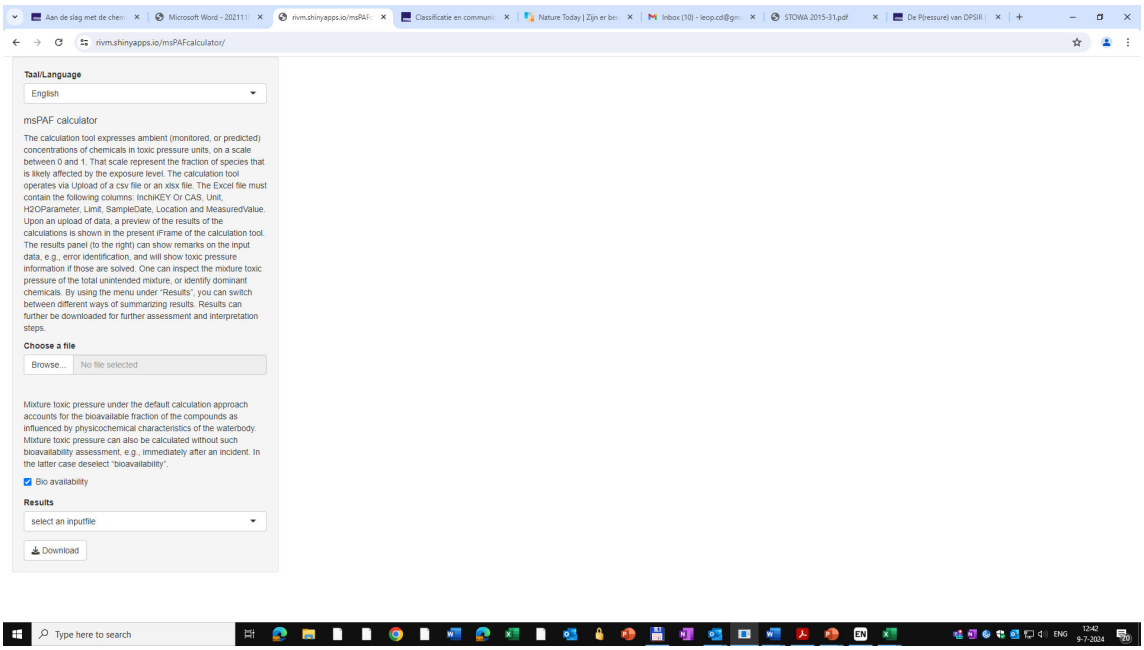


Figure 8. The calculation tool is available as iFrame within the website https://www.sleutelfactortoxiciteit.nl/key-factor-toxicity-introduction. An iFrame is a HTML entity that is embedded in a webpage, but that liaises to another source (here: an R-Shiny application that runs elsewhere). The right-hand side of the page is empty at the start, when no input data have yet been loaded.

3.6 Step 5: Landing page and buttons

The calculation tool uses five buttons:

1. Taal/Language – Select either English or Dutch language when using the tool.

The remainder of the Guidance follows the English choice here.

2. Choose File – Browse.
   Here an input file is chosen (the prepared .csv file)
3. Bioavailability – Choose whether bioavailability of certain elements should be accounted for when calculating the PAF-values. Default is: take bioavailability modification by physicochemical characteristics of the sample into account. The assessment can be run with 100% of the chemicals being bioavailable, as happens on the short term after an incident with substantial chemical emissions.
4. Results – This button show "Select an inputfile" as status when no data set is yet loaded.
   Once you have uploaded a file the results are calculated, and various pull-down options appear:
   a. Warnings: possible problems in the input file are summarized
   b. PAFtable: the toxic pressure of each substance per sample is shown separately (columns PAF-Acute and PAF-chronic), with associated calculation metrics in the other columns.
   c. msPAFacute: the toxic pressure (msPAF-EC50)[3] is shown for the total mixture ("Acute.All") and for a number of substance groups ("Acute: Ammonium/Ammoniak", "Acute.Bestrijdingsmiddel" (for pesticides), "Acute.Metals" (for metals), "Acute.OverigOrg." (for other organic substances), and "Acute.PAKs" (for PAHs).

[3]Species loss will likely occur if values increase above this toxic pressure ; these msPAF-EC50 values are used to classify the water sample into the classes 'yellow', 'orange' or 'red'.

d. msPAFchronic: the toxic pressure (msPAF-NOEC)[4] is expressed as explained under "c" for the total mixture and the various compound groups.
e. msPAFqualitative: both types of toxic pressure data (c and d) summarized as a 5-step qualitative classification score as shown in **Error! Reference source not found.**.

5. Download: here the results of the calculations are exported to an Excel file. The exported Excel has a set of worksheets on the identification of the whole analysis, and the various outputs described above.

3.7 Step 6: Read the input data

The *'Browse'* button under *Choose a file* is the button to select the input file. When this button is pressed, a pop-up window appears where you can select the input file (see Figure 9). Choose the input file with which the analyses should be done.

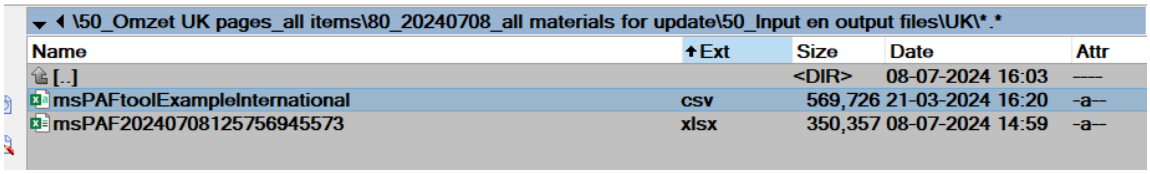


Figure 9.Selecting the .csv inputfile from the data files for water systems analyses. Shown (blue bar) is the example filename for the English-language input format file, whilst the other file is the Excel-file with the automatic generated output filename.

When the input file has been successfully loaded, the text "Upload complete" will appear under the 'Browse' button (Figure 10):

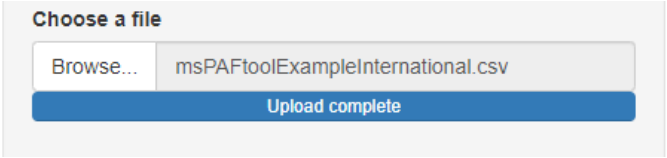


Figure 10. Message that appears when a .csv file has been successfully uploaded.

3.8 Step 7: Evaluate the Warnings and resolve input issues

After the file has been successfully read in, the 'Select an input file' notion under Results changes to 'Warnings' and associated to this, the comments that were generated by the tool when reading the measurement data appear on the right side of the screen (Figure 11).

[4] Species are hindered if values increase above this toxic pressure (they are exposed above their no-effect level); these msPAF-NOEC values are used to divide the water sample into the classes 'blue' and 'green'.

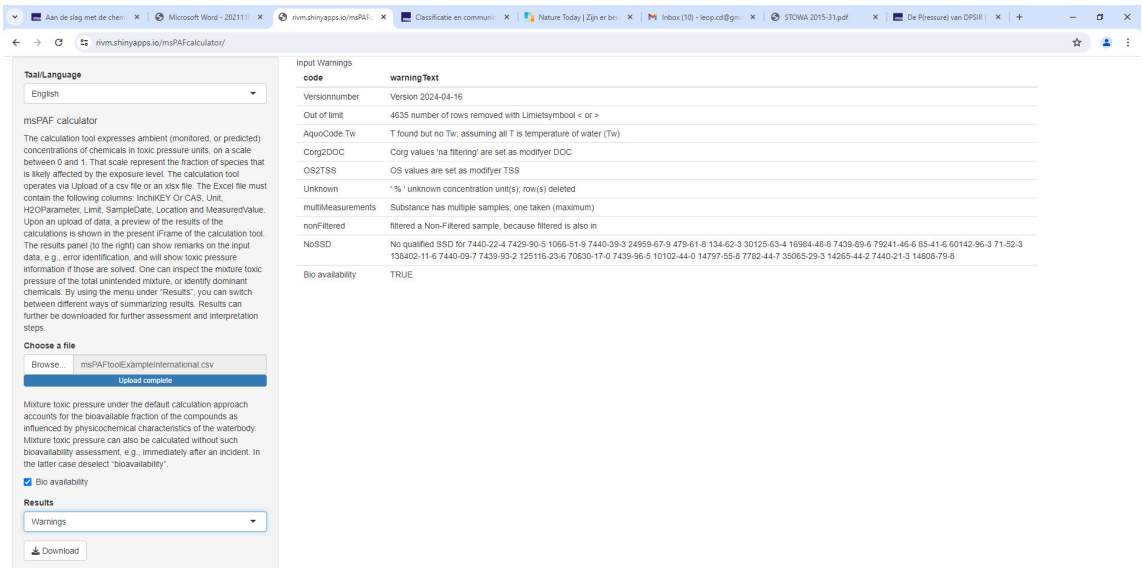


Figure 11. results screen that appears initially if a dataset (.csv-file) has been successful uploaded. The "Results" tab (left) has changed to "Warnings", which are listed in the right-hand part of the screen.

The most important step that is now to be done is to check the Warnings, and the apparent input problems. The user reviews the alerts and, if possible, resolves the alerts that have been flagged:

- This is possible if, for example, a substance with CAS code is found that is not in the calculation tool (e.g., "Illegal CAS 7440-09-07"; there may be a typing error (which can be corrected), unless the substance is missing in the calculation tool.

The tool executes a checksum assessment on the CAS-number, to identify typing errors. That is, the CAS-number is designed so that it has a logical checksum. Errors yield "Illegal CAS". Parameters for which there is no SSD model yield "NOSSD" (no SSD) as remark. If the CAS-number is correct but if there is no good SSD model for the compound, the tool reports "substances with CAS not in chemistry list".

Solving a matter may not be possible so that some calculations cannot be made, such as because of "<" values for the concentration of a substance.

The above example gives a number of error messages, but nevertheless the tool can be run for the non-erroneous input values. Eventually, the proper functioning of the calculation tool can be checked by selecting the PAF table or msPAFacute/msPAFchronic tables under 'Results', and checking the tab "Input data" in the output Excel with the data that were entered. If an output table is generated, the model run has been successful. If not, it is necessary to look at which data needs to be supplemented on the basis of the text under 'warningText'. When the required data has been completed in the Input file, the tool can be run again. You can do this by using the adapted .csv file re-upload.

3.9 Step 8: Evaluate on the screen the toxic pressure output

By pressing the other buttons under 'Results', the output that is calculated is shown on the right side of the screen for visual inspection. Outcomes concern successively the various ways of gaining insight into the toxic pressure patterns across the samples, that is, by looking at PAF-values per compound, per group and for total mixtures.

3.9.1 PAFtable

By pressing the PAFtable button, a series of output data is displayed on the right side of the screen (Figure 12).

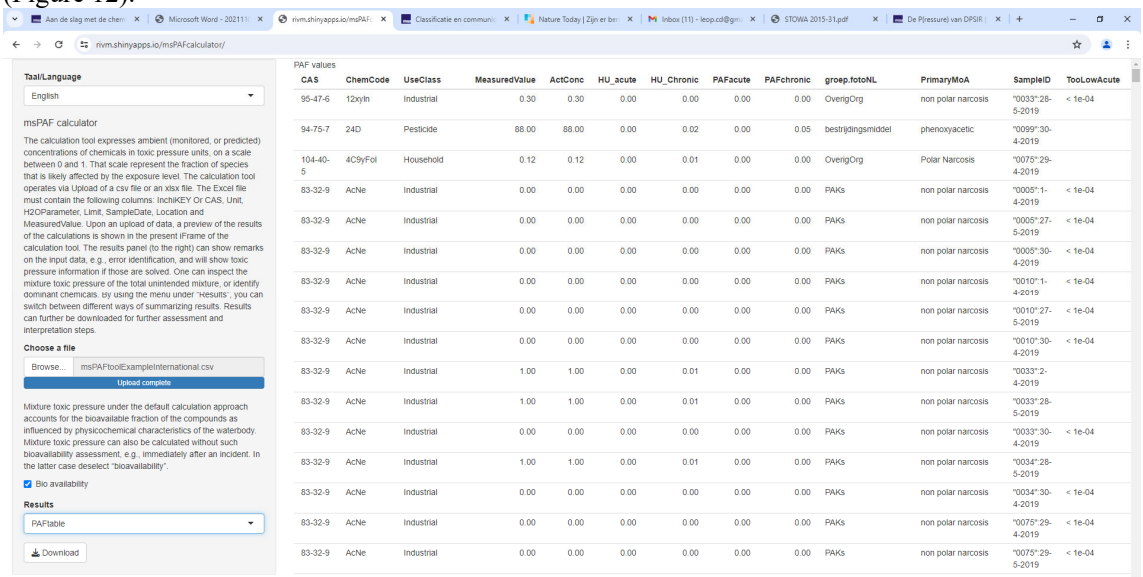


Figure 12. Screenshot of the Results-screen for the English-language version output for the PAFTable selected as output.

The output columns are a summary of the input data, so that they can be checked, and provide insight into the toxic pressure per substance, such as acute (would the concentration of this substance lead to species loss) and chronic (would the concentration of this substance lead to nuisance). The summarized columns have the following meaning:

CAS	The identification of the chemical contaminant with the CAS standard
ChemCode	The identification of the chemical contaminant with the AquoCode standard
UseClass	In this calculation tool, the classification of substances into use classes, such as Industrial, Household, etc.
MeasuredValue	The measured concentration, as input data point
ActConc	The calculated bioavailable concentration
HU_acute	The Hazard Unit (HU), calculated as a concentration in the sample divided by the Avg10Log_acute, so that numbers HU>1 indicate that the average species is exposed above the EC50 level (and thus there is contamination with effects at that level, for the average-sensitive species)
HU_Chronic	The Hazard Unit (HU), calculated as a concentration in the sample divided by the Avg10Log_chronic, so that numbers HU>1 indicate that the average species is exposed above the NOEC level (and therefore there is contamination with effects at that level, for the average-sensitive species)
PAFacute	Conversion of the HU_Acute to the fraction of species exposed above their EC50 level, where elevated values indicate increased barriers to ecological status (basis for classification into "yellow", "orange" or "red".
PAFchronic	Conversion of the HU_Chronic to the fraction of species exposed above their NOEC level, with elevated values indicating increased barriers such as the onset

	of effects on growth or reproduction ("nuisance"), and basis for classification into "blue" or "green".
PrimaryMoA	Categorization of the compound by mode of action (here is number code; substances with the same mechanism of action have the same code)
SampleID	The combination of Meetobject.LocalID and Start Date that identifies the Sample, here "location "0005" and sample time ("2019-04-01")
TooLowAcute	Negligible PAF-value, which is not further used in calculations or interpretation (see Release Notes for further explanation)
TooLowChronic	Negligible PAF-value, which is not further used in calculations or interpretation (see Release Notes for further explanation)
Avg10Log_acute	Median of the log-transformed EC50 data for the species for which the compound has been tested (midpoint of the sigmoidal lognormal SSD-EC50)
Dev10Log_acute	Standard deviation of the log-transformed EC50 data for the species for which the compound has been tested (slope of the sigmoid log-normal SSD-EC50 at the midpoint)
Avg10Log_chronic	Median of the log-transformed NOEC data for the species for which the compound has been tested (midpoint of the sigmoidal lognormal SSD-NOEC)
Dev10Log_chronic	Standard deviation of the log-transformed NOEC data for the species for which the compound has been tested (slope of the sigmoid log-normal SSD-NOEC at the midpoint)

Note: keep track in interpretation of the choice for the check box on “Bioavailability”. Report specifically whether the resulting values are calculated with (default) bioavailability correction, or without such correction.

All results will be available in a separate Worksheet if the Results are exported, and can then be analysed further in the context of the goals of the water system analysis that is being made.

3.9.2 msPAFacute

By pressing the msPAFacute button, a series of output data is displayed on the right side of the screen (Figure 13).

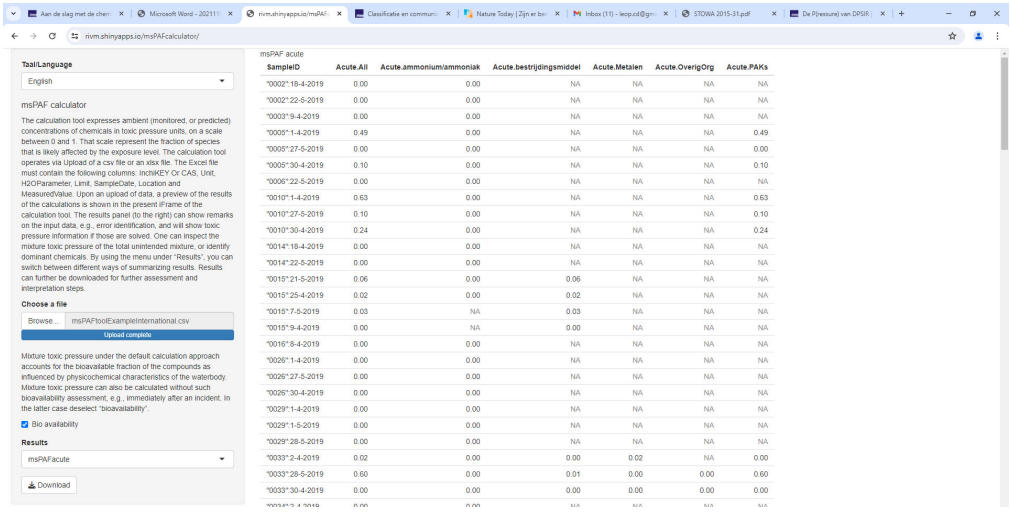


Figure 13. Screenshot of the Results-screen for the English-language version for the msPAFAcute button selected as output.

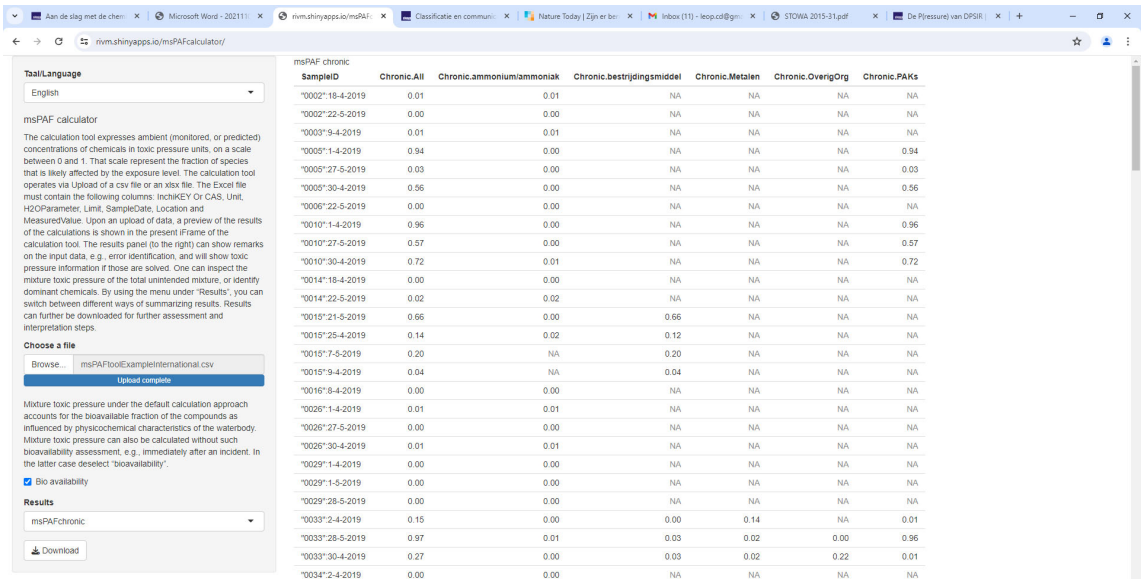
SampleID — The combination of Meetobject.LocalID and Start Date that identify the sample; an msPAF value is calculated per sample.
Acute.All — msPAF-EC50 of all substances measured in the sample.
Acute.ammonium/ammoniak — msPAF-EC50 of ammonia only (based on measurements of ammonium and/or ammonia, where the toxic pressure is derived from the most toxic substance, ammonia)
Acute.bestrijdingsmiddel — msPAF-EC50 of pesticides only
Acute.Metal — msPAF-EC50 of metals only
Acute.OverigOrg — msPAF-EC50 of only the other organic contaminants (non-PACs and non pesticides)
Acute.PAKs — msPAF-EC50 from the PAHs only

The subdivision into substance groups makes it possible to determine which substance group in the sample contributes most strongly to effects, with the effects here referring the likelihood of species loss.

Note: keep track in interpretation of the choice for the check box on “Bioavailability”. Report specifically whether the resulting values are calculated with (default) bioavailability correction, or without such correction.

3.9.3 msPAFchronic

By pressing the msPAFchronic button, a series of output data is displayed on the right side of the screen:



SampleID	Chronic.All	Chronic.ammonium/ammoniak	Chronic.bestrijdingsmiddel	Chronic.Metalen	Chronic.OverigOrg	Chronic.PAKs
"0002":18-4-2019	0.01	0.01	NA	NA	NA	NA
"0002":22-5-2019	0.00	0.00	NA	NA	NA	NA
"0003":9-4-2019	0.01	0.01	NA	NA	NA	NA
"0005":1-4-2019	0.94	0.00	NA	NA	NA	0.94
"0005":27-5-2019	0.03	0.00	NA	NA	NA	0.03
"0005":30-4-2019	0.56	0.00	NA	NA	NA	0.56
"0006":22-5-2019	0.00	0.00	NA	NA	NA	NA
"0010":1-4-2019	0.96	0.00	NA	NA	NA	0.96
"0010":27-5-2019	0.57	0.00	NA	NA	NA	0.57
"0010":30-4-2019	0.72	0.01	NA	NA	NA	0.72
"0014":18-4-2019	0.00	0.00	NA	NA	NA	NA
"0014":22-5-2019	0.02	0.02	NA	NA	NA	NA
"0015":21-5-2019	0.66	0.00	0.66	NA	NA	NA
"0015":25-4-2019	0.14	0.02	0.12	NA	NA	NA
"0015":7-5-2019	0.20	NA	0.20	NA	NA	NA
"0015":9-4-2019	0.04	NA	0.04	NA	NA	NA
"0016":8-4-2019	0.00	0.00	NA	NA	NA	NA
"0026":1-4-2019	0.01	0.01	NA	NA	NA	NA
"0026":27-5-2019	0.00	0.00	NA	NA	NA	NA
"0026":30-4-2019	0.01	0.01	NA	NA	NA	NA
"0029":1-4-2019	0.00	0.00	NA	NA	NA	NA
"0029":1-5-2019	0.00	0.00	NA	NA	NA	NA
"0029":28-5-2019	0.00	0.00	NA	NA	NA	NA
"0033":2-4-2019	0.15	0.00	0.00	0.14	NA	0.01
"0033":28-5-2019	0.97	0.01	0.03	0.02	0.00	0.96
"0033":30-4-2019	0.27	0.00	0.03	0.02	0.22	0.01
"0034":2-4-2019	0.00	0.00	NA	NA	NA	NA

Figure 14. Screenshot of the Results-screen for the English-language version output for the msPAFchronic selected as output.

SampleID — The combination of Meetobject.LocalID and Start Date that identify the sample; an msPAF value is calculated per sample.
Chronic.All — msPAF-EC50 of all substances measured in the sample.
Chronic.ammonium/ammoniak

msPAF-EC50 of ammonia only (based on measurements of ammonium and/or ammonia, where the toxic pressure is derived from the most toxic substance, ammonia)

Chronic.bestrijdingsmiddel
msPAF-EC50 of pesticides only

Chronic.Metal msPAF-EC50 of metals only

Chronic.OverigOrg msPAF-EC50 of only the other organic contaminants (non-PACs and non pesticides)

Chronic.PAKs msPAF-EC50 from the PAHs only

The subdivision into substance groups makes it possible to determine which substance group in the sample contributes most strongly to effects, with the effects here referring the likelihood of species experiencing 'nuisance' from the exposure to the chemical(s).

Note: keep track in interpretation of the choice for the check box on "Bioavailability". Report specifically whether the resulting values are calculated with (default) bioavailability correction, or without such correction.

3.9.4 msPAFqualitative

By pressing the msPAFqualitative button, a series of output data is displayed on the right side of the screen (Figure 15).

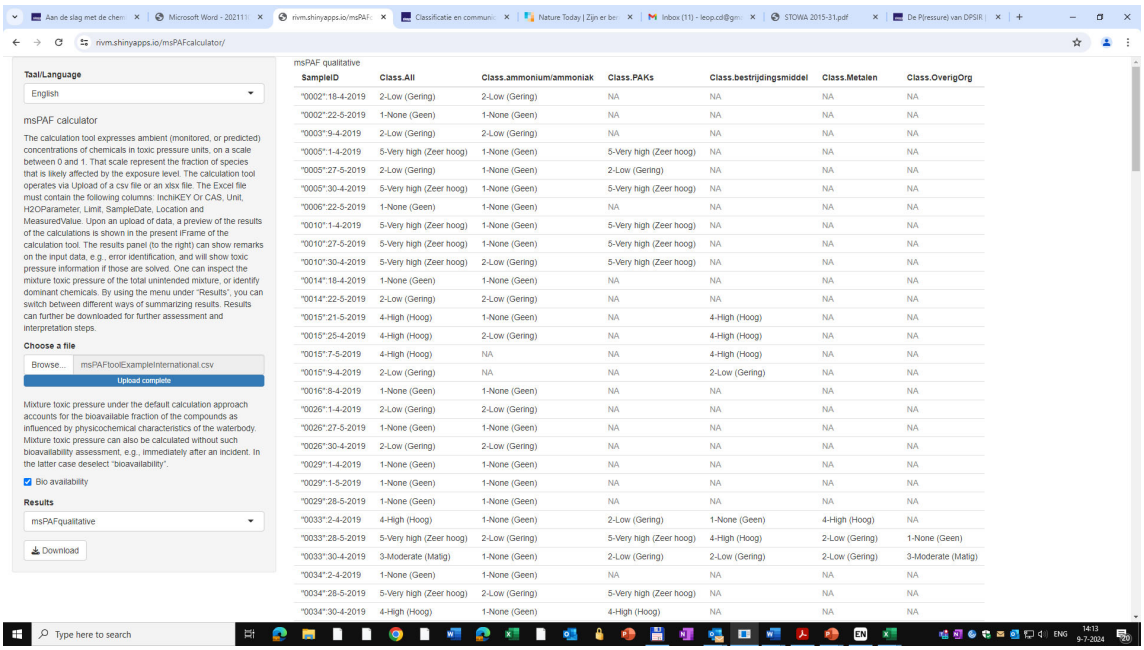


Figure 15. Outputs of the calculation tool in the five chemical pollution classes as defined in Figure 2.

SampleID The combination of Meetobject.LocalID and Start Date that identify the sample; an msPAF value is calculated per sample.

Class.All The classification of the toxic pressure of all substances together

Class.ammonium/ammonia
The classification of the toxic pressure of ammonia

Class.PAKs	Toxic pressure classification of all PAHs
Class.Bestrijdingsmiddelen	The classification of the toxic pressure of all pesticides
Class.Metalen	The classification of the toxic pressure of all metals
Class Overig.Org	The classification of the toxic pressure of organic substances that are not part of the other groups.

Note: keep track in interpretation of the choice for the check box on “Bioavailability”. Report specifically whether the resulting values are calculated with (default) bioavailability correction, or without such correction.

3.10 Step 9: Export the toxic pressure output

By pressing the 'Download', the entire set of calculation results is exported to Excel (Figure 16). Further data processing can be done there.

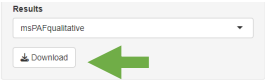


Figure 16. Outputs of the calculation tool can be downloaded as Excel, with all the information shown so far presented in separate Worksheets.

The Excel file has a unique name, which is based on the 'msPAF **day/time**.xlsx' identification (such as an export in the year 2024, on July 8th (2024708) at 12:57 hr (Figure 17):

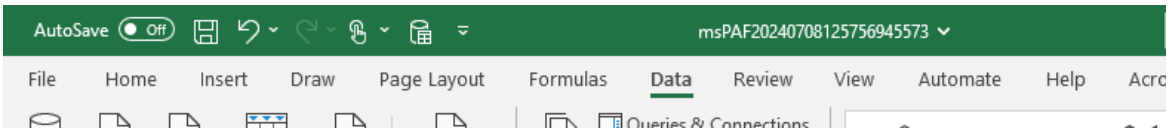


Figure 17. The download button creates an export Excel file with a unique name, which encompasses a day-time code (here: July 8th, 2024, at 12:57 hr).

A number of Tabs are produced in each Excel export, expanding on and partly similar to the selection buttons on the screen (Figure 18):

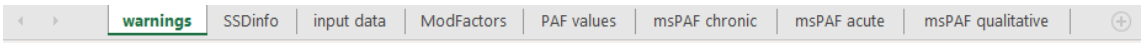
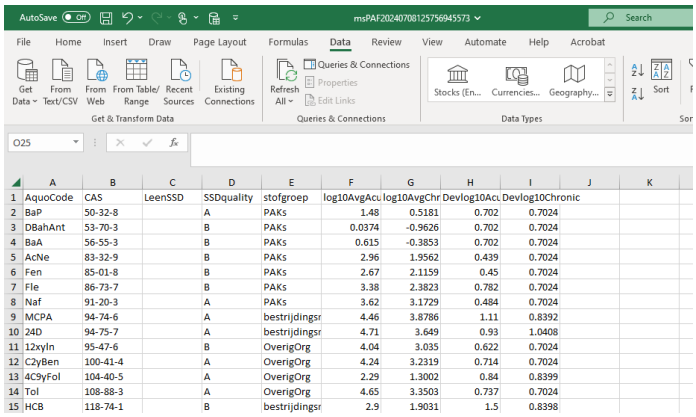


Figure 18. Identification of the Worksheets in the Excel export of an analysis run.

- The 'Warnings' worksheet as shown earlier in the web tool are repeated
- The ‘SSDinfo’ worksheet lists the *Species Sensitivity Distributions* (SSD) information used (these are the models that summarize the ecotoxicity of a substance, in the sigmoid relationship between concentration on the X-axis and potentially impaired fraction species on the Y-axis (Figure 19):

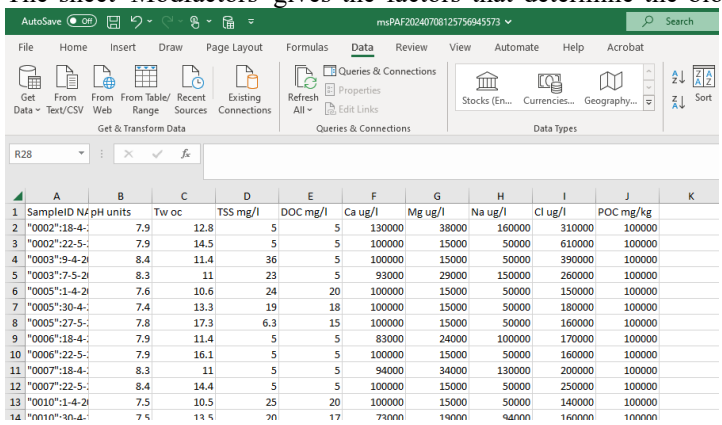


	A	B	C	D	E	F	G	H	I	J	K
1	AquoCode	CAS	LeenSSD	SSDquality	stofgroep	log10AvgAcu	log10AvgChr	Devlog10Acu	Devlog10Chronic		
2	BaP	50-32-8		A	PAKs	1.48	0.5181	0.702	0.7024		
3	DBahAnt	53-70-3		B	PAKs	0.0374	-0.9626	0.702	0.7024		
4	BaA	56-55-3		B	PAKs	0.615	-0.3853	0.702	0.7024		
5	AcNe	83-32-9		B	PAKs	2.96	1.9562	0.439	0.7024		
6	Fen	85-01-8		B	PAKs	2.67	2.1159	0.45	0.7024		
7	Fle	86-73-7		B	PAKs	3.38	2.3823	0.782	0.7024		
8	Naf	91-20-3		A	PAKs	3.62	3.1729	0.484	0.7024		
9	MCPA	94-74-6		A	bestrijdingsr	4.46	3.8786	1.11	0.8392		
10	24D	94-75-7		A	bestrijdingsr	4.71	3.649	0.93	1.0408		
11	12xyln	95-47-6		B	OverigOrg	4.04	3.035	0.622	0.7024		
12	C2yBen	100-41-4		A	OverigOrg	4.24	3.2319	0.714	0.7024		
13	4C9yFol	104-40-5		A	OverigOrg	2.29	1.3002	0.84	0.8399		
14	Tol	108-88-3		A	OverigOrg	4.65	3.3503	0.737	0.7024		
15	HCB	118-74-1		B	bestrijdingsr	2.9	1.9031	1.5	0.8398		

Figure 19. The exported Excel-worksheet for the information on the SSD-models of the compounds that are present in the input .csv file.

- AquoCode: the Aquocode of the substance
- CAS: the CAS number of the substance
- 'LeenSSD': sometimes there is no SSD of a compound, but of an almost identical substance. In those cases, this 'loan SSD' is used to derive the toxic pressure of the measured substance; In those cases, the name of the 'loan substance' is listed here.
- SSDquality: the toxic pressure is determined only if the SSD is of good quality. This is shown with the codes A and B (very good and good SSDs), C (moderate SSD, can produce unexpectedly high PAF values, as in Figure 3b) and D (no SSD available).
- 'Stofgroep': this is the substance group employed in the tool to group substances.
- Log10AvgAcute, ditto Chronic, and ditto StDev's: see above (the parameters of the SSD-EC50 and the SSD-NOEC)

- The 'Input data' worksheet shows a copy of the entered data, where the calculation tool has harmonized all units; the user can check here whether all input data has really been copied and unit-harmonized correctly.
- The sheet 'Modfactors' gives the factors that determine the bioavailability of the chemicals.



	A	B	C	D	E	F	G	H	I	J	K
1	SampleID NA	pH units	Tw oc	TSS mg/l	DOC mg/l	Ca ug/l	Mg ug/l	Na ug/l	Cl ug/l	POC mg/kg	
2	"0002":18-4-	7.9	12.8	5	5	130000	38000	160000	310000	100000	
3	"0002":22-5-	7.9	14.5	5	5	100000	15000	50000	610000	100000	
4	"0003":9-4-2	8.4	11.4	36	5	100000	15000	50000	390000	100000	
5	"0003":7-5-2	8.3	11	23	5	93000	29000	150000	260000	100000	
6	"0005":1-4-2	7.6	10.6	24	20	100000	15000	50000	150000	100000	
7	"0005":30-4-	7.4	13.3	19	18	100000	15000	50000	180000	100000	
8	"0005":27-5-	7.8	17.3	6.3	15	100000	15000	50000	160000	100000	
9	"0006":18-4-	7.9	11.4	5	5	83000	24000	100000	170000	100000	
10	"0006":22-5-	7.9	16.1	5	5	100000	15000	50000	160000	100000	
11	"0007":18-4-	8.3	11	5	5	94000	34000	130000	200000	100000	
12	"0007":22-5-	8.4	14.4	5	5	100000	15000	50000	250000	100000	
13	"0010":1-4-2	7.5	10.5	25	20	100000	15000	50000	140000	100000	
14	"0010":30-4-	7.5	13.5	20	17	73000	19000	94000	160000	100000	

The relevant columns are:

- SampleID: identification of the sample for which the modifying factors are listed
- pH units (dimensionless)
- The water temperature (Tw) in degrees Centigrade

  - Total Suspended Solids (TSS) in mg/L (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
  - Dissolved Organic Carbon (DOC) (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
  - Calcium Concentration (Ca) (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
  - Magnesium Concentration (Mg) (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
  - Sodium Concentration (Na) (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
  - Chlorine Concentration (Cl) (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
  - Particulate Organic Carbon (POC) mg/kg (measured value, or default value for a typical Dutch water system in cases where a measured data point is missing)
- The PAFvalues worksheet: as described, at the ViewSelect button
- The msPAF chronic worksheet: as described, at the ViewSelect button
- msPAF acute worksheet: as described, at the ViewSelect button.
- msPAF qualitative worksheet: as described, at the ViewSelect button

Always check that your raw input (.csv input file) and the harmonised input in the export worksheet that copies the 'Input data' show an exact match. As a check, one can ‘subtract’ the reported values from the .csv-input values for each cell, so that the resulting worksheet (inputs minus reported outputs per sample line) would show zero-values everywhere, whereas differences would show up as non-zero values. Note: non-zero values may be due to unit-harmonisation, which can be checked as logical cause of order-of-magnitude differences.

The PAFtable shows the calculated values of the toxic pressure (of each substance, both acute and chronic) for all place/time sample codes entered (each line).

The msPAFacute shows the mixture toxic pressure for all place/time sample codes under the modeling "acute", where the numbers give insight into the fraction of species that would be severely affected (EC50 level), and where these values can eventually be presented (summarized) as the colours yellow, orange and red.

The msPAFchronic shows the mixture toxic pressure under the modeling "chronic" for all place/time sample codes, where the numbers give insight into the fraction of species that are affected (exposure above their no-effect level, NOEC), and where these values can be presented (summarized) as the colours blue and green.

The msPAFqualitative shows the mixture toxic pressure qualitatively expressed for all place/time sample codes. It distinguishes the following levels **None, Low, Moderate, High and Very high** pollution, these levels correspond to the classes of chemical pollution described in Figure 2.

4 From output data to interpretation for the WFD targets

4.1 From raw data to a meaningful summary of results

The output of the calculation tool gives the toxic pressure values per place/time per substance. Values for 'acute' are classified into the yellow/orange/red classification, and 'chronic' into blue and green classification. These colours rate the water quality in five chemical pollution classes, rated from none to very high pollution (see Figure 2).

The most important principle and practical utility of the calculation tool is represented in this classification, as the five classes of chemical pollution levels are calibrated to the five Ecological Status Classes of the Water Framework Directive.

These data can be plotted on a map, per substance, per substance group or for the total toxic pressure.

- This shows and ranks the water bodies regarding the likely magnitude of impacts of exposure to the chemical(s) present in the water bodies.

Further, the results per site can be analysed, so as to identify from the PAF- and msPAF tables the rank order of the relative importance of the different substance groups or substances that contributes strongly to the effects caused by a local mixture.

The targets of a water system analysis, and the sets of measured substances, are often not fully consistent and complete, and constant over time. It is recommended to evaluate whether the measured set of substances is representative of the potentially present substances, as derived from local emissions plus upstream sources. Section 2.2 provides methods to help in this evaluation.

4.2 Interpretation for cases where chemicals may be missing

However, the goals of the water system analysis, and the measured substances, are often not 1:1 related, or in other words: complete. The substances being monitored do not have to be complete, as described in Section 2.2 "Representativeness: evaluating locally occurring substances". Also, due to the cost of monitoring, they can be collected in cycles – with years with a more 'complete' compound package alternating with years with a limited package. If there are partially, or not continuously, substance packages that may not be representative, targeted data analysis steps are needed to properly summarize the data.

> For example, if in six years there is an alternation of a large-small-small-large-small-small measurement package, which means that the toxic pressure that is *calculated* will be higher-lower-lower-lower-higher-lower, but that does not mean that the toxic pressure *in the field* varies equally with the calculated values. The toxic pressure in the field will be more constant, if, for example, the land use in the sample years is actually constant. The fact that a lower toxic pressure is calculated in the years with a smaller measurement package must be handled by a good, targeted data analysis and summary. The report by Postma et al. (2021) provides an extensive example of this. It makes sense with a large-small-small measurement package to see the low values as underestimating the actual toxic pressure, which will have the high value for all six years.

When interpreting the results for a water system exploration, it is therefore important that the user properly summarizes the data in Excel into a spatial map image of the toxic pressure mixture. This can be done by carefully looking at what data is available, how the substance packages differ or do not differ between places or moments. The user can then make a targeted analysis to properly summarize

the data. If a map image of the toxic pressure mixture is shown, that map image should provide a summary of the ecotoxicity of the water system and needs to be best interpreted to the best of the ability of the assessor. The interpretation is therefore "more" than calculating the toxic pressure per line in the input database: the entire map, with knowledge of the hydrological relationships and knowledge of the economic activities (sources of emissions) in relation to the toxic pressure variation, is the basis for interpretations.

4.3 Different goals of the water system analysis

4.3.1 Questions

For chemical contaminants, the water system analysis should be helpful in understanding the possible problem with chemical pollution:

- The "where questions":
  - Where does it occur?
  - Where does it come from?
  - Where is it going?
- The "when questions":
  - Are there any trends over time?
  - Are they increasing?
  - Are they decreasing?
- The "what to do questions":
  - Where and when are measures needed?
  - Which sites need protection, in the context of novel economic activities/emissions, and which sites need remediation because of yellow/orange/red classifications of chemical pollution classes?
  - Which measures can be used (cost) effectively?
  - Against which substance groups and/or substances?
  - Can measures also be taken at the level of land use, because substances are often emitted together?
- The “were measures effective -question”.
  - Was a measure against a point source effective? E.g., an improved WWTP-treatment technique.
  - Was a measure against a diffuse source of emissions effective?

All these possible goals also determine the way in which (first) the monitoring is set up, and (b) the raw output data is analysed. The next sections provide some example results, obtained with application of the calculation in a water system analysis.

4.3.2 Spatial variation in toxic pressure: map images as the end result

Figure 20 shows recent maps of toxic pressure in the Netherlands (data 2013-2018). This illustrates the summary of results as a map image of a water system analysis. A map mainly answers questions about the places where the toxic pressure is increased, and which substance groups contribute most strongly to this.

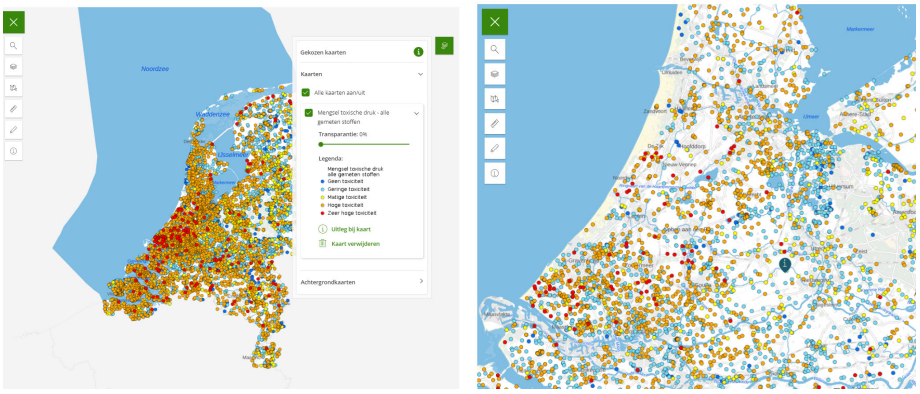


Figure 20. Examples of presenting the summarized results of toxic pressure calculations as spatial maps. Left: all of the Netherlands. Right: detailing for 'low Netherlands'. Source: Atlas of Natural Capital and SFT2 website. These maps are zoom-able, and one can put focus on specific compound groups. Note: the mapped results are always an under-estimate of the local toxic pressure, as not all chemicals that are locally present may have been part of the monitoring results. It is important, to check whether the under-estimation may be negligible (major compounds have been measured), or not.

4.3.3 Trend analysis as the end result

New economic activities can lead to a deterioration in water quality, and measures can be taken to improve it. It is therefore desirable that the Key Factor Toxicity analyses can provide insights into changes over time, to evaluate either deterioration trends or efficacy of measures. **Figure*21*** shows an example, where water quality has improved since 1980. The variation around the trend line is caused by, for example, the weather (wet and dry years, with more and less leaching of metals from the soil and more and less dilution)

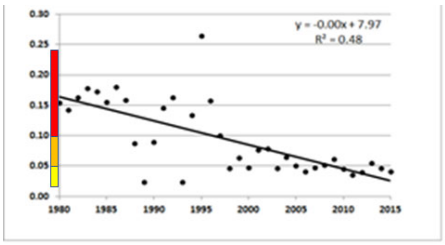


Figure21. Improvement of water quality at a single monitoring point in De Dommel, caused by long-term measures against metal contamination at the landscape scale. The measures appear to be effective, as the metal toxic pressure declines over time.

Colophon

The Guidance for Version 1.0 of the calculation tool was written as part of the Project Toxicity of the Knowledge Impulse on Water Quality, and it is now updated for Version 2.0 because of questions posed to the Helpdesk and user-experiences. In that Knowledge Impulse, the national Dutch government, the Dutch provinces, the Dutch water boards, the drinking water companies and various knowledge institutes have been working together to gain more insight into the quality of groundwater and surface water and the factors that influence this quality. This was aimed at informing water managers and other parties to take the right measures to improve water quality and increase biodiversity.
In the program, parties brought together existing and new knowledge and made this knowledge (better) applicable for practice. In this way, they strengthened the basis of the water quality policy. The program started in 2018 lasted four years. It was funded by the Ministry of Infrastructure and Water Management, STOWA, water boards, provinces and drinking water companies.
The content of this Guidance (for Version 1.0) has been submitted for peer review to various experts and end users in the past. Their suggestions for improvement have been incorporated into Version 1.0. The updated approaches in version 2.0 have been checked internally by RIVM-colleagues that were not involved in the project/design. The updated approaches will be evaluated by end users.
The knowledge presented in this note is based on the most recent insights in the field. Nevertheless, when applying them, the results should be considered critically at all times. The author(s) and STOWA cannot be held liable for any damage resulting from the application of the ideas from this publication.

Version control

Updated versions of this document shall be published as necessary. This happens when knowledge and insights increase, and the calculation tool is adjusted, or when the usage change is tightened (respectively version 1.0, 2.0, 3.0, etc., and 1.1, 1.2, ... 2.1, 2.2, etc.).

- Version 2.0, August 1, 2024

Literature

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