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Toxic pressure in the Dutch delta measured with bioassays

Trends over the years 2000-2009

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Rijksinstituut voor Volksgezondheid
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Abstract

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Trends over the years 2000-2009

During the years 2000-2009, the effects of toxic substances on the ecosystem in Dutch inland waters were measured with a complementary method, i.e., by means of so-called bioassays. This approach provides information on the effects of unknown chemicals in water which are overlooked by traditional analytical techniques. The latter cover only a small portion of the large number of chemicals that are present in surface water. Moreover, classical chemical techniques do not provide any insight into the auxiliary effect that several toxic substances may have.

The results from the bioassays confirm that damage to the aquatic ecosystem during the last decade (2000-2009) due to the presence of toxic substances has decreased, with improved water quality as a result. Toxic pressure in the river Rhine in the year 2000 was already very low but has decreased yet further. Toxic pressure in the water of the rivers Meuse and Scheldt was significantly higher than that in the river Rhine ten years ago, but has also decreased in the last decade. The results also indicate that the toxic pressure is higher upstream and decreases downstream.

Bioassays measure the reaction of five organisms to toxic chemicals in the water. Trends became apparent when the results of five bioassays in several water bodies collected over ten years were combined. Information derived from these data is more accurate because the multitude of data has reduced the spread in the outcome.

Responses to the bioassays provided insight into the nature of the toxic compounds. The cocktail of toxic substances in the river Rhine was found to consist of non-polar chemicals, i.e., substances without a specific mode of action which affect all aquatic organisms. In the other rivers, pesticides are probably responsible for the observed effects. In the summer of 2002, the river Meuse must have been polluted by both known (albeit banned for more than ten years at that time) and unknown herbicides, as revealed by a comparison of chemical measurement and bioassay results.

Key words:
bioassays, trends, toxic pressure, inland waters, the Netherlands, last decade

Rapport in het kort

Toxische druk in de Nederlandse Delta, gemeten met bioassays
Trends over de jaren 2000 - 2009

Van 2000 tot en met 2009 zijn met behulp van een additionele methode, zogeheten bioassays, de effecten van giftige stoffen op het ecosysteem in Nederlands oppervlaktewater gemeten (toxische druk). Deze methode geeft meer informatie over de effecten van onbekende chemische stoffen in water dan de traditionele chemische technieken. Deze meten namelijk slechts een klein deel van het grote aantal chemicaliën dat in oppervlaktewater zit. Bovendien geven ze geen inzicht in het eventuele versterkende effect dat meerdere stoffen bij elkaar kunnen hebben.

De bioassays bevestigen het vermoeden dat het ecosysteem in water het afgelopen decennium steeds minder door chemische stoffen is aangetast, waardoor de waterkwaliteit is verbeterd. De toxische druk in het water van de Rijn was in 2000 al gering en neemt verder af. In het water van de Maas en de Schelde was de toxische druk tien jaar geleden aanmerkelijk hoger, maar die is sindsdien flink afgenomen. Ook blijkt het oppervlaktewater in Nederland stroomafwaarts minder giftige stoffen te bevatten.

Bioassays peilen de reactie van vijf levende waterorganismen op chemische stoffen in het water. Door alle gegevens van tien jaar metingen met bioassays te combineren, worden trends duidelijker zichtbaar. Bovendien zijn de resultaten nauwkeuriger, want de veelheid aan data verkleint de spreiding in de uitkomsten.

De reacties van de bioassays geven inzicht in de soort chemische stof. Zo wordt duidelijk dat de Rijn voornamelijk ‘niet-polaire’ stoffen bevat, oftewel stoffen zonder specifieke werking waarop alle organismen reageren. In de andere rivieren zijn bestrijdingsmiddelen waarschijnlijk verantwoordelijk voor de waargenomen effecten. In 2002 bleek dat de Maas tijdens de zomer sterk verontreinigd moet zijn geweest met bekende (hoewel al meer dan tien jaar verboden) én onbekende onkruidbestrijdingsmiddelen. Dat laatste werd duidelijk door chemische metingen met bioassays te vergelijken.

Trefwoorden:
bioassays, trends, toxische stoffen, zoet oppervlaktewater, Nederland, afgelopen decennium

Summary

Since the year 2000 the effects of toxic substances in Dutch inland water bodies have been monitored with a set of bioassays. Toxic effects have diminished in water sampled from the rivers Rhine, Meuse, and Scheldt. This trend seems to be a continuation of the improvement observed in the monitoring program of the preceding decade. Differences between the rivers indicate that the unknown cocktail in the rivers Meuse and Scheldt causes more effects in the bioassays than water sampled from the river Rhine. The nature of the toxic cocktail is also different. The river Rhine seems to be more affected by non-polar organic chemicals with a non-specific mode of action, whereas organic substances with a more specific mode of action are probably more dominant in the other rivers. The river Meuse seems to be contaminated by insecticides and the river Scheldt by herbicides. The multitude of data allowed regrouping of the bioassay results on a monthly basis, and a seasonal pattern is apparent. There is an increased effect during the summer that can only be ascribed to an enhanced presence of herbicides. This pattern is most pronounced for the river Meuse but is also noticeable for the river Rhine.
Decreased toxic effects observed at downstream locations of these rivers may possibly be ascribed to mixing with rather clean run-off water and water from small brooks and ditches. Removal mechanisms (sedimentation, volatilization, and (bio)degradation) may also play a role.

Measuring exclusively the effects of toxic substances in surface water can be used in addition to, or as an alternative for, traditional chemical techniques. Chemical monitoring has several shortcomings. It can never cover the large number of chemicals potentially present in surface waters, and *combined* effects of substances (synergistic, additive or antagonistic) are not included. The biomonitoring procedure consists of a method to extract and isolate the toxic fraction from surface water. Subsequently, the (acute) effect of toxic substances is determined by means of a battery of organisms from different trophic levels. Such an approach makes it feasible to interpret the data using a species sensitivity distribution to indicate the potentially negative influence of toxic substances on the ecological status. In the Netherlands, the experimental procedure for monitoring by means of bioassays has not been changed during the past decade and has been applied within the framework of a monitoring program carried out for the last ten years. The results offer an excellent opportunity to evaluate the trend in toxic pressure in Dutch freshwater water bodies.

A comparison between chemical monitoring and biomonitoring in the river Meuse demonstrated a synchronous pattern between the herbicides Diuron and Atrazine on one hand and effects determined with the Pulse Amplitude Modulation (algae) bioassay (PAM) on the other. It also revealed a period of six months in the year 2002 that appeared to be highly toxic for the PAM (algae) bioassay and which could not explained by the measured concentrations of Diuron and Atrazine. Another herbicide must have been present at relatively high concentrations during that episode, which is not unlikely given that the two above-mentioned herbicides have been banned for ten years.

Conclusions: The introduced methodology to analyze bioassay data over a longer period enables assessment of the damage in Dutch aquatic ecosystems due to the presence of unknown toxic substances. The data show an acceptable variance and provide environmental policy-makers with information that is fairly interpretable. Continuation of biomonitoring of the river Meuse is necessary to compliment chemical monitoring as a means to safeguard water quality. The biomonitoring

frequency of the river Rhine may be lowered. Sampling locations downstream on the major rivers and the frequency of sampling may be reconsidered.

1 INTRODUCTION

Water quality is traditionally measured by means of chemical analyses of a selected list of chemicals, in accordance to the principles of the so-called 'Good Chemical Status'. This approach has several disadvantages, including the problem of the number of chemicals present in surface water being much larger than the number of chemicals that can be analyzed. In addition, the effects of combinations of substances are rarely known. Measuring net toxicity by means of bioassays performed with sentinel organisms is useful as complementary method because it can be applied to monitor the combined effects of all (unknown) substances present, including interactions. When such monitoring can be conducted so as to quantify the fraction of species that might be affected by an unknown mixture, there is a link to the Water Framework Directive concept of Good Ecological Status – specifically to the difficulty of handling 'unknown' chemical mixtures in this context.

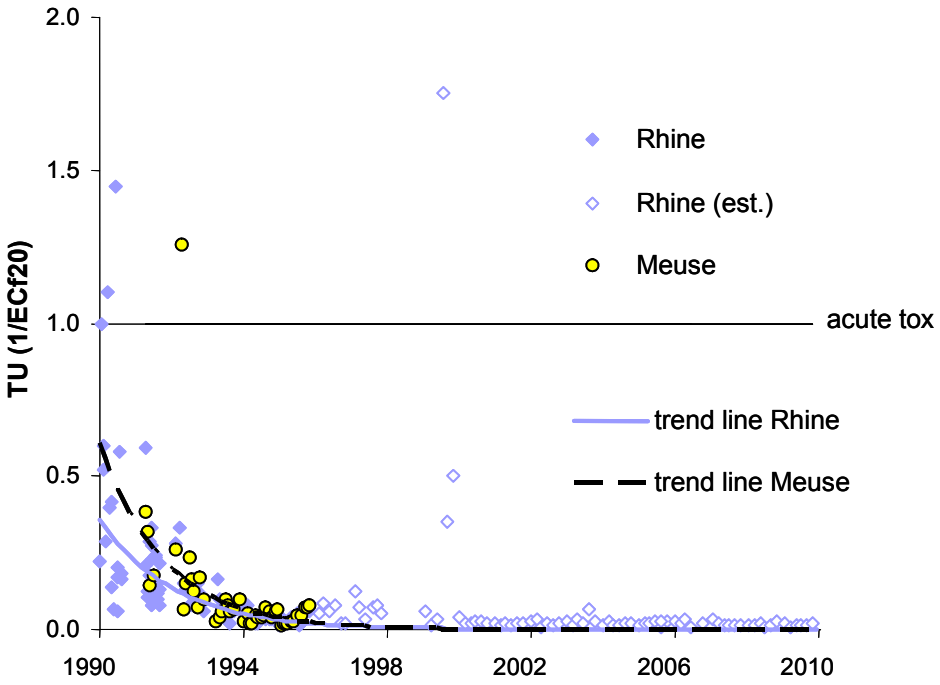

Figure 1 Trends in toxicity in Toxic Units (TU)[1] in the rivers Rhine and Meuse derived from Microtox® bioassays; Rhine (est.) refers to extrapolated data

Toxicity data determined with one bioassay, i.e. the Microtox® test, have been collected for more than two decades. The trend lines in Figure 1 show that during the first half of the 1990s the net toxicity in the rivers Rhine and Meuse decreased to a large extent. They also show that the river Meuse appears to have been more

[1] Toxic units in Figure 1 are calculated from ECf20 data. ECf20 means the factor by which the water sample has to be concentrated to observe 20 % effect. Because these ECF20 data are different from the ECf50 endpoints measured nowadays, ECf20 data were used that rely on overlap data. A river-specific conversion factor was derived from these data. Data specific for the river Rhine were used in Figure 1.

toxic than the river Rhine during that time period. Hence, raw data with a single sentinel species can show clear trends in net ecotoxicity of unknown mixtures. Given the known success in providing summary information on net mixture toxicity during the 1990s (Figure 1), it was decided to run further biomonitoring for the next ten years. During the past ten years, toxicity has been monitored in Dutch inland waters on a regular basis by the National Institute of Public Health and the Environment (RIVM) in cooperation with the Centre of Water management (formerly called Institute for Inland Water Management and Waste Water Treatment). At the respective locations where the rivers Rhine and Meuse enter the Netherlands, samples were collected 6 times per year, each year, for 10 years. 14 other locations were also monitored bimonthly, but only during 3 years of this 10-year period. Consequently, a valuable dataset has been collected which provides the opportunity to analyze trends over the last decade and to make a comparison with other information. Several approaches are possible:

1. time series can be used to determine a trend in toxic effects measured in individual bioassays;
2. the median value of 30 bioassay results in a year (6 samples, each tested with 5 different bioassays) can be analyzed, which gives only 10 points per decade with confidence margins;
3. pT, which has been named 'toxic potency' in the past but is now referred to as toxic pressure, can be calculated from 5 bioassays according to the SSD technique (Species Sensitivity Distribution, see Posthuma et al., 2002) for each individual water sample.

pT, which is a model construct relying on toxicity measurements, has a great advantage because of a low uncertainty regarding interpretation. The 'fraction of disappeared species' or 'potentially affected fraction of species' is easier to grasp than results of bioassays in terms of 'concentration factors' or 'toxic units'. By looking at trends represented by toxic pressure data it is possible to interpret whether there is an up- or downward trend in the potential of a water sample to be toxic to sentinel organisms (and thus also to local species), in either space or time.

Durand et al. (2009) recently evaluated the methodology for toxicity measurements in water samples by means of bioassays. The procedure for preparing water samples to enable assessment of toxic pressure has not been changed since 2000. Briefly, after the organic substances are concentrated using solid phase extraction, the substances are eluted with acetone. The acetone is then removed by distillation, with the aim of returning the organic substances into an aqueous environment – but in a concentrated form. We should be aware of the restrictions of the method: it is not suitable for metals in water. 5 bioassays are carried out to determine the toxicity of the concentrate. The toxicity of this unknown cocktail is determined by diluting the concentrated sample to an appropriate extent to determine the 50% effect in each toxicity assay. The toxicity of the sample is then expressed as the concentration factor of the original water sample that corresponds with this observed effect. For example, an ECf50 of 10 determined in a bioassay means that 50% of the organisms in the test exposed to a tenfold concentrated sample show an effect, while an ECf50 of 100 indicates an effect after the original sample has been concentrated 100-fold. The data are then analyzed according to SSD to estimate a single value for pT of this unknown mixture, which represents the percentage of species exposed above their chronic no-effect concentration (NOEC), according to De Zwart and Sterkenburg (2002). Note that throughout this report toxic pressure in water samples refers to organic chemicals that may contribute to toxic effects on bioassays. For a more detailed summary of this conventional pT method, the reader is referred to Appendix F.

In this report, several approaches are used to analyze the data and visualize the trend in toxic pressure in freshwater during the last decade.
The main questions to be answered are:

- Which trends in time (years), season or space are visible? Has toxicity decreased over time (have measures taken in the past had any effect and/or are measures needed)?
- What is the added value of biomonitoring toxic pressure in inland waters with respect to conventional chemical monitoring?
- How can future monitoring be optimized in a cost-effective manner to provide environmental policy-makers with sufficient information to manage water quality?

2 METHODS

Water samples were taken and processed according to an extraction procedure described by Durand et al. (2009). The raw data for this study derived from each water sample are the so-called 50% effect concentration factors, ECf50, determined with five bioassays. The concentration factor is the factor the original water sample has to be concentrated to observe an acute toxic effect. The ECf50 is thus the factor the original water sample has to be concentrated to observe an acute toxic effect. Higher ECf50 values indicate lower toxicity. The five bioassays (listed below) are selected to represent three important trophic levels in an aquatic ecosystem (bacteria, algae, and invertebrates):

- Microtox® (bacteria);
- PAM (algae);
- Thamnotoxkit F™ (crustaceans);
- Rotoxkit F™ (rotifers);
- Daphnia IQ (crustaceans).

2.1 Logistic aspects

The underlying rationale for the period selected for analyzing trends, the different time frames in the considered sampling series and the choices made in the treatment of data are given below.

2.1.1 Period

Since the early 1990s water quality in the Netherlands has been regularly assessed within the framework of a biological monitoring program using a procedure which has similarities to the current methodology. In 2000 a new procedure was introduced to extract micro-pollutants from freshwater samples (Durand et al., 2009).
This revised procedure has proven to be more efficient in terms of hydrophobic (narcotic) chemicals (Struijs and Van de Kamp, 2001). Consequently, results before 2000 are less suitable to include in a trend analysis because the extract of the unknown mixture of organic contaminants in a water sample would have had a different composition and effect in bioassays due to differences in the extraction procedure. Therefore, data obtained prior to 2000 are less comparable with data obtained during the last ten years and therefore unsuitable for inclusion in a trend analysis. Figure 1 suggests that the largest change in water quality must have occurred in the decade immediately preceding 2000.

2.1.2 Locations and sampling frequency

All samples were taken at locations in the catchments of the rivers Meuse, Rhine, and Scheldt. These locations are given per river catchment in Table 1 in the order of upstream (border) to downstream. In bold are the locations where rivers enter the Netherlands and coordinates X and Y refer to a special 'Dutch only' grid (RD; Rijksdriehoekscoordinaten[2]). Appendix A provides a topographical map with river catchments and sampling locations.

Samples were taken 6 times per year. Only the border locations for the rivers Rhine and Meuse (Lobith and Eijsden, respectively) were sampled every year; sampling at

[2] In the RD grid, the east–west coordinate has a value between 0 and 300 and the south–north has a value between 300 and 620. The Onze Lieve Vrouwen church in Amersfoort is the 'center' and has the coordinate 155000;463000

the other locations occurred at 2- or 3-year intervals in the 10-year period covered by this analysis. The sample scheme is given in Table 2.

Table 1 Characterization of sample sites in the Netherlands

Location	Basin	X	Y	Name/characterization
Lobith	Rhine	203500	429750	border Germany
Wolderwijd	Rhine	166990	484771	lake (IJssel Lake)
Vrouwenzand	Rhine	155400	535900	lake (IJssel Lake)
Markermeer	Rhine	143610	504350	lake (IJssel Lake)
Ketelmeer	Rhine	172600	513700	lake (IJssel Lake)
Nieuwegein	Rhine	136180	448300	canal (Lek)
Maassluis	Rhine	76700	437253	canal (Nieuwe Waterweg)
Amsterdam	Rhine	122374	488080	Northsea canal
Eijsden	Meuse	177000	310000	border Belgium
Belfeld	Meuse	205750	370220	upstream of a barrage
Keizersveer	Meuse	121070	414560	Bergse Maas
Bovensluis	Meuse	93200	411900	lake (Hollands Diep)
Haringvlietsluis	Meuse	63400	427600	lake, sluice
Steenbergen	Meuse	75750	406440	lake (Volkerak)
Schaar van Ouden Doel	Scheldt	75825	374070	border Belgium
Sas van Gent	Scheldt	44241	359102	canal

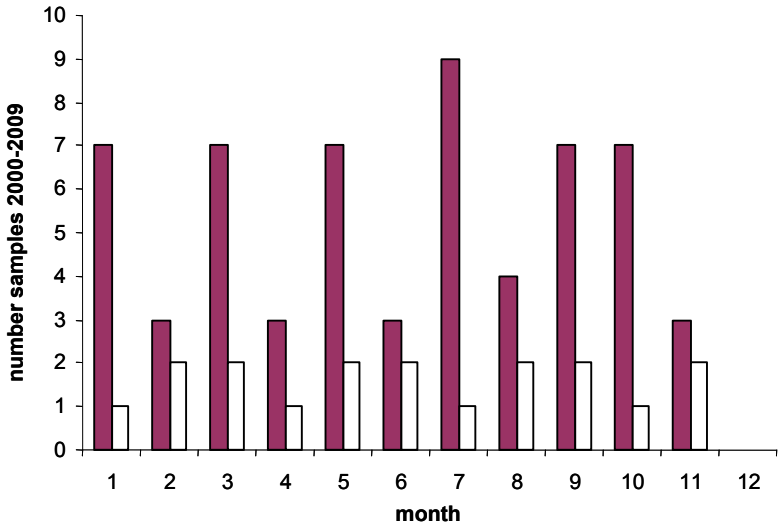

Figure 2 Number of samples collected on the Dutch border per site distributed over the months January to November (1-11). Solid bars (total N = 60) for Rhine and Meuse each; open bars (total N = 18) for the river Scheldt.

Figure 2 shows that the sample scheme was bimonthly but not regularly applied, due to logistic reasons. No sample was ever taken in the month of December (month 12).

Table 2 Sampling scheme (bold: the locations where rivers enter the Netherlands)

Location	00	01	02	03	04	05	06	07	08	09
Lobith	6	6	6	6	6	6	6	6	6	6
Wolderwijd						6			6	
Vrouwenzand	6				6			6		
Markermeer	6				6			6		
Ketelmeer	6					6			6	
Nieuwegein	6									
Maassluis	6						6			6
Amsterdam							6			6
Eijsden	6	6	6	6	6	6	6	6	6	6
Belfeld	6				6					
Keizersveer	6				6			6		
Bovensluis						6			6	
Haringvlietsluis	6					6			6	
Steenbergen	6					6			6	
Schaar v Ouden Doel	6					6			6	
Sas van Gent							6			6

2.2 Treating bioassay data

The ECf50 value for a specified bioassay is dimensionless and characterizes the unknown toxic cocktail in a surface water sample with respect to that bioassay. It is the analog of the toxicological effect parameter EC50 or LC50 (effect, respectively, letal concentration for 50 % of the organisms with respect to a test organism) in units of chemical concentration. As only acute effects can be measured in the bioassays used, ECf50 values always pertain to acute effects. Durand et al. (2009) provide detailed information on the experimental procedure. The maximum value of the endpoint in a bioassay is a concentration factor of 1000, meaning that a water sample has to be concentrated 1000-fold to find 50% effect in a bioassay. Several calculation methods are employed to aggregate bioassay data of freshwater samples.

2.2.1 Species Sensitivity Distribution of concentration factors

The method described earlier (see Durand et al., 2009, among others) is referred to as pT or conventional pT and is distinct from 'trend-pT' which is introduced in this report for the first time.

Conventional pT or point-estimated pT*.* According to Durand et al. (2009), the pT of one water sample is calculated from five ECf50 values by means of the SSD method (see Appendix F for a summary of this method). This procedure results in one pT value per sample; however, there is a wide confidence interval which varies widely and apparently haphazardly. This is a statistical effect of curve fitting procedures based on only 5 organisms rather than on an ecological phenomenon. For the rivers Rhine and Meuse on the Dutch border, this approach yields 6 data points each year, which gives 60 pT values over the whole period. All of these 60 pT points have, however, wide confidence intervals (see Figure 5).

Trend-pT. The aim of this new procedure is to reduce the confidence limit in pT in order to obtain results more useful for environmental policy-makers. One disadvantage to this approach is that the method is only applicable if time series of ECf50 are available. The 5 log-transformed concentration factors (ECf50) of a specific sample are considered to be normally distributed (De Zwart, 2002). In addition, the shape of the SSD with respect to a river is assumed to remain stable

(constant) over the whole period of 60 sampling dates (10 years). This is a valid assumption if the characteristics of the river under consideration at the sampling site (e.g. the river Rhine entering the Netherlands) and experimental procedures have not changed to a large extent. The characteristics of a river include physical, chemical, and weather conditions. In this context, chemical characteristics are considered to be constant if the *composition* of the chemical cocktail does not change considerably while the concentration of the cocktail may gradually change in time.
The average of 5 log ECf50 values may show a trend over the 60 sampling dates which can be identified. The combination of this trend and the presumed constant standard deviation of 5 data sets on log ECf50 provides a possibility to compute pT with uncertainty margins that are interestingly narrower than those obtained when calculated according the conventional pT method (per sampling date): instead of a two-dimensional SSD curve, a three-dimensional curving plane is examined in an integrated time series (see for a more detailed explanation Appendix B). Where this curving plane is dissected at location log ECf50 = 1 (a concentration factor of 10), perpendicularly to the axes of log ECf50 and year, areas are obtained that separate the unprotected from the protected fraction of species in the set of distribution functions. These areas diminish over time when toxic stress decreases, as shown for the river Meuse in Figure B1 of Appendix B. These demarcated areas indicate the fraction of species that experience effects at a concentration factor below 10 due to chronic exposure, which essentially is pT. Applying this technique, the trend-pT (e.g. the right panels of Figure 6) is calculated as a function of time, with uncertainty envelopes that are computed from the uncertainty in both the trend line (*location*) and the *shape* of the distribution (Figure B1 in Appendix B). These uncertainty envelopes will become narrower as pT decreases over time. With lower pT, the uncertainty in pT appears to be considerably lower than that indicated by the 5%-95% confidence bars as conventionally determined for a single point *not* being part of a time series.
This technique will, however, fail to see a trend if pT is very low. In the year 2000, pT in the river Rhine was already far below 1% (Struijs et al., 2000). For such a low toxic pressure, (log-transformed) ECf50 data could be used according to Equation 1 to see a trend in the river Rhine.

2.2.2 *The median of all concentration factors in one year*

All ECf50s of a specific sample location can be aggregated for 1 year by calculating the *median*. This approach aggregates 6 samples and is based on 30 bioassays results (5 bioassays in 6 samples). The approach may be satisfactory for analyzing long-term trends. For Eijsden (Meuse) or Lobith (Rhine), this approach gives 1 data point per year or 10 points in the considered monitoring period of one decade. All ECf50s are log transformed and averaged and the 95% confidence limit subsequently computed. Assuming that the set is log normally distributed, the median ECf50$_k$ for year *k* is calculated as:

$$\text{median}\, ECf50_k = 10^{\overline{\log ECf50_k}}$$

$$\overline{\log ECf50_k} = \frac{\sum \log ECf50_k}{n_k} \qquad \textbf{Eqn 1}$$

where n_k (≤ 30) represents the number of bioassays for a location in year *k*. In 2006 and 2007, the set of bioassays was incomplete because Rotoxkit F™ and Thamnotoxkit F™ bioassays failed in 2 out of 6 samples taken from the rivers Rhine

and Meuse. Therefore, all bioassays of these samples were rejected, leaving n_k = 20 (instead of 30) during the years 2006 and 2007.
An increasing ECf50 indicates a decreasing toxicity and, therefore, we may use the reciprocal of median ECf50 to indicate toxic units (TU). For a series with high ECf50s, both conventional and trend-pT may be too low (for example pT < 0.1%) to show a trend over time. In such cases, the median ECf50 (or TU) with only 1 point per year is a good alternative to indicate a trend. The method of median ECf50 is also applied to compare different locations with each other.

2.3 Influence of seasons and weather

2.3.1 Season dependency

Emissions due to agricultural and industrial activities are expected to vary with the season, with the former being more dependent on the season than the latter. The application of pesticides produces a chemical load that is more strongly dependent on season than industrial activities, which show reduced emission during the summer holidays.
The season dependency of flow rates of the river Rhine, Meuse or Scheldt is accounted for by taking the median flow rate in month *m*. This step is performed to avoid distortion by outliers in the daily flow rate for each specific month. The median flow rate for a specific month (*m*) is calculated as the median of all flow rates on a daily basis (which is the average flow rate on a day) in month *m* over the period 2000-2009.

2.3.2 Concentration factors adjusted for the variability of the weather

The main reason for taking the flow rate into account is its high daily variability due to weather conditions. Variations within a month due to weather conditions may deviate by a factor of three from the monthly average or median flow. On the other hand, the seasonal fluctuations of both average (or median) flow rate and chemical emissions are slow and moderate in amplitude. The time window of seasonal variations is in the order of months, whereas fluctuations in flow rate due to weather conditions are in the order of days and weeks (see Figure 3). Fluctuations in flow rates are presumably much greater than fluctuations in the chemical emissions. Therefore, exceptional rainfall or drought is conceived as an accidental but natural dilution or concentration factor, respectively, caused by the variability of the weather. A correction for such events may facilitate a better interpretation of the trends.

Daily flow rates of the rivers Meuse, Scheldt, and Rhine were provided by the service desk (servicedesk-data@rws.nl) of the Department of Waterways and Public Works (Ministry of Transport, Public Works and Water Management). The average daily flow rate of the three major rivers on the Dutch borders is then used over the analyzed period and the *median flow rate* calculated for each month (Table 3). The *actual flow rate* at the day of sampling is retrieved from the dataset. In Figure 3, small circles show the actual daily flow rates during sampling in the period 2000-2009. The deviation from the median flow rate of the rivers Rhine and Meuse is most pronounced during the first six months of each year and, therefore, the concentration factor may be adjusted to account for this. For example, if the flow rate on the day of sampling is a factor of two higher than the median flow rate for that month, the toxic cocktail is 'naturally diluted'. This will be measured as a lower toxicity in the bioassays. If not only a sample-specific interpretation is made, but also a broader trend analysis over seasons is needed, the concentration factor derived for bioassay *j* in the laboratory can be corrected by the ratio of the actual daily (FR_a) and median monthly flow rate (FR_m):

$$ECf50_J(\text{corrected}) = ECf50_J \frac{\text{FR}_\text{m}}{\text{FR}_\text{a}} \qquad \textbf{Eqn 2}$$

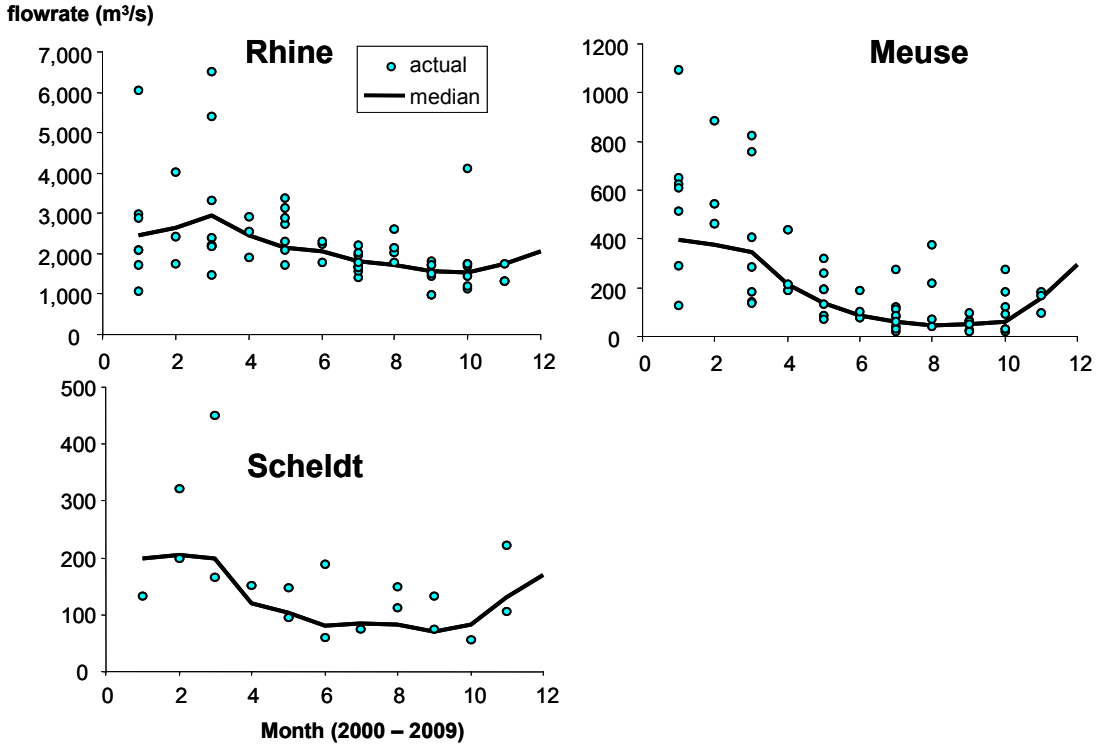

Figure 3 Actual daily flow rates (FR_a) during sampling (circles) and median monthly flow rates (FR_m) represented as drawn lines.

Table 3 Median monthly flow rates (m^3/s) between 2000 and 2009. The first column also provides the number of flow rates from which the median value was computed (in parenthesis).

Month	Rhine	Meuse	Scheldt
January (279)	2594	432	200
February (255)	2709	438	206
March (279)	2919	410	198
April (270)	2471	229	121
May (279)	2199	160	105
June (270)	2077	85	80
July (279)	1787	70	84
August (279)	1731	69	82
September (270)	1633	59	70
October (279)	1620	69	83
November (270)	1793	154	130
December (279)	2023	265	170

2.4 Comparison with chemical monitoring

The Department of Waterways and Public Works (Ministry of Transport, Public Works and Water Management) is responsible for carrying out a Dutch national water quality monitoring program. Within the framework of this program, physical and chemical parameters are measured on a routine basis at various locations, among which are all locations being sampled for the bioassay monitoring. After validation, the data of the monitoring program are made available through the public database Waterbase (DONAR, 2010).
In the ideal situation, trends found in bioassay monitoring are compared with trends found with traditional routine chemical monitoring. However, there is no long-term time evaluation study of integrated chemical data available. Yearly, a general report on water quality is made available by the Ministry of Transport, Public Works, and Water Management (e.g. in Water in Beeld, 2010, with data from 2008).
However, data from individual compounds from the chemical MWTL monitoring program can be used to compare long-term time trends in the concentrations of these individual (DONAR, 2010) compounds with the trends found in the bioassay monitoring. Data for several compounds were downloaded from the Waterbase website – in particular, data on herbicides, insecticides and other organic micro-pollutants. However, for most compounds, no data above the reporting limit have been measured. Only for some herbicides (Diuron, Atrazine, Simazine, and Bentazon) are data above the reporting limit available for comparison with bioassay data.

2.5 Dealing with failures or non-response

For both the rivers Rhine and Meuse, there would have been 300 ECf50 datasets available to analyze the trend (5e bioassays per sample and 6 samples per year over the 10-year period). Due to accidental loss of the sub-samples for the Rotoxkit F™ and Thamnotoxkit F™ bioassays in July and October 2006 and February and April 2007, the dataset was reduced to 280 because all 5e bioassays were eliminated from these 4 samples.

Some water samples apparently contain a toxic mixture that has such a low toxicity for a bioassay that the ECf50 value can only be quantified as 'higher than 1000'. The reciprocal value of such a ECf50 is analogous to the detection limit for an analytical method. Thus, so-called censored data referred to as 'ECf50 > 1000' can be treated as 'below the limit of detection 0.001'. Depending on the fraction of non-response data in a dataset (*frac*), a surrogate value can be evaluated. In most cases, 1 or 2 bioassays out of 5 are censored; thus, *frac* is below 0.5. A substituted value (x_i) for ECf50 > 1000 is calculated according to Swaving and De Vries (2000):

$$x_i = LOD \cdot \left(\frac{LOD}{x_{0.9}} \right)^{frac} \qquad \text{Eqn 3}$$

$$frac \leq 0.5$$

with the limit of detection (LOD) equal to 0.001 and $x_{0.9}$ the 90th percentile of the dataset consisting of reciprocal ECf50 numbers.

3 RESULTS

Those concentration factors for which a 50% effect is found in the bioassays (ECf50) constitute the raw data in this study. For the rivers Rhine, Meuse, and Scheldt, the ECf50s determined for five different bioassays per water sample, are given in Appendix A, together with the sampling date and the flow rate on that date. The median flow rate for the relevant month is also given. The bioassay results from twelve downstream locations (i.e., downstream of the border locations) on the rivers Rhine and Meuse are also listed in Appendix A.

3.1 Trends in time: Rhine, Meuse, and Scheldt

A high concentration factor (ECf50) indicates a state of low toxicity. All ECf50 data obtained using the Microtox®, PAM (algae), and Daphnia IQ bioassays in samples taken from the rivers on the Dutch border were below 1000. An ECf50 occasionally reached the limit of the concentration factor, namely, 1000. Only for the Thamnotoxkit F™ and Rotoxkit F™ tests are some censored data part of the dataset for which, according to Equation 3, a concentration factor higher than 1000 is assigned (substituted data). In 2007, the river Rhine had only 1 test result (out of 6 water samples) that surpassed 1000 (Thamnotox F™), and in 2008, there were more censored data: 5 of 6 samples for the Rotoxkit F™ test and all 6 samples for the Thamnotox F™ test. In 2008 in the river Scheldt, the concentration factor exceeded 1000 in 1 of 5 samples according to Rotoxkit F™ bioassay and in 2 samples according to the Thamnotox F™ test. In the river Meuse, the results of only 2 Thamnotox F™ bioassays (out of 6) surpassed 1000. The substituted concentration factors are presented in bold type in the tables of Appendix A.

The ECf50 data per test are displayed in the reverse mode in Figure 4 on a logarithmic Y-axis to indicate decreasing toxicity with a downwards trend (i.e., with increasing ECf50). In 2 of 6 water samples in 2006 and 2007, the bioassays of Thamnotox F™ and Rotoxkit F™ failed and, consequently, instead of a total of 270 values, Figure 4 depicts 262 ECf50 values for the rivers Meuse and Rhine (see missing symbols in Figure 4).

The trend lines calculated from the scattered points in Figure 4 indicate a decreasing toxicity in all bioassays on samples from the rivers Meuse and Rhine over the past decade, with the exception of that for Daphnia IQ. The results for the Daphnia IQ test are displayed on a linear (non-reverse) scale in Figure C1 of Appendix C. This graph shows that between 2002 and 2005 the toxicity of Rhine water for Daphnia IQ is at least twofold lower than that in the periods 2000-2001 and 2006-2008. For the river Meuse, the difference was almost a factor of 3 in these same periods. Although only results for 3 years are available, a similar pattern for the Scheldt is likely: the average of ECf50 is equal to 284 in 2005 (low toxicity) but to 76 and 98 in 2000 and 2008, respectively. This episode of relatively low toxic pressure for Daphnia IQ can not be explained by changes in the quality of performance in the laboratory (no match with change in personnel or methodology) nor to the quality of the Daphnia IQ cultures and auxiliary materials.
A decreasing toxicity of Scheldt water in all bioassays, including that for Daphnia IQ, is visible in Figure 4, while in the rivers Meuse and Rhine, the toxicity for Daphnia IQ over the whole 10-year period (including the episode of 2002-2005) shows a slight increase. The trend lines in Figure 4 are linear with respect to ECf50, and the curvature is due the logarithmic Y-axis. The period when the Thamnotoxkit F™ and Rotoxkit F™ bioassays failed in the Rhine and Meuse samples are indicated by an X.

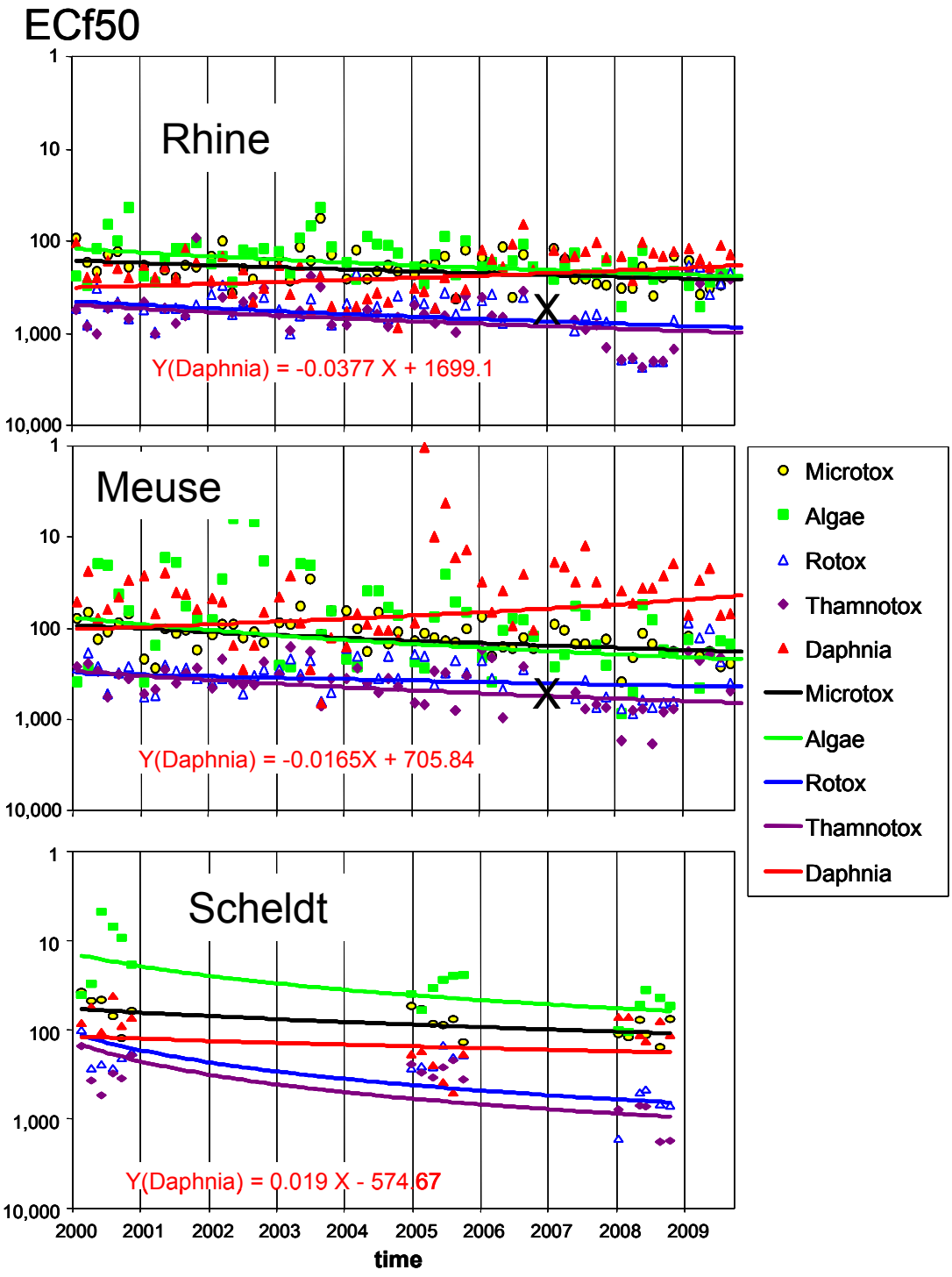

Figure 4 Concentration factors for which a 50% effect is found (ECf50) in five bioassays determined in water samples collected from three rivers where they enter the Netherlands.

The significance of this toxicity is questionable; however, the toxicity for Daphnia IQ clearly does not show a decrease, as in the other bioassays. In the years 2000 and 2001 the average of ECf50 (Daphnia IQ) is 47 for the river Meuse while the average of ECf50 values over the period 2006-2008 is *lower* (41). For the river Rhine these numbers are 199 and 145, respectively, which again may indicate a small increase of toxicity (see Figure C1 Appendix C).

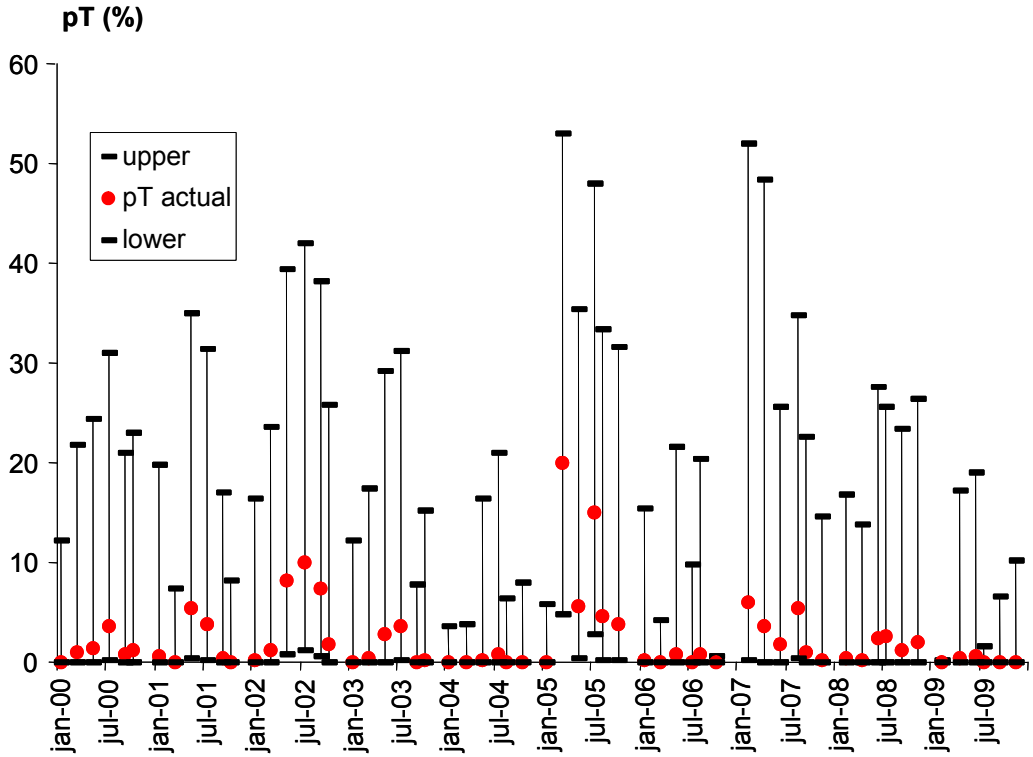

Figure 5 Toxic pressure, in this report referred to as conventional pT (see Appendix F for explanation) of the river Meuse (Eijsden). Error bars are 5%- 95% confidence limits.

Conventional pT is computed from five ECf50 values. Consequently, the 5%-95% confidence envelope is very wide, as shown by Figure 5 for the river Meuse. The wide margins in Figure 5 make the results difficult to understand and almost useless for policy-makers. Treating the raw data (ECf50s) in an alternative manner using the Trend-pT method introduced in this report (right panels of Figure 6) provides an approach to reduce the uncertainty in pT. By taking time into account, the procedure benefits from the fact that the width of the distribution of the five ECf50 values per water sample does not change significantly over the years (see detailed explanation in the preceding section and in Appendix B).

The results of this approach show a declining trend of toxic pressure (trend-pT) in the rivers Meuse and Scheldt (right panels of Figure 6; see also Sas van Gent, Appendix C). The dotted lines in the right panels (Meuse and Scheldt in Figure 6) demonstrate that the 5%-95 % confidence interval is becoming narrower over the course of time, which can also be explained by a decreasing tendency for negative outliers to occur. A declining trend in pT for the river Rhine, however, is not detectable because the pT is too low. As an alternative, a toxic effect at a sampling

location is presented as the median of 30 ECf50 values in a year (with confidence limits). When plotted over several years, as in Figure 7, a declining toxicity is also apparent for the river Rhine.

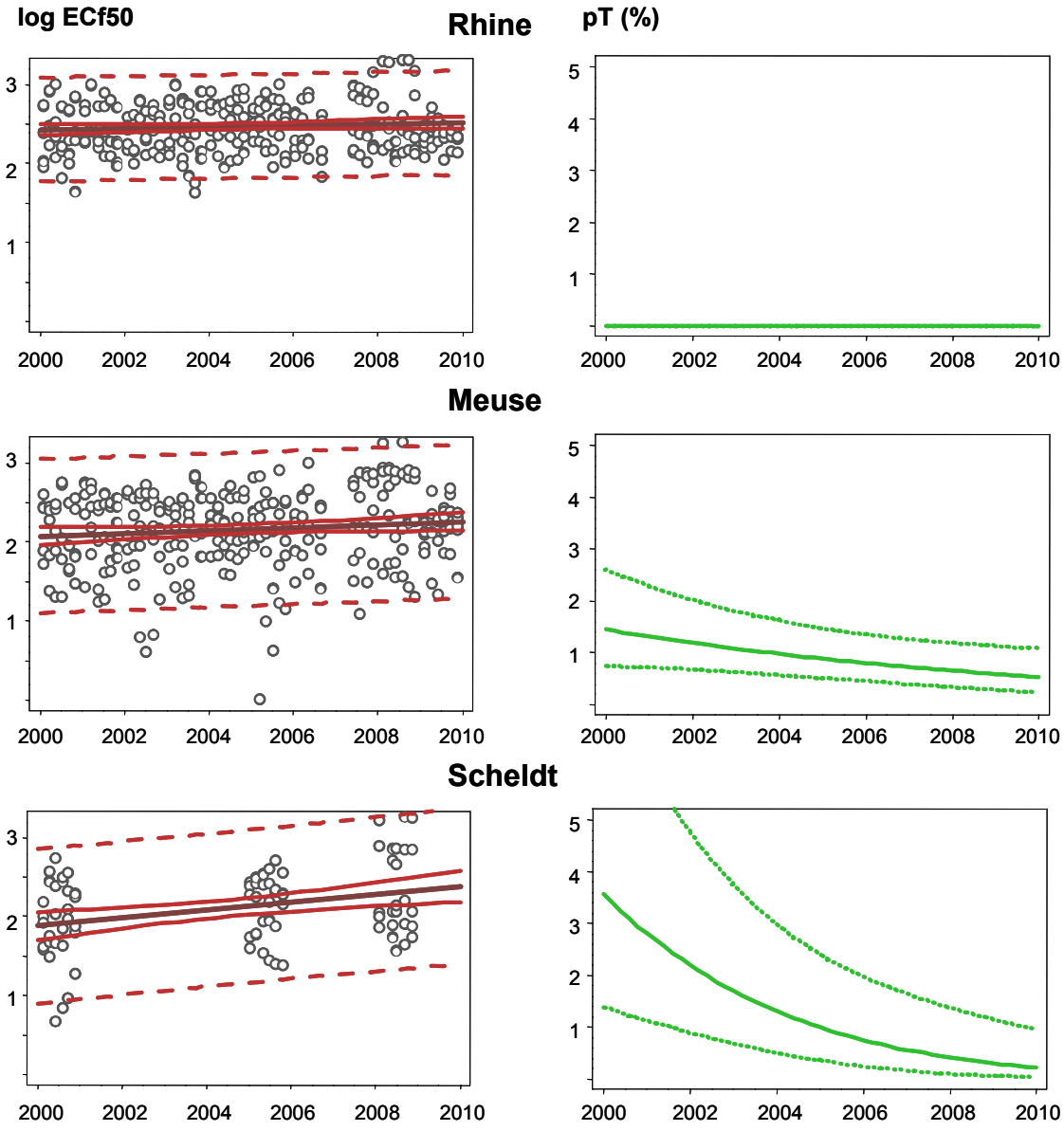

Figure 6 Trends in effect-concentration factors (left panel) and trend-pT (right panel) and uncertainty margins (see Table E1 in Appendix E)

Based on these results, it is possible to draw the conclusion that the toxic pressure was declining in the rivers Rhine, Meuse and Scheld during the first decade of this century.

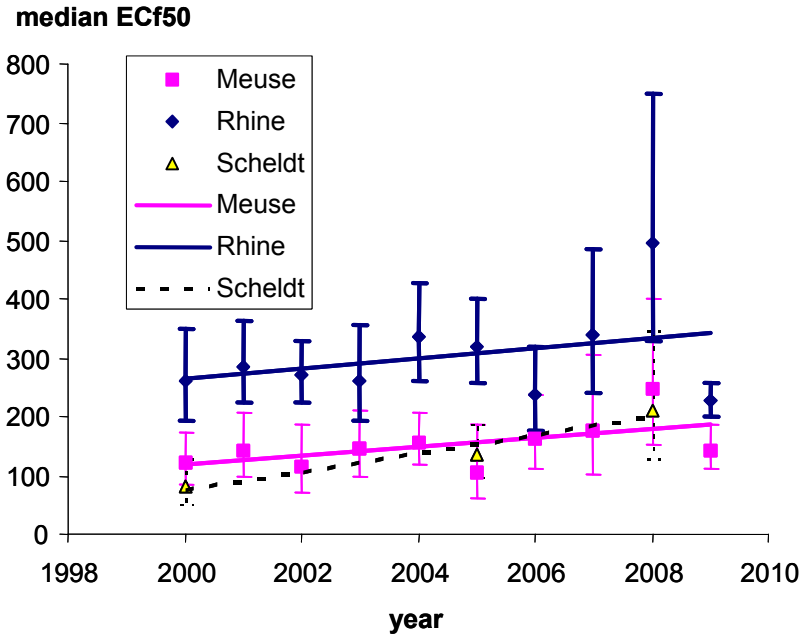

Figure 7 Median effect-concentration factors and trend lines. The vertical bars are 5-95% confidence intervals

3.2 Spatial trends

3.2.1 Difference between the rivers Rhine and Meuse

Figures 4 and 6 (left panel) show that the toxicity of the river Meuse is systematically higher (concentration factors are systematically lower) than that of the river Rhine. It can also be concluded that the nature of the toxicity differs. The narrower spread ($\hat{S}$, see Table E1, Appendix E) in 280 ECf50 values obtained using the different bioassays suggests that, relative to the river Meuse, non-polar organic chemicals are the dominant form of chemical pollution in the river Rhine. Apparently, the cocktail in the river Meuse contains more polar organic compounds and pesticides with a different and more specific mode of action. Vaal and Folkerts (1998) demonstrated that the variability among bioassays is rather high for these type of pollutants, which have a more specific mode of action (which means that one or few types of organisms, such as algae, is extremely sensitive, while other test organisms are not), compared to (non-polar) organic substances with a narcotic (or non-specific) mode of action (all organisms are affected by narcotic chemicals).

Non-polar organic compounds seem to dominate the toxic pressure in Rhine water. These chemicals are more efficiently extracted from the water sample than the more polar toxicants (Struijs and Van de Kamp, 2001). The latter seem to be more relevant for the river Meuse and, therefore, we may conclude that toxic stress is underestimated for the river Meuse and that toxic effects in this river are also underestimated compared to the river Rhine in which toxicity was already low.

3.2.2 Sampling at downstream locations on the major rivers (Rhine and Meuse)

Trends from upstream to downstream are shown in Figure 8 with median ECf50 values from 2004 or 2005 and error bars representing 95% confidence intervals. Because not all locations were sampled in the same year, it is not possible to compare locations within one specific year. Therefore, the years 2004 an 2005 were chosen for this comparison. Time trends per site for these locations and the location on the map are shown in Appendix A. In the river Meuse, the toxicity decreases

from the border (Eijsden) to downstream locations (Belfeld and Keizersveer) and decreases still further at locations where the Meuse water is mixed with (less toxic) water of the river Rhine (Bovensluis, Volkerak and Haringvliet). Figure 8 shows that the toxicity in the river Rhine still declines downstream even though it is low at the Dutch border.

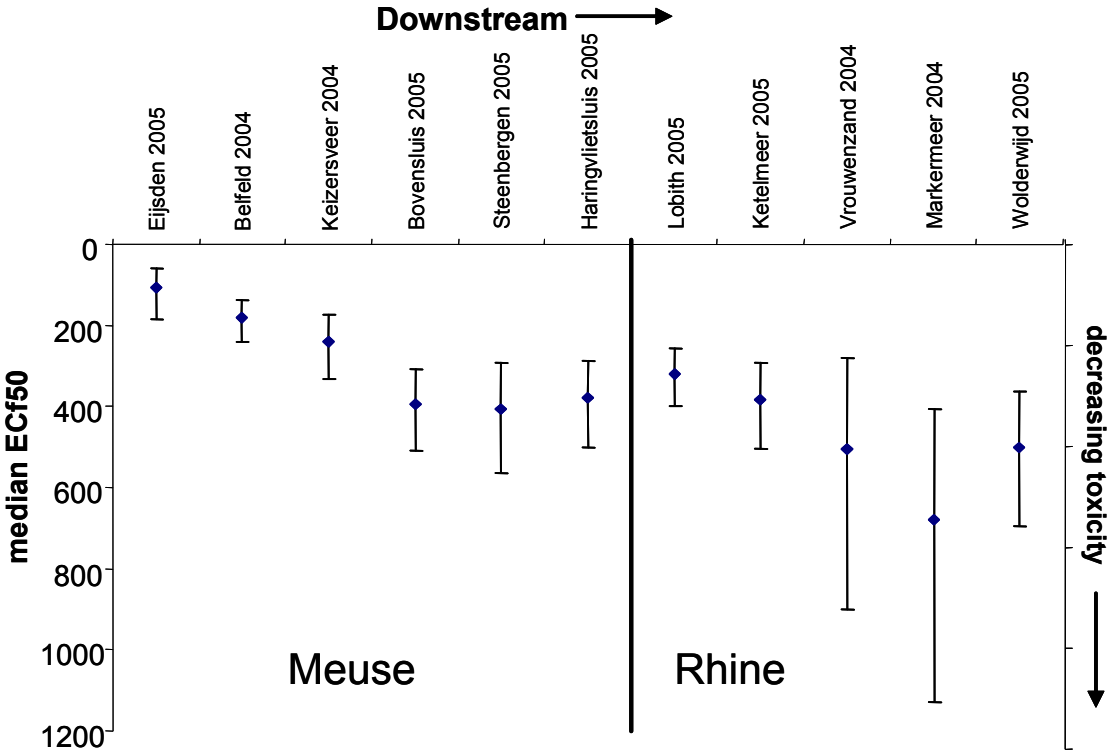

Figure 8 Median ECf50 values decrease downstream. Data were calculated according to Equation 1; error bars represent 95% confidence limits

3.2.3 *Differences in the nature of toxicants between the rivers Meuse and Scheldt*

Although toxic stress in the rivers Meuse and Scheldt is comparable and significantly higher than that in the river Rhine, it is apparently caused by different substances that differ in their mode of action. This can be inferred from a comparison of the *average* ECf50 values of the most sensitive bioassays (Microtox®, PAM (algae), and Daphnia IQ). Despite the fact that samples were taken from the river Scheldt only in the years 2000, 2005 and 2008 and that the average is calculated from 18 ECf50 values per bioassay (compared to 60 ECf50 values for the river Meuse), the results do show a clear pattern. In the river Scheldt, the average values of ECf50 for Microtox® and PAM (algae) bioassays are 86 and 40, respectively, which is lower than that for the river Meuse (137 and 149, respectively). Apparently, the river Scheldt is systematically more toxic for the organisms being tested. For Daphnia IQ, we see the opposite (see Appendix C): the toxicity is higher (average ECf50 = 74) in the river Meuse than in the river Scheldt (average ECf50 = 153). This again indicates differences with respect to the nature of the toxicity between the two rivers.

3.3 Toxicity in Rhine, Meuse, and Scheldt corrected for the actual flow rate

To eliminate as much as possible the factor 'accidental' daily dilution or concentration due to highly varying weather conditions, the ECf50 values can be corrected for the daily flow rate. This step will facilitate the interpretation of long-term trends, eliminating the need to consider the factor daily dilution. Concentration factors for the three rivers were scaled to the flow rate on the day of sampling relative to the flow rate that is typical for the relevant month. The latter is the median of all flow rates for each separate month over the period 2000-2009. The concentration factor adjusted accordingly is denoted as the ECf50 (corrected). In analogy to Figure 4, the results are presented in Appendix D. A common factor linking Figure 4 and Figure D1 in Appendix D is that the ECf50 values for Daphnia IQ have a slight tendency to decrease in the rivers Meuse and Rhine but not in the river Scheldt. Thus, the flow rate correction did not neutralize this tendency. The ECf50 (corrected) is more scattered; however, Figure D2 (Appendix D) illustrates that all data for ECf50 (corrected) for the river Meuse are less skewed than the set of actually measured ECf50 values. In other words, the set of 280 concentration factors (4 series of 5 were eliminated from the analysis because of incompleteness) bears a stronger resemblance to a log normal distribution when all factors are corrected for the flow rate. Nevertheless, a graph of conventional pT (corrected) analogous to Figure 5 is still not useful because of large confidence intervals (not shown). Treating ECf50 (corrected) analogously to Figure 6 (right panel) yielded a trend-pT plot for the river Meuse, as given by Figure 9. This plot indicates that in the year 2000 trend-pT (corrected) is almost a factor of four higher than trend-pT without correction for flow rate (compare Figures 9 and 6). The variability ($\hat{S}$) is larger when the data are corrected for the flow rate. $\hat{S}$ of trend-pT corrected equals 0.599 compared to 0.491 when the ECf50 data are not corrected for the flow rate (see Appendix C).

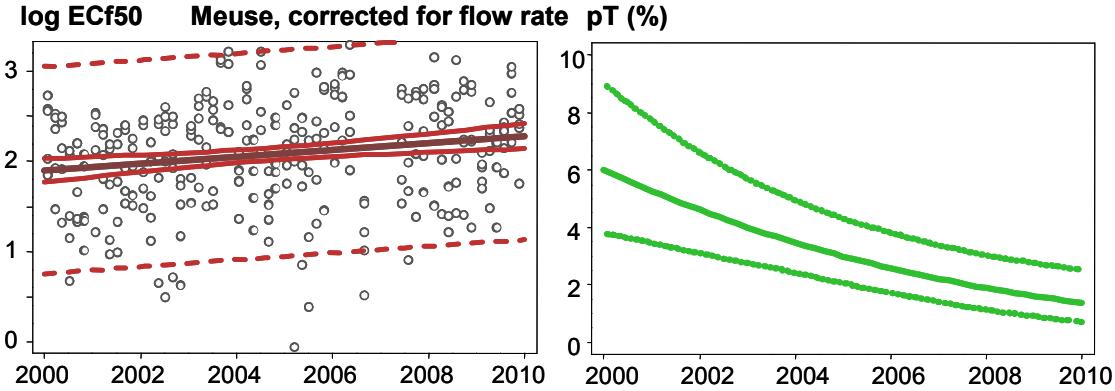

Figure 9 Toxic pressure determined for the river Meuse, trend represented as log ECf50 (left panel) and pT and confidence limits (right panel). ($\hat{S}$ = 0.580; n = 250).

3.4 Seasonal influence

Anticipating that both the flow rates of river water and the emissions of toxicants to the water depend on the season, we compared average monthly ECf50 values for the three rivers over the 10-year period. For the locations Lobith (Rhine) and Eijsden (Meuse), the average is calculated from only 3 measurements in the months February, April, June, and November (see Figure 2). The number of measurements

is indicated in Figure 10 by *n* given for Microtox® but also apply for the bioassays PAM (algae) and Daphnia IQ. Average ECf50 data for the river Rhine are based on actually determined data; average ECf50 (corrected) accounts for the flow rate. Squares represent the average of n original ECf50 data; circles are flow-corrected data.

3.4.1 Rhine

Figure 10 shows that the pattern for PAM (algae) in the Rhine resembles that of the river Meuse (Figure 11). Relatively low ECf50 values are found from May to November, indicating higher toxicity in the summer months. Over the whole year the river Rhine has ECf50 values that are a factor of two or three higher than those for the river Meuse. A seasonal trend is far from pronounced according to the Microtox® and Daphnia IQ bioassays. Scaling to the flow rate does not significantly change this pattern.

3.4.2 Meuse

Figure 11 shows that during the months of February and March the toxicity measured in the Microtox® assay is roughly a factor of two lower than that during the rest of the year. The PAM (algae) test shows a more pronounced 'toxic period' from May until November and an approximately fourfold lower toxicity during the winter and early spring. Flow rate correction reduces the scattering of relatively high ECf50 numbers. This scattering of uncorrected ECF50 values should be ascribed to weather fluctuations during the spring.
For the Daphnia IQ test, the picture is more complicated: a higher toxicity is shown during the first 6 months of the year and probably also during the last 2 months; however, during the months in between, the river Meuse seems significantly less toxic for Daphnia IQ. One month does deviate considerably in the summer period: in August, the ECf50 value is as low as 40 and the ECf50 (corrected) is even 20. This is not an artifact because an ECf50 value of 40 is the average of 104, 17, 25, and 12, while an ECf50 (corrected) equal to 20 is the average of 32, 30, 5, and 12. The flow rate corrected series of ECf50 values in August is even the lowest of the year. Before and after this peak, in July and September, the toxicity for Daphnia IQ is relatively low, which suggests that the emission of substances that are toxic for Daphnia IQ is relatively low from July until October but that in the month of August there could have been an emission that affects Daphnia IQ.

3.4.3 Scheldt

Only a few data points were available to calculate the average ECf50 value for each month (Figure 12). Only the results of the PAM (algae) test show some similarity with those of the other rivers. Remarkably, in terms of both actual and corrected ECf50 values, according to the Microtox® and Daphnia IQ tests the month of October seems to be a non-toxic intermezzo.

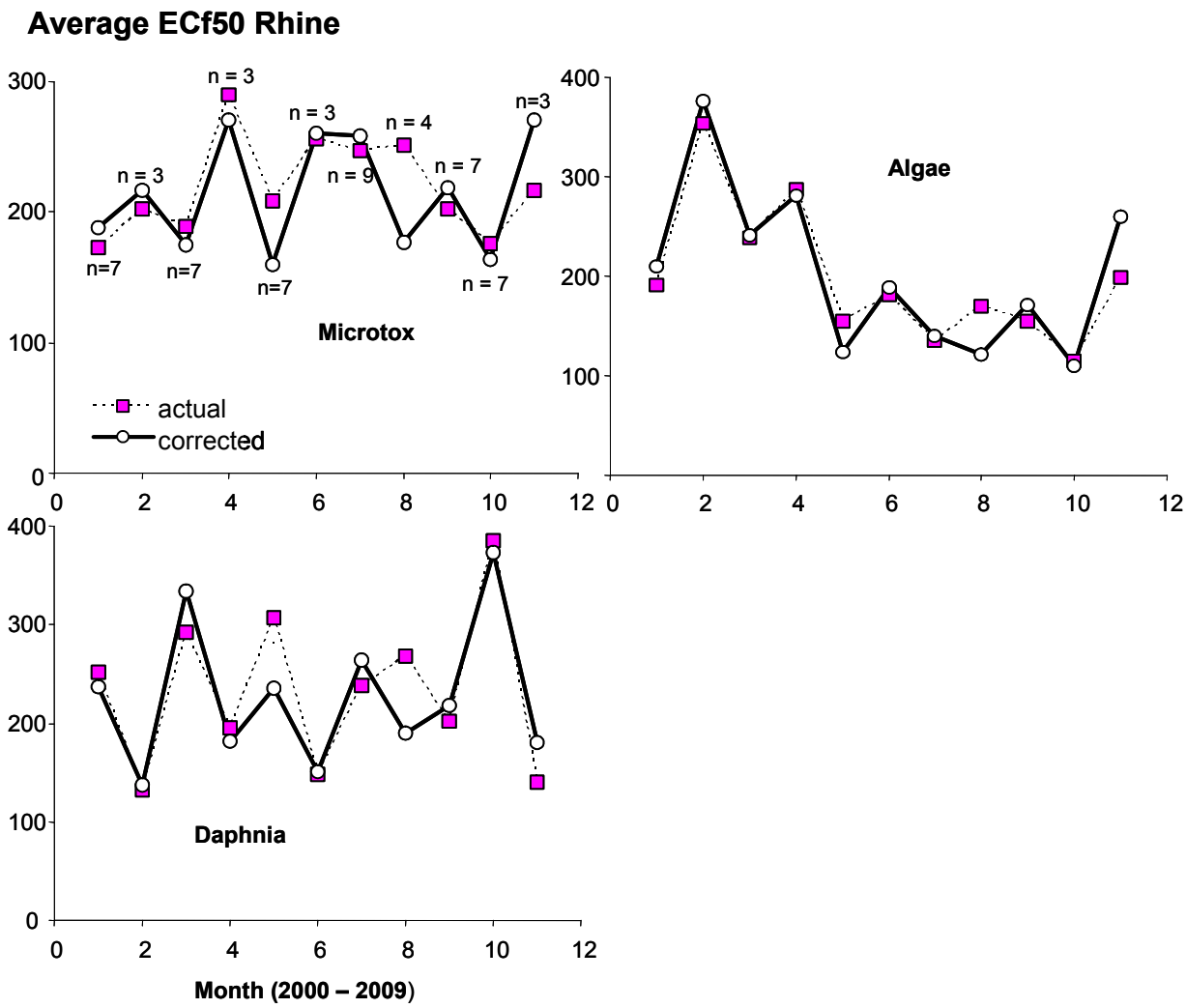

Figure 10 Seasonal dependence ECf50 data for the river Rhine

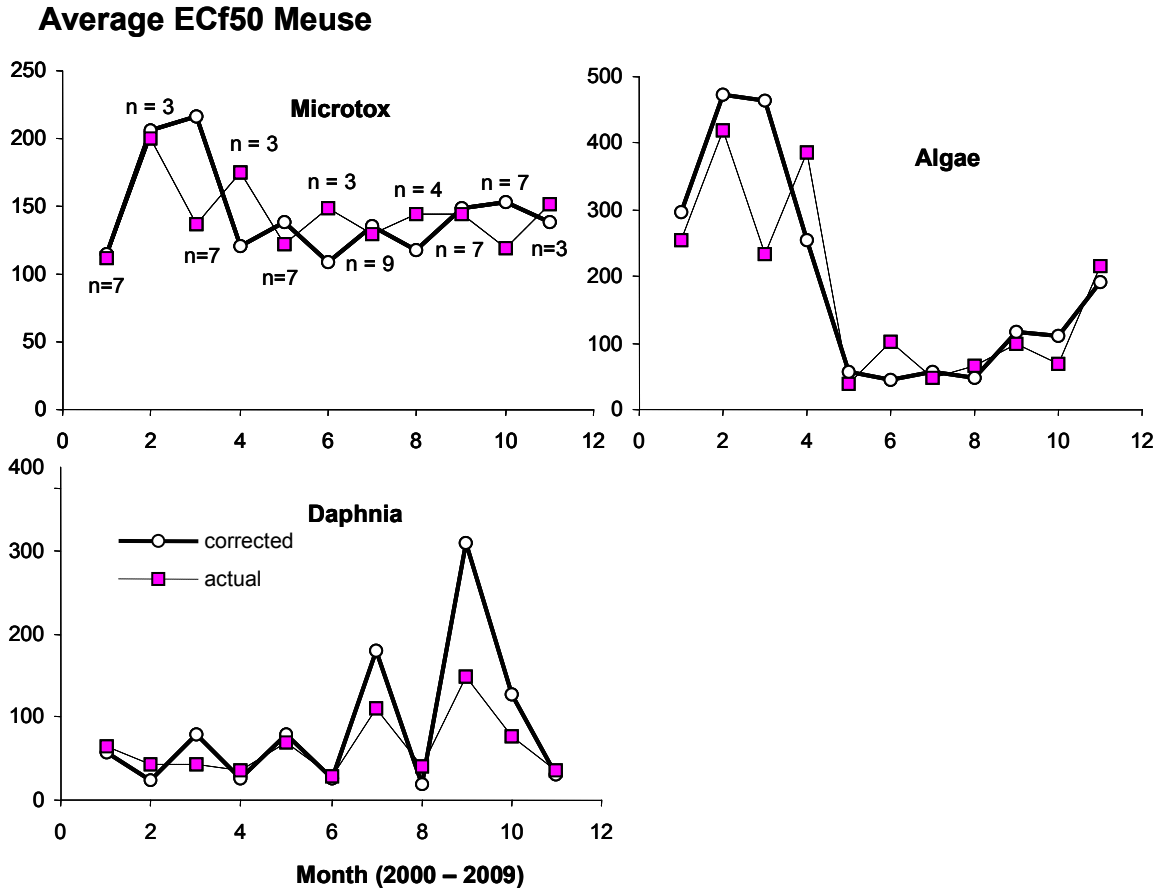

Figure 11 Seasonal dependence ECf50 data for the river Meuse.

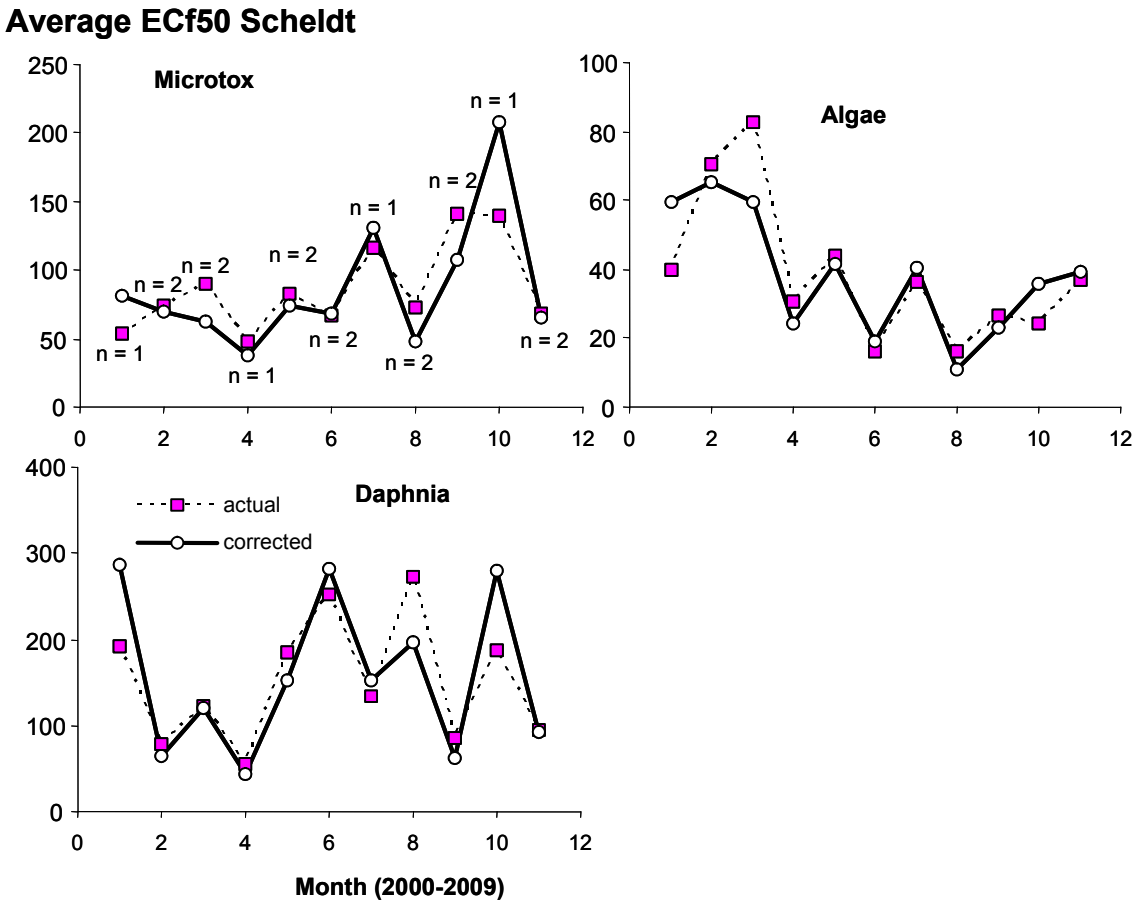

Figure 12 Seasonal dependence ECf50 data for the river Scheldt.

3.5 Comparison with chemical monitoring: the case of herbicides in the river Meuse

The Helpdesk Water (http://www.helpdeskwater.nl/service-functies/english/) is primarily designed to respond to questions from people who are (professionally) involved in water policy, water management and water safety issues in the Netherlands. This helpdesk was created by the Dutch government, provinces, municipalities, and the union of local water boards. Yearly reports (by *Water in Beeld*, the coordinating organization) provide water quality information on, for example, the concentrations of toxic chemicals in water bodies. In some cases, the results are combined in trend figures. Concentrations of some herbicides measured in the MWTL national monitoring program (Waterbase) can be compared to toxicity measured in bioassays. At the sampling locations where the three large rivers enter the Netherlands (Lobith, Eijsden, and Schaar van Ouden Doel), the herbicides Diuron and Atrazine were found by the MWTL monitoring program to have the highest concentrations among the organic compounds.
Figure 13 depicts the concentrations of these two herbicides in the river Meuse during the past decade. A seasonal pattern of concentration can be clearly seen for both herbicides. This is also the case at the other two border locations and also for the herbicides Simazine and Betazon (data not shown). It is also evident that the height of the seasonal peak in the summer decreases over time. When these trends are compared to the toxicity measured in the PAM (algae) bioassay, which is assumed to be most sensitive to herbicides, a very clear association is visible:

increasing concentrations of herbicides are positively correlated with a higher toxicity according to the PAM (algae) bioassay. The PAM (algae) bioassay measured an exceptionally high toxicity in 2002 (data points May 0.16, July 0.24, September 0.15, not shown in graph) compared to the other years. It is possible that another unknown compound, which is not measured routinely in the program for chemical monitoring, is responsible for this high toxicity.
Although high levels of metals are occasionally measured in the river waters (see Waterbase), metals are not extracted in the extraction procedure for the bioassays, so they cannot be the cause of the observed toxicity in the bioassays.

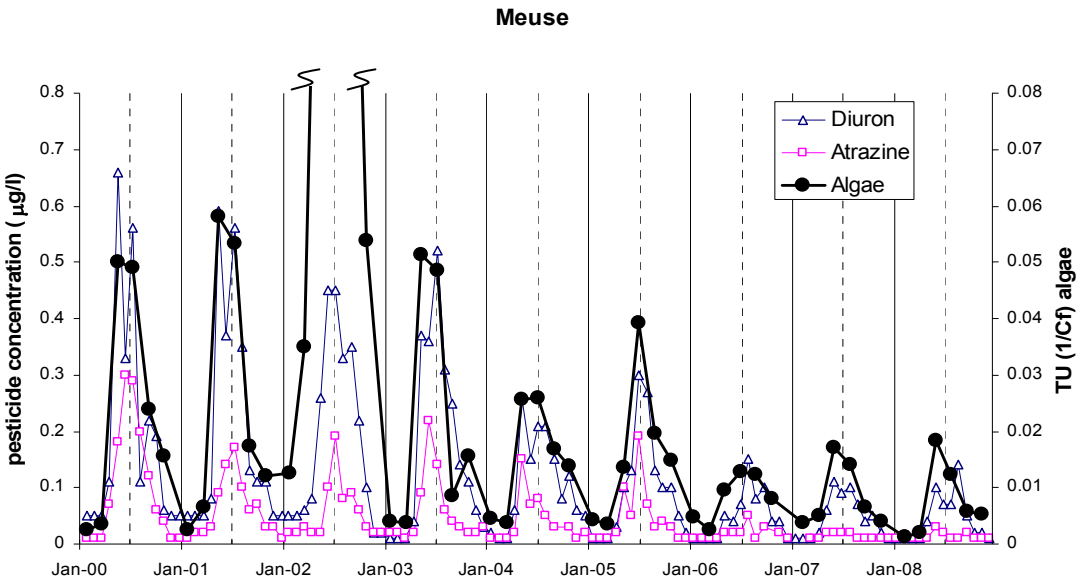

Figure 13 Chemical monitoring in the river Meuse at sampling location Eijsden (daily concentrations of herbicides Diuron and Atrazin in µg/L from Waterbase) compared with the results of the PAM bioassay monitoring test (toxicity to PAM (algae) in TU=1/ECf50).

4 INTERPRETATION

The data from this study are derived from monitoring over the past ten years. Prior to 2000, however, Dutch inland waters were already being monitored, although the methodology differed from that presently being used. A number of conclusions will be presented and discussed in the following sections the most encompassing of which is that toxicity in the rivers Rhine, Meuse, and Scheldt has been steadily decreasing for more than two decades.
The results of the analysis reported here also allow other conclusions to be drawn. New insight has been gained on spatially explicit differences in water quality and on the influence of seasons and weather, leading to the conclusion that the toxicity in the Dutch delta decreases further downstream. Aggregation of monthly raw data indicates that the toxicity for algae is higher in the summer in all rivers, although, it is more pronounced for the river Meuse.
Alternative computation techniques that were introduced to analyze trends are discussed below. An alternative procedure to increase the applicability of the pT concept to relatively unpolluted surface waters is discussed.
A number of recommendations are made to improve the cost-effectiveness of monitoring the quality and ecological health of Dutch inland waters.

4.1 Continuation of a trend

The results of this analysis show that toxic stress in the rivers Rhine, Meuse, and Scheldt have *continued* to decrease during the last decade. They also indicate that the river Meuse is more polluted with toxic substances than the river Rhine. These two conclusions are based on a comparison of the trend over the past ten years with monitoring series of the Microtox® test and ecological observations over a period of time before 2000. Figure 1 shows that the cocktail of organic chemicals caused more toxic effects in the river Meuse than in the river Rhine even in the 1990s. The decline in toxic stress during the 1990-2000 period for both the river Rhine and Meuse was rather impressive. Results over the period 2000-2009 confirm a continuous decline according to the trend line which is apparently logarithmic (Figure 1). The trend with respect to the Microtox® assays of water sampled from the river Rhine and Meuse also corresponds to the observed restoration of river water during the last twenty years, especially in the river Rhine. The number of fish species was at a dramatic low level during the 1970s (Lelek and Kohler 1990), an analysis of the development of the benthic macro-fauna in the 20th century revealed a drastic decline in the number of species from the mid-1950s to the early 1970s (Tittizer et al., 1994) which has increased considerably during the last two decades. The Microtox® bioassay, which is a test with the bacteria *Vibrio fischeri*, can, to certain extent, be considered representative for fish because this test is representative for the citric acid cycle (Krebs cycle). Cronin et al. (1991) found a strong correlation between data from Microtox® bioassays and fish data. The overall conclusion that can be drawn is that decreased exposure to toxic substances during the last twenty years has contributed to the improved ecological health of the rivers Rhine and Meuse.

4.2 Spatially explicit trends

Spatial differences can be viewed in several ways. First, toxic stress in the major rivers entering the Netherlands can be compared. Additionally, examination of the data provides some information on the nature of the unknown toxic cocktail and how these rivers differ from each other. Secondly, a trend in water quality from upstream to downstream can be investigated, although this is only possible for a

short period (2004-2005) as many locations downstream were only sampled during these 2 years.

Averaging ECf50 data over the whole period of 10 years, *per bioassay*, leads to a comparison of rivers, as summarized in Table 4. The average ECf50 value per bioassay is based on 60 ECf50 values for both the river Rhine and Meuse and 20 ECf50 values for the river Scheldt (see also Appendix C). This rather unrefined approach of making a distinction may conceal some tendencies and differences among rivers as will be explained in a subsequent section in which the role of seasons and weather conditions are taken into account.

Table 4 Toxicity in the water of 3 rivers compared qualitatively, based on 10-year average ECf50 values per bioassay per river, which are s-hown in Appendix C.

River	**intensity toxicity**	**toxic for**	**toxicity possibly due to**
Rhine	low	All bioassays	Non-polar organic chemicals
Meuse	high	Daphnia IQ	insecticides
Scheldt	high	Microtox® PAM (algae)	herbicides

Computation of the yearly median of ECf50s (Figure 8) for all locations either in 2004 or in 2005 reveals a declining toxicity of the rivers Rhine and Meuse as water progresses downstream. This tendency is, however, only reliable if both years are comparable, which we assume in this case. A lower toxicity downstream may be explained by processes that are characteristic of many processes occurring in the delta rivers. Several of the delta rivers show a low decrease in toxicity over a distance of more than 150 km. Mixing may take place when rivers become interconnected. The relatively toxic water of the river Meuse, with a median flow rate of 150 m^3/s, is mixed with a much higher amount of rather clean water from the river Rhine (median flow rate is 1980 m^3/s). It can be shown, however, that even water of the river Rhine, which has a low toxicity at the Dutch border, becomes less toxic downstream. One explanation may be provided by removal processes associated with chemical fate. For example, sedimentation causes the removal of toxicants from the water column (possibly resulting in their accumulation in the sediment). Other likely removal processes are (bio)degradation and, in IJssel Lake, for example, volatilization due to a long hydraulic retention time. Mixing with run-off water and water of small streams may cause further dilution. The nature of the toxicants in water from the river Rhine is still recognizable downstream. The spread in ECf50 values (see Table E1, Appendix E) remains around 0.322 (Lobith), which indicates the dominating role of non-specific compounds. At sample locations that are hydraulogically the most remote from Lobith, such as Markermeer and Vrouwenzand, the spread increases significantly. Both of these sampling sites are located on IJssel Lake where a long hydraulic retention time allows removal processes, such as the volatilization of non-polar compounds. The relatively enhanced spread in ECf50 data for these sampling locations, especially Vrouwenzand, suggests that non-specific compounds (probably pesticides) have become relatively more important.
The conclusions can be drawn that 1) the toxicity of freshwater is highest upstream, diminishing at locations further downstream probably due to a variety of natural processes that are typical for a river delta and 2) that there are no large additions of toxicity to the large rivers within the Netherlands.

The toxicity of water samples is underestimated due to incomplete recovery in the extraction procedure. Incompleteness of the recovery is higher for polar than for

non-polar substances. As a consequence, toxic pressure in the river Meuse, which is already higher than that in the river Rhine, is even more underestimated.

4.3 Seasonal influences and weather conditions

Table 4 was constructed on the basis of the raw data (ECf50s) without considering the influence of weather and season. However, a number of patterns become apparent if these latter factors are taken into account. An enhanced toxicity for algae during the summer months is apparent for both the river Scheldt and river Rhine and very apparent for the river Meuse. Herbicides probably affect the rivers Rhine and Meuse between April and October. Such seasonal influences became visible after subdivision of ECf50 values into monthly segments (Figures 10-12). A number of patterns emerge even for the relatively unpolluted river Rhine, from which it can be concluded that non-polar organic chemicals with a non-specific mode of action (affecting the different bioassays in a more or less similar way) are not the only chemicals that affect this river. However, this effect is low because the ECf50 value for PAM (algae) is still high (approximately 120, see Figure 10), although significantly lower than that in the winter. A clearer 'summer effect' is displayed by Figure 11 (river Meuse), showing over a period of 5 months a rather constant ECf50 level of approximately 60 for PAM (algae), which indicates a toxicity for these organisms that cannot be ignored. Unraveling seasonal influences leads to an interpretation that substantially deviates from the summary of Table 4. Nevertheless, water of the river Scheldt on the Dutch border is most toxic for PAM (algae) during the summer (ECf50 ≈ 30). Averaging ECf50s of the different bioassays over the whole year already leads to the interpretation that herbicides are likely the greatest contributors to toxic stress in the river Scheldt, where the PAM (algae) shows the lowest mean ECf50. These tentative conclusions should be taken with caution, however, as they are based on only 18 ECf50 values.
Measured toxic effects for algae can be linked to chemical monitoring data, as shown in Figure 13 which depicts a surprisingly good correspondence between these two datasets for the river Meuse, grouped on a monthly basis (Figure 11). Although Atrazine has been prohibited in the European Union since 2004 (Decision nr. 2004/248/EG, 10 March 2004), and the use of Diuron was restricted in Belgium in 1999 and prohibited since 2002, both herbicides are present in the river Meuse and Scheldt at measurable levels. Monitoring based on bioassays confirms the seasonal fluctuation in Diuron and Atrazine levels. The strong deviation in 2002 in the Meuse, when the toxicity for algae was very high, can, however, not be explained from measured concentrations of Diuron and Atrazine alone. Values for ECf50 for PAM (algae) in May, July, and September were as low as 6, 4, and 7, respectively, indicating, according to Durand et al. (2009), acute effects for PAM (algae). This comparison leads to the conclusion that measuring toxicity by means of different bioassays covering several trophic levels in the water column provides additional information on the nature of the toxic loads.

The correction of the ECf50 for the actual flow rate, ECf50 (corrected), takes into account a natural dilution (or concentration) of the water sample due to a daily varying flow rate which may deviate from the expected flow rate, based on the average or median flow rate in the relevant month. It does not add significantly more relevance to the information already obtained by dividing the ECf50 values by months and taking the average. This is illustrated in Figures 10-12. On the other hand, a comparison of Figures 6 and 9 suggests that for the river Meuse a correction of trend-pT for the actual flow rate does have a significant influence on the trend. The emission reduction in the Meuse catchment on the border (Eijsden) is possibly underestimated if we do not account for the accidental flow rate. Moreover, toxicity in general in the river Meuse is probably more underestimated than that in the river

Rhine because of the suspected nature of the toxic compounds present (specific vs. narcotic substances, respectively).
After correction for the flow rate, the trend-pT level for the river Rhine remains too low to see a trend.

4.4 Alternative computation techniques

A low trend-pT for the river Rhine is caused by a low concentration of toxic chemicals and a low variability among the bioassay test results, which indicates that the toxicants are predominantly non-polar chemicals with a non-specific mode of toxic action. Furthermore, the design of pT, which applies a factor of ten to extrapolate from acute to chronic effects but does not routinely account for limitations in the recovery of compounds in the concentration procedure (see Appendix F). If an extra factor of two were to be incorporated to account for incompleteness of the concentration procedure (as proposed by Durand et al., 2009), a higher trend-pT value is calculated because the extrapolation factor would be twenty instead of ten. Higher trend-pT values may be more useful for revealing a trend. Nevertheless, for reasons of consistency we adhere to the current design of pT and have introduced the concept of median ECf50 as an alternative technique to identify a trend. If the period is sufficiently long, for example, ten years, as in this study, a long-term trend can be observed using median ECf50 values.
In conclusion, the alternative time-integrated trend-pT analysis, as opposed to the conventional pT value per sampling date presented in Durand et al. (2009), yields a clear result (right panels of Figure 6) that is also interpretable by water managers and policy-makers. Together with the chosen indication level of a (conventional) pT of 5% in Durand et al. (2009), this method of data analysis provides a simple and clear picture that allows water managers to prioritize actions or compare sites.

4.5 Possible future monitoring activities

Due to the continuous introduction of new chemicals into ecological systems, in this analysis we did not rely exclusively on positive trends based only on bioassays performed in the last decade or only on routine chemical monitoring programmes. Figure 13 shows the additional value of both methods combined. Bioassays provide insight into the grade of toxicity of water samples, sometimes specified to a certain group of chemicals (e.g. herbicides showing specific effects in the PAM (algae) bioassay), and chemical monitoring facilitates in the identification of the compounds causing the toxicity.
The methods of data analysis used in this report yield an easily interpretable manner of presenting long-term trends in water quality. This provides water managers with the tools to make decisions on monitoring and measures to improve water quality.
In terms of future monitoring of Dutch waters using bioassays, based on our results we recommend lowering the frequency of monitoring the river Rhine to once every three years (because of consistent low toxicity), while for the river Scheldt it would be preferable to increase the frequency to annual monitoring due to the observed fluctuating toxicity with several higher outliers during the past years. Monitoring of the river Meuse remains useful in the current monitoring set-up because of the continuing presence of the banned compounds Diuron and Atrazine and the occasional unexplainable peaks in toxicity found in this river. The addition of the Ems catchment to the monitoring program would complete the set of Dutch river catchments and provide a complete picture of water quality in the Netherlands.

5 CONCLUSIONS

- Decreasing trends were observed with respect to toxic pressure in all rivers entering the Netherlands, both in time and in space (downstream).
- The river Rhine is less polluted by toxic chemicals than the rivers Meuse and Scheldt.
- The toxic compounds in the river Rhine are of a different kind than those in the rivers Meuse and Scheldt. The river Rhine seems to be predominantly contaminated with substances with a non-specific mode of action. The concentration factors to reach a specified toxic effect are rather close to each other.
- The organic chemicals in the rivers Meuse and Scheldt generally have a more specific mode of action, as inferred from a greater spread in toxic responses in the bioassays. There is also a difference in the predominant toxic mode of action between the river Meuse and the river Scheldt based on the observed difference between responses to the Microtox® test on one hand and the PAM (algae) and Daphnia IQ tests on the other.
- Underestimation of toxic stress is more likely for the rivers Meuse and Scheldt than for the river Rhine because the narcotic (non-specific) chemicals that prevail in the latter are more efficiently recovered in the pre-treatment of water samples than polar substances with a specific mode of action.
- An alternative computation technique is successfully used to increase the applicability of the pT concept on long-term datasets. Water managers and policy-makers will also be able to interpret the data.
- A higher toxic response is systematically measured during the summer months. This is probably due to enhanced concentrations of herbicides, as indicated by higher responses in the PAM (algae) bioassays.
- An eye-catching correlation for the river Meuse was observed between concentrations of herbicides (Atrazine and Diuron) and the PAM (algae) bioassay response. Both herbicides had already been banned during the period under consideration. Nonetheless, a surprisingly high toxic effect only during the summer of 2002 could not be explained from the measured concentrations of even these herbicides. Other herbicides, until now unidentified, must have been present.

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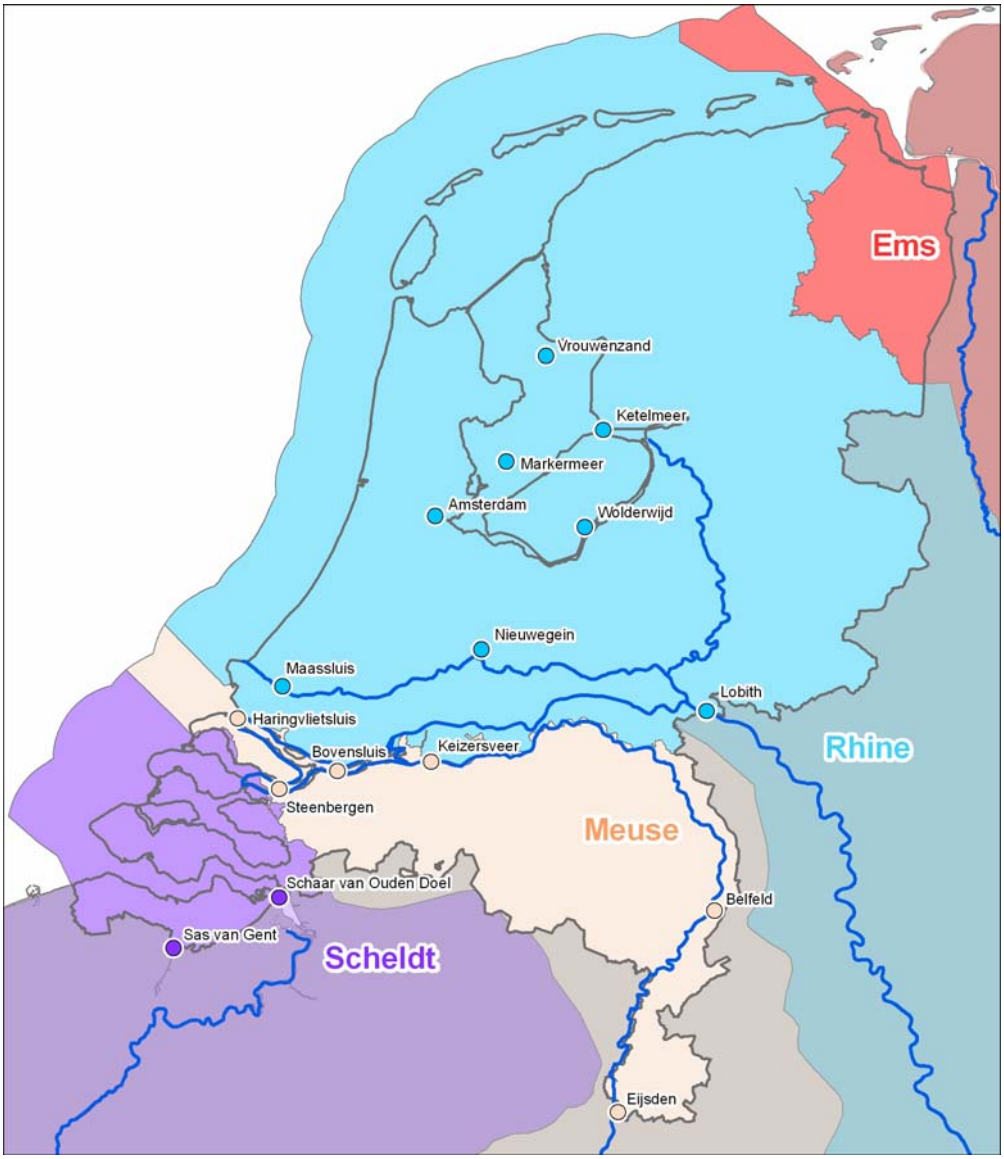
Appendix A Information on toxicological test data

Geographical map of sampling locations



Concentration factors for 50% acute effect (ECf50)

The river Rhine (Lobith)

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date d-m-y	Flow rate (m^3/s)		Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
	actual	median					
25-01-00	2095	2463	91	243	534	548	105
21-03-00	3321	2934	169	303	805	834	250
16-05-00	2296	2162	214	121	330	1000	250
11-07-00	1961	1809	142	66	517	531	163
05-09-00	1806	1568	130	98	465	481	196
31-10-00	1701	1546	193	43	686	702	250
23-01-01	2075	2463	184	242	552	459	187
20-03-01	6494	2934	282	279	974	1000	250
15-05-01	2735	2162	205	157	525	530	191
10-07-01	2214	1809	254	123	534	784	250
04-09-01	1458	1568	186	123	612	637	122
30-10-01	1759	1546	188	103	504	94	173
23-01-02	2989	2463	146	178	380	294	267
20-03-02	2399	2934	99	144	307	415	145
15-05-02	3141	2162	369	275	626	608	365
10-07-02	1697	1809	197	127	462	456	210
04-09-02	1721	1568	258	136	552	410	463
30-10-02	4122	1546	169	119	403	324	331
22-01-03	2893	2463	152	129	550	631	184
19-03-03	2215	2934	272	224	1000	956	375
14-05-03	1707	2162	116	93	642	583	187
09-07-03	1420	1809	164	69	427	243	557
03-09-03	992	1568	56	43	240	313	236
29-10-03	1145	1546	142	115	809	816	522
20-01-04	6049	2463	256	168	468	799	570
16-03-04	1471	2934	125	262	224	550	519
11-05-04	3367	2162	263	88	545	561	427
06-07-04	1556	1809	215	107	508	616	362
31-08-04	2037	1713	181	111	686	851	467
25-10-04	1754	1546	214	135	389	678	878
18-01-05	1720	2463	230	188	443	500	323
15-03-05	2188	2934	186	292	468	612	350
10-05-05	2900	2162	177	141	542	772	534
05-07-05	1659	1809	145	88	364	657	250
30-08-05	2613	1713	420	228	626	996	411
25-10-05	1188	1546	124	102	490	389	336
18-01-06	1085	2463	153	192	438	408	124
15-03-06	5402	2934	193	168	375	651	157
10-05-06	2077	2162	115	206	777	675	194
05-07-06	1672	1809	410	163	**ND**	**ND**	107
30-08-06	1785	1713	143	110	409	350	67
25-10-06	1454	1546	200	188	**ND**	**ND**	205
15-02-07	4013	2625	119	258	**ND**	**ND**	126
11-04-07	1909	2465	159	188	**ND**	**ND**	153

Date d-m-y	Flow rate (m^3/s)		Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
	actual	median					
07-06-07	2252	2045	261	137	948	726	149
02-08-07	2143	1713	263	231	642	897	128
27-09-07	1502	1568	286	221	617	814	104
22-11-07	1735	1758	297	167	747	**1445**	154
13-02-08	2413	2625	326	508	**1942**	**1942**	149
09-04-08	2911	2465	330	170	**1903**	**1903**	274
04-06-08	2301	2045	189	127	**2393**	**2393**	105
30-07-08	1767	1809	396	262	**2031**	**2031**	138
24-09-08	1734	1568	254	230	**2016**	**2016**	149
19-11-08	1321	1758	149	212	735	**1484**	133
11-02-09	1739	2625	161	296	184	221	120
08-04-09	2538	2465	380	505	192	292	158
03-06-09	1782	2045	320	281	375	237	191
29-07-09	2032	1809	304	213	290	242	112
23-09-09	997	1568	247	233	221	257	143
18-11-09	1328	1758	204	217	221	292	135

The river Meuse (Eijsden)

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date d-m-y	Flow rate (m^3/s)		Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
	actual	median					
25-01-00	289	395	79	392	278	268	52
21-03-00	283	347	68	275	186	250	24
16-05-00	133	139	135	20	261	302	79
11-07-00	275	63	109	20	529	568	61
05-09-00	97	49	89	42	310	315	45
31-10-00	184	63	70	64	260	354	30
23-01-01	649	395	222	392	564	537	27
20-03-01	822	347	278	153	545	463	70
15-05-01	258	139	103	17	252	286	25
10-07-01	122	63	114	19	299	405	41
04-09-01	61	49	105	57	278	314	42
30-10-01	123	63	173	82	354	278	63
23-01-02	623	395	120	80	400	449	48
20-03-02	756	347	91	29	354	218	51
15-05-02	194	139	91	6	363	408	158
10-07-02	83	63	130	4	531	412	289
04-09-02	64	49	108	7	339	412	156
30-10-02	277	63	146	19	281	239	66
22-01-03	516	395	86	253	290	321	45
19-03-03	184	347	92	267	219	159	27
14-05-03	85	139	57	19	322	363	89
09-07-03	39	63	29	21	228	178	289
03-09-03	25	49	121	117	627	708	671
29-10-03	19	63	64	65	500	363	127
20-01-04	1095	395	64	219	169	354	164
16-03-04	141	347	103	261	199	279	68.5
11-05-04	319	139	179	39	404	397	90.9
06-07-04	19	63	67.7	39	354	502	106
31-08-04	221	48	152	60	200	366	104
25-10-04	89	63	109	72	354	437	133
18-01-05	611	395	140	238	196	657	88
15-03-05	408	347	116	277	204	678	1
10-05-05	194	139	126	74	424	299	10
05-07-05	112	63	136	25	306	306	4
30-08-05	39	48	146	51	229	799	17
25-10-05	31	63	103	67	306	329	14
18-01-06	126	395	76.4	207	228	175	31
15-03-06	138	347	205.1	382	353	207	68
10-05-06	69	139	159.3	105	463	988	39
05-07-06	31	63	164.5	79	**ND**	**ND**	95
30-08-06	375	48	128.8	81	285	269	25
25-10-06	32	63	166.1	122	**ND**	**ND**	106
15-02-07	883	378	90.6	261	**ND**	**ND**	19
11-04-07	188	213	106	195	**ND**	**ND**	22
07-06-07	78	84	150	58	588	506	32
02-08-07	73	48	150	71	374	759	12

Date d-m-y	Flow rate (m^3/s) actual	median	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
27-09-07	62	49	153	154	750	676	31
22-11-07	182	157	134	254	572	737	52
13-02-08	461	378	386	859	775	**1750**	39
09-04-08	438	213	214	514	859	818	53
04-06-08	186	84	115	55	607	786	36
30-07-08	88	63	148	82	736	**1840**	37
24-09-08	52	49	186	173	657	820	27
19-11-08	168	157	179	186	640	758	20
11-02-09	546	378	123	134	87	179	72
08-04-09	212	213	203	448	130	225	30
03-06-09	100	84	181	191	102	193	22
29-07-09	62	63	269	137	238	213	73
23-09-09	21	49	244	149	402	482	69
18-11-09	96	157	143	203	233	160	35

The river Scheldt (Schaar van Ouden Doel)

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date d-m-y	Flow rate (m^3/s)		Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
	actual	median					
15-02-00	321	206	38	40	100	154	84
11-04-00	151	121	48	31	274	372	56
06-06-00	189	80	46	5	242	550	108
04-08-00	149	82	70	7	274	311	42
19-09-00	132	70	124	9	212	353	92
17-11-00	223	130	62	19	177	192	74
12-01-05	133	200	54	40	272	245	191
07-03-05	165	198	59	60	261	305	174
02-05-05	148	105	87	34	267	339	254
27-06-05	61	80	88	28	150	262	395
23-08-05	111	82	76	25	204	218	502
19-10-05	56	83	140	24	195	363	188
04-02-08	200	206	111	101	**1652**	780	72
31-03-08	450	198	121	106	**ND**	**ND**	72
26-05-08	95	105	78	54	506	714	114
01-07-08	75	84	116	36	464	728	135
17-09-08	75	70	159	44	699	**1796**	80
10-11-08	105	130	76	55	703	**1746**	114

The river Scheldt downstream (Sas van Gent)

ND = not determined

Date d-m-y	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
03-01-06	51	46	191	178	79
27-02-06	56	58	240	240	57
24-04-06	51	39	ND	ND	65
19-06-06	62	11	480	427	91
14-08-06	87	11	244	231	58
09-10-06	152	19	218	218	68
26-01-09	64	74	175	205	112
23-03-09	73	84	274	172	45
18-05-09	100	51	231	151	110
13-07-09	184	25	185	220	89
07-09-09	138	36	196	220	181
02-11-09	177	50	273	229	153

Locations downstream on the river Rhine

Ketelmeer

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date d-m-y	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
04-02-00	100	212	481	418	148
31-03-00	229	179	418	**1386**	250
26-05-00	172	58	500	**1663**	250
21-07-00	404	120	995	**1459**	250
18-09-00	315	132	545	**1465**	180
10-11-00	138	117	500	594	250
06-01-05	418	188	500	**1329**	789
03-03-05	255	305	**1681**	**1681**	452
28-04-05	119	115	471	572	209
23-06-05	111	171	408	612	303
18-08-05	294	156	345	824	348
13-10-05	246	169	864	870	503
28-02-08	215	485	**ND**	**ND**	199
24-04-08	347	372	**1693**	**1693**	233
19-06-08	360	198	**1831**	**1831**	265
14-08-08	382	220	**1831**	**1831**	221
09-10-08	322	186	666	**1475**	124
04-12-08	405	289	**1949**	**1949**	153

Vrouwenzand

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date d-m-y	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
12-01-00	174	122	238	903	250
08-03-00	84	197	592	898	250
29-06-00	150	197	2052	2052	250
23-08-00	58	147	288	538	250
18-10-00	46	180	642	684	250
13-12-00	132	134	654	1497	250
14-01-04	289	203	372	1341	605
10-03r-04	311	224	546	1317	604
04-05-04	314	258	3247	3247	3247
30-06-04	2	248	9271	9271	27
25-08-04	151	166	707	1449	499
20-10-04	234	199	956	1365	741
06-02-07	488	232	ND	ND	231
03-04-07	104	332	ND	ND	343
31-05-07	87	325	2309	2309	577
25-07-07	69	251	720	1595	370
19-09-07	90	232	820	941	208
14-11-07	339	290	1602	1602	341

Markermeer

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
14-01-00	289	175	364	**1381**	250
10-03-00	234	230	227	**1344**	250
30-06-00	208	206	**1878**	**1878**	250
25-08-00	127	166	199	493	188
20-10-00	276	225	**1814**	**1814**	227
15-12-00	450	270	**1720**	**1720**	250
12-01-04	356	296	373	**1258**	950
09-03-04	571	281	**1517**	**1517**	827
03-05-04	196	288	520	**1817**	**1817**
28-06-04	4	341	**7403**	**7403**	72
23-08-04	657	287	**1488**	**1488**	785
18-10-04	706	268	**2584**	**2584**	**2584**
08-02-07	498	256	**ND**	**ND**	263
05-04-07	920	380	**ND**	**ND**	266
01-06-07	444	331	**2506**	**2506**	**2506**
27-07-07	433	365	420	**1210**	922
21-09-07	248	286	**1802**	**1802**	219
16-11-07	548	325	**1532**	**1532**	378

Wolderwijd

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
4-01-05	174	160	490	496	679
28-02-05	232	285	3571	3571	3571
26-04-05	302	248	626	785	318
21-06-05	343	117	536	1444	512
16-08-05	270	164	667	1388	501
10-10-05	251	188	761	835	659
29-01-08	477	453	1362	1362	496
26-03-08	332	370	1629	1629	275
20-05-08	376	313	726	1285	269
15-07-08	335	112	ND	ND	159
9-09-08	275	222	2222	2222	108
4-11-08	368	297	1783	1783	207

Nieuwegein

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
23-02-00	161	240	127	722	250
20-04-00	58	95	409	587	170
14-06-00	154	61	553	888	250
9-08-00	241	79	642	4059	146
4-10-00	206	104	923	912	250
29-11-00	118	115	547	884	255

Amsterdam

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
26-01-06	146	134	561	705	161
23-03-06	163	160	575	575	123
18-05-06	154	77	**ND**	**ND**	133
13-07-06	309	74	186	188	114
7-09-06	176	87	**ND**	**ND**	130
2-11-06	235	79	**ND**	**ND**	130

Maassluis

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
3-02-00	114	155	529	786	139
30-03-00	185	317	529	**1371**	250
25-05-00	173	50	509	**1703**	250
20-07-00	142	51	520	505	180
14-09-00	158	97	233	660	78
9-11-00	117	89	403	490	193
25-01-06	188	170	**2382**	**2382**	94
22-03-06	177	207	768	984	146
17-05-06	200	106	641	651	155
12-07-06	227	107	623	311	140
6-09-06	159	111	386	279	165
1-11-06	417	119	**ND**	**ND**	232
7-01-09	64	74	175	205	112
4-03-09	73	84	274	172	45
13-05-09	100	51	231	151	110
8-07-09	184	25	185	220	89
17-09-09	138	36	196	220	181
11-11-09	177	50	273	229	153

Locations downstream on the river Meuse

Belfeld

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
22-02-00	108	237	115	323	55
18-04-00	55	74	430	491	25
13-06-00	127	19	455	805	210
8-08-00	237	45	380	689	66
3-10-00	163	39	214	481	139
28-11-00	375	269	742	**1400**	154
20-01-04	113	236	234	379	353
16-03-04	76	174	182	262	57
11-05-04	283	37	477	509	117
6-07-04	130	43	289	368	288
31-08-04	162	55	707	381	64
25-10-04	142	70	267	443	214

Keizersveer

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
23-02-00	108	316	577	582	37
19-04-00	125	72	447	638	94
14-06-00	143	17	495	712	238
8-08-00	352	39	437	972	86
5-10-00	186	38	282	629	197
29-11-00	165	117	353	821	49
20-01-04	336	180	59	436	372
16-03-04	160	211	181	393	127
11-05-04	177	34	552	824	209
6-07-04	181	38	354	462	363
31-08-04	213	49	438	913	545
25-10-04	218	74	459	548	741
13-02-07	214	187	**ND**	**ND**	131
10-04-07	216	196	**ND**	**ND**	71
31-05-07	236	50	506	604	98
1-08-07	236	95	712	849	154
26-09-07	275	103	675	849	61
21-11-07	187	175	695	912	104

Bovensluis

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
31-01-05	296	382	561	853	412
29-03-05	300	320	962	**1507**	93
23-05-05	414	140	612	798	274
18-07-05	331	111	846	851	503
12-09-05	307	190	546	730	325
7-11-05	239	110	437	974	327
28-01-08	311	442	**ND**	**ND**	118
25-03-08	245	519	**2067**	**2067**	133
19-05-08	227	173	**ND**	**ND**	129
14-07-08	225	171	**2028**	**2028**	171
8-09-08	434	161	**2207**	**2207**	126
3-11-08	291	201	**2018**	**2018**	158

Steenbergen

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
11-01-00	171	55	252	514	250
7-03-00	180	66	806	**1626**	250
2-05-00	61	102	483	554	250
27-06-00	237	48	950	**1702**	250
23-08-00	129	36	674	863	250
17-10-00	134	45	500	**1745**	202
31-01-05	327	123	515	666	684
31-03-05	251	167	992	**1931**	**1931**
26-05-05	262	93	678	851	284
21-07-05	205	90	599	878	489
15-09-05	340	94	534	**1498**	452
10-11-05	211	78	570	**1572**	405
28-01-08	116	158	**2306**	**2306**	138
27-03-08	262	401	**2030**	**2030**	138
19-05-08	272	210	**1796**	**1796**	294
17-07-08	275	137	**2096**	**2096**	203
11-09-08	351	108	716	**1469**	307
3-11-08	284	126	902	**1480**	169

Haringvlietsluis

ND = not determined; bold are estimated concentration factors of censored data (ECf50 > 1000)

Date	Microtox®	PAM (algae)	Rotoxkit F™	Thamnotoxkit F™	Daphnia IQ
11-02-00	115	204	155	627	212
7-04-00	382	243	**1754**	**1754**	250
30-06-00	112	41	336	557	250
31-07-00	79	35	707	619	222
22-09-00	192	71	769	**1609**	173
17-11-00	181	111	522	664	250
4-01-05	202	233	388	956	**1362**
2-03-05	196	211	786	**1377**	358
26-04-05	254	121	447	747	248
21-06-05	292	197	339	908	118
16-08-05	331	96	640	941	247
19-10-05	380	130	591	**1415**	619
29-01-08	291	498	**ND**	**ND**	186
26-03-08	295	446	**ND**	**ND**	164
20-05-08	219	196	**1954**	**1954**	182
15-07-08	498	238	**1755**	**1755**	257
9-09-08	356	166	**2081**	**2081**	156
4-11-08	517	219	**1810**	**1810**	240

Appendix B A Statistical Procedure to Estimate Trend-pT and its Confidence Limits

Linear Regression Assumptions

Figure B1 below (River Meuse, flow corrected) displays a scatter diagram of the common logarithm ($\log_{10}$) of the 50% Effect Concentration factor data plotted over time (years) with several curves superimposed illustrating the statistics of the linear trend model used. In this model, we ignore the identity of the individual species (tests), and consider them as part of a single concentration factor distribution at each measurement point in time. The analogy with species sensitivity distributions (SSDs) has been made in the main text.

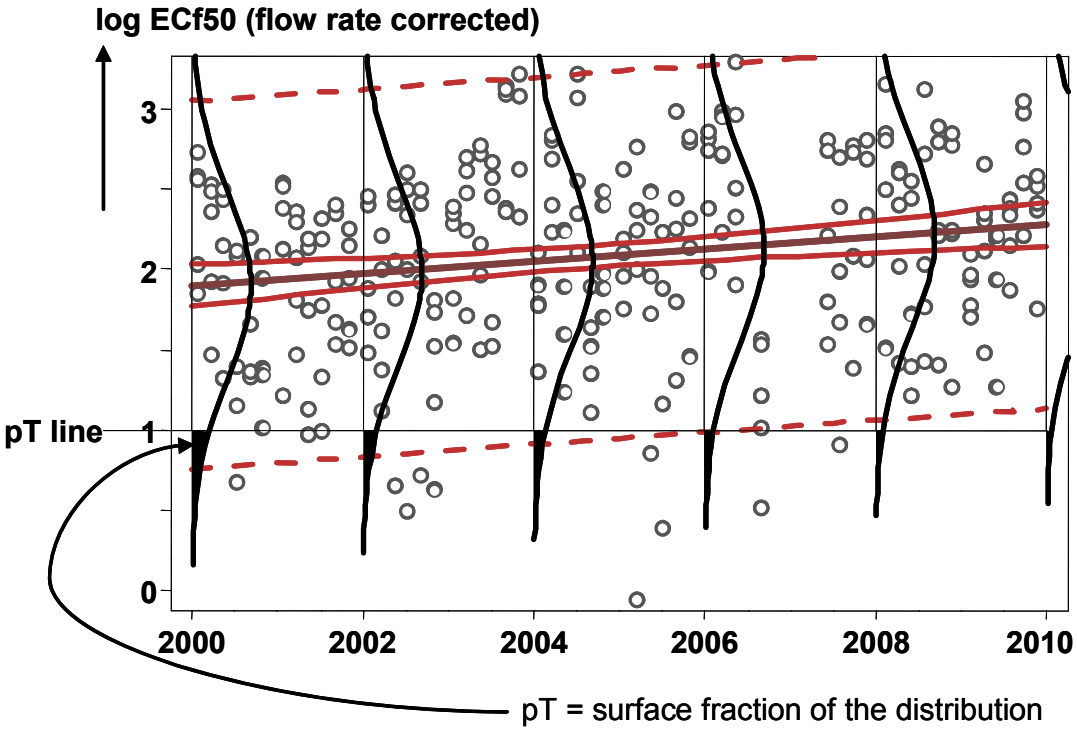


Figure B1 Three-dimensional representation of the procedure for the calculation of pT. The shaded surface decreases as the distribution curves move upwards on the log ECf50 axis.

Like the classical single toxicant-related SSDs (e.g. Aldenberg and Jaworska, 2000), we assume these distributions to have a normal (Gaussian) distribution. However, we now consider the means of these SSDs to be located on a straight line modelling the linear trend of the distribution means over time. Furthermore, we adopt the standard regression assumption that the standard deviation of the $\log_{10}$ ECf50 data distribution around the line is constant over time, though unknown.

Denoting

$$\begin{cases} x = time - 2000 \\ y = \log_{10}(ECf_{50}) \end{cases}$$

with *x* expressed in years, starting at the year 2000, and *y* the common logarithm of the 50% Effect Concentration factor.

Figure B1 is a plot of *y* against *time*, but the linear regression will model *y* as a linear function of *x*, the time elapsed since the year 2000:

$$y = \beta_0 + \beta_1 \cdot x$$

causing β_0 to be the intercept at $time = 2000$. The slope of the line, β_1, is in units of 1/year. The β -coefficients of this linear model are unknown and will be estimated from the data through regression.

The *i*-th observation y_i at (shifted) time point x_i is taken as the sum of the linear (trend line) part of the model and a random deviation from the line, ε_i:

$$y_i = \beta_0 + \beta_1 \cdot x_i + \varepsilon_i, \quad (i = 1,2,...,n)$$

where the 'errors', ε_i, are interpreted as random values from a Normal distribution with constant standard deviation σ:

$$\varepsilon_i \sim \text{Normal}(0,\sigma) .$$

In classical regression theory (Draper and Smith, 1998), the least-squares estimate of the β -coefficients: $\hat{\beta} = (\hat{\beta}_0, \hat{\beta}_1)$ is calculated (in matrix-vector notation) as:

$$\hat{\beta} = (x^T x)^{-1} x^T y$$

where *x* is the (*n*×2) design matrix with *n* rows: $\begin{pmatrix} 1 & x_i \end{pmatrix}$, and *y* is the column data vector with *n* entries y_i.

The fitted line, in matrix-vector notation is: $\hat{y} = x\hat{\beta}$, i.e. per data point:

$$\hat{y}_i = \hat{\beta}_0 + \hat{\beta}_1 \cdot x_i, \quad (i = 1,2,...,n)$$

.

This is the straight line in Figure B1, plotted over 'all' values on the x-axis. The fitted equation for this case is

$$y = 1.901 + 0.0380 \cdot x$$

Table E1 in Appendix E lists the estimates for all cases, together with their standard error. The standard error of the slope is 0.0117. The upward slope turns out to be

significant with a *p*-value of 0.001, or 0.1%. We may conclude that the $\log_{10}$ ECf_{50} has a tendency to increase over the years in this case.

An estimate of the square of the residual error is calculated as:

$$\hat{s}^2 = (y-\hat{y})^T(y-\hat{y})/\nu = \frac{1}{n-2}\cdot(y-x\hat{\beta})^T(y-x\hat{\beta})$$

called the *mean square error due to regression*, and $\nu = n-2$, the *degrees of freedom*. The square root of the mean square error, $\hat{s}$ (also indicated as *s-hat* in the text) is an estimate of σ of the error distribution: $\text{Normal}(0,\sigma)$. The important point is that this so-called *pooled* estimate is based on all data points, not on individual 5-test pT samples, making the estimate much more precise.

In Figure B1, we have visualized the Normal distributions at the even years: 2000, 2002, …, 2010, projected onto the *x*, *y*-plane, as a quasi-3D plot. Note that the means of the normally distributed $\log_{10}$ ECf50 are located precisely at the slightly increasing trend line. The standard deviations of these Normal distributions are all equal to the estimate: $\hat{s}$. The value in Figure B1 is equal to 0.580 with a standard error of 0.025.

An *acute* version of pT could be defined as the (estimated) fraction of species around or below a 50% Effect Concentration factor of 1.0, i.e. 0.0 on the $\log_{10}$ scale of the vertical axis in Figure B1. The interpretation is that, without concentrating the water samples in order to increase the amount of potential toxicants, the samples already show 50% (acute) toxic effect. To assess a 50% effect value with a *chronic* toxicity interpretation, we estimate the fraction of species below a 50% Effect Concentration factor of 10.0, which corresponds to a value of 1.0 on the $\log_{10}$ scale. This means that one has to concentrate the water samples 10 times to find a 50% acute effect. The species with a 50% effect at lower concentration factors are likely to be (potentially) affected, chronically.

In this way, we obtain point estimates of the fraction of species below 1.0 as the cumulative probabilities of the jointly fitted Normal distributions (shaded areas below the horizontal pT line in Figure B1). Since the means given by the trend line in this case move upward, the water samples need considerably more concentration to exhibit 50% effect in the species, so that the pT values in Figure B1 seem to *decrease* over time.

Confidence and Prediction Limits

Standard regression theory also yields confidence limits of the regression line and prediction limits of the data, both at given points in time. At any chosen time point on the *x*-axis, denoted x_0, with trend line prediction

$$\hat{y}_0 = \hat{\beta}_0 + \hat{\beta}_1 \cdot x_0$$

the two-sided 95% confidence limits of the value of the trend line at time x_0 are at:

$$\hat{y}_0 \pm t_{n-2}(0.975) \cdot \hat{s} \cdot \sqrt{\frac{1}{n} + \frac{(x_0 - \overline{x})^2}{\sum_{i=1}^{n}(x_i - \overline{x})^2}}$$

(Draper and Smith, 1998, p. 80, 81). The term $\hat{s}$ multiplied by the square root expression is the *standard error* of $\hat{y}_0$ at x_0. The *t*-value is the 0.975 percentage point of a Student-t distribution with *n* - 2 degrees of freedom, say roughly 2.0 for bigger datasets.

When plotted with x_0 varying over the whole time axis, we get the familiar hollow shape as shown by the continuous lines that envelope the trend line in Figure B1. The equation reveals that the interval widens with the distance between x_0 and the mean of the time data points, $\overline{x}$. At time point $x_0 = \overline{x}$ the confidence interval has minimum size equal to

$$\hat{y}_0 \pm t_{n-2}(0.975) \cdot \frac{\hat{s}}{\sqrt{n}}$$

Note that a linear regression line passes through the point $(\overline{x}, \overline{y})$, that is the mean (average) of both *x* and *y* data points. We observe that the confidence interval gets quite narrow for large datasets, as is the case in Figure B1.

Confidence intervals for the data scatter around the line, also called *predictive limits*, are also drawn in Figure B1 (dashed lines). The equation is very similar to the confidence limits of the fitted value above:

$$\hat{y}_0 \pm t_{n-2}(0.975) \cdot \hat{s} \cdot \sqrt{1 + \frac{1}{n} + \frac{(x_0 - \overline{x})^2}{\sum_{i=1}^{n}(x_i - \overline{x})^2}}$$

(Draper and Smith, 1998, p. 81, 82). Note the extra 1 under the square root sign. These bands seem to be almost linear, but the equation implies that at x_0-points far from the mean $\overline{x}$, a similar hollow shape will appear.

pT Uncertainty

In assessing the uncertainty of the line, as well as the uncertainty of the pT-predictions, we use a more elaborated interpretation of linear regression, based on Bayesian Statistics (Box and Tiao, 1973/ 1992, Tanner, 1996, Gelman et al., 2004). In this theory, all three unknown parameters: β_0, β_1, σ are considered as random variables, and their, so-called, *posterior* distributions calculated from the data allow the uncertainty of any quantity in the model, e.g. fits, predictions, and pT-values to be evaluated.

The remarkable fact is that all equations given above, in particular the linear fit and confidence limit equations, remain valid in the Bayesian viewpoint. The confidence limit interpretation changes into evaluations of predictive (posterior) distributions. Using the posterior distributions for the parameters makes the assessment of the pT uncertainty more straightforward than through confidence limits.

In Bayesian linear regression, the posterior uncertainty of σ is distributed as a scaled *inverse chi* distribution with *n* - 2 degrees of freedom (Box and Tiao, 1973/ 1992, p. 117, Tanner, 1996, p. 18, Gelman et al., 2004, p. 356). The scale factor is $\sqrt{n-2}\cdot\hat{s}$. The mode (highest density value) of this distribution is at

$$\sqrt{\frac{n-2}{n-1}}\cdot\hat{s}$$

which is almost equal to the above residual standard deviation, $\hat{s}$, for large datasets.

Simulating posterior values of σ becomes straightforward, when random numbers are available, drawn from the classical chi-squared distribution with the same degrees of freedom[3]: take the inverse (power of -1), take the square root, and multiply by the scale factor $\sqrt{n-2}\cdot\hat{s}$. By taking the standard deviation of these simulated posterior values, we obtained the standard errors of $\hat{s}$ in Table E1. In the case of Figure B1 (Meuse, flow corrected), we have $\hat{s}=0.580$ and a simulated Bayesian standard error of 0.025. Figure B2 displays the posterior probability density of σ. The mode is at 0.579, quite close to $\hat{s}$.

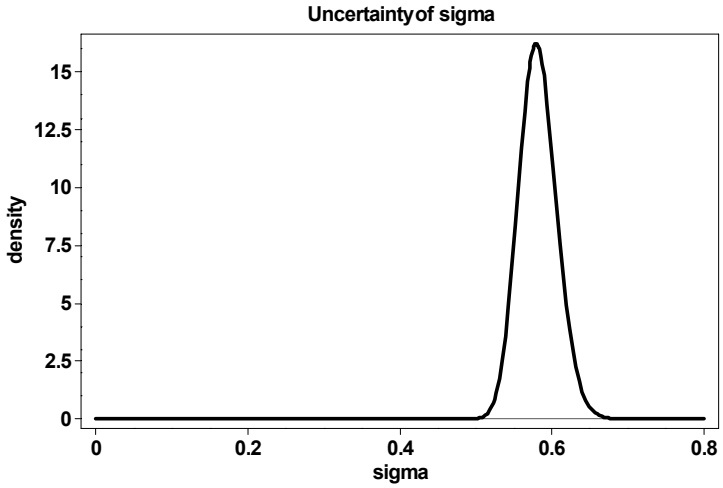


Figure B2 Bayesian posterior density of the standard deviation of log_{10} ECf50 Normal distributions of residuals around the trend line in Figure B1.

Using standard non-informative prior distributions for the parameters β_0, β_1, σ, that is: uniform regression coefficients and uniform ln(σ), one obtains an efficient algorithm to simulate the posterior distributions of the regression coefficients β_0 and β_1 (Tanner, 1996, p. 19, Gelman et al., 2004, p. 356) as follows:

$$\tilde{\beta} \sim \text{MVNormal}\left(\hat{\beta},\ (x^T x)^{-1}\cdot\tilde{\sigma}^2\right)$$

[3] This could be done in MS Excel® through the RAND and CHIINV functions.

The explanation is that for a *fixed* (non-random) value of σ, the posterior distribution of β is multivariate Normal, with mean vector $\hat{\beta}$ (i.e. the classical least squares estimate of the regression coefficients!), and, also classical, covariance matrix $(x^T x)^{-1} \cdot \sigma^2$.

To implement this, we first draw $m = 5000$ values of $\tilde{\sigma}$ from the density in Figure B2, and, treating each $\tilde{\sigma}$-component $\tilde{\sigma}_j$ as a 'given' σ, we draw the two regression coefficients from the multivariate Normal with the specified mean vector and covariance matrix, to obtain a matrix of regression coefficients $\tilde{\beta}$ with $m = 5000$ rows $\tilde{\beta}_j = \left(\tilde{\beta}_{0j} \quad \tilde{\beta}_{1j}\right)$.

The combined matrix with equal number of rows: $\left(\tilde{\beta}_{0j} \quad \tilde{\beta}_{1j} \quad \tilde{\sigma}_j\right)$ is a simulation of the joint posterior distribution of regression coefficients and the $\log_{10}$ ECf_{50} standard deviation around the line.

Note that, considering the uncertainty of the simulated $\tilde{\beta}$ coefficients alone, we essentially have a collection of $m = 5000$ regression lines (this is not visualized in Figure B1). However, the uncertainty of these regressions at each point in time is the same Student-t distribution as used to calculate the 5% and 95% classical *confidence limits* displayed in Figure B1.

The same is true for the predictive limits (dashed lines in Figure B1). At any point in time x_0, we simulate the predictive uncertainty by drawing a *single* random Normal value $\tilde{y}_{0j}$ for each row $\left(\tilde{\beta}_{0j} \quad \tilde{\beta}_{1j} \quad \tilde{\sigma}_j\right)$ of the simulated joint parameter distribution:

$$\tilde{y}_{0j} \sim \text{Normal}\left(\tilde{\beta}_{0j} + \tilde{\beta}_{1j} \cdot x_0,\ \tilde{\sigma}_j\right) \quad (j = 1, 2, ..., m)$$

(Gelman et al., 2004, p. 358). This results in a simulated predictive distribution with the same Student-t distribution as used for the calculation of the *predictive limits* above (dashed lines in Figure B1). It follows that the Normal curves in Figure B1 are in fact close approximations to Student-t distributions, which is justified given the large sample size: *n* = 280. The degrees of freedom are 278.

A point estimate (single value) of pT at any point in time x_0, as depicted in Figure B1, could be obtained as a cumulative probability of these Student-t distributions evaluated at $y = 1.0$ (or from their approximating Normal distributions). However, we prefer to assess the full Bayesian uncertainty of pT from the posterior simulation.

Instead of drawing a sample of Normal values $\tilde{y}_{0j}$ at time point x_0, together building up a Student-t distribution, we retain each row $\left(\tilde{\beta}_{0j} \quad \tilde{\beta}_{1j} \quad \tilde{\sigma}_j\right)$ of the simulated joint parameter distribution as a *separate* Normal distribution. For each of these *m* Normal distributions, pT is calculated as the Normal cumulative probability at $y = 1.0$:

$$p\tilde{T}_{0j} = \text{CDF}_{\text{Normal}\left(\tilde{\beta}_{0j} + \tilde{\beta}_{1j} \cdot x_0,\ \tilde{\sigma}_j\right)}(1.0) \quad (j = 1, 2, ..., m)$$

This leads to m = 5000 simulated pT_0 values at any time point x_0. This procedure is repeated over a grid of such time points. The simulated joint parameter set is reused each time.

These simulated pT values can be interpreted as the uncertainty of pT for the linear trend model. At each point in time, we can calculate percentiles, e.g. median, 5th and 95th percentiles and connect these to form percentile curves. The results are shown in Figure B3 for the same case as in Figures B1 and B2.

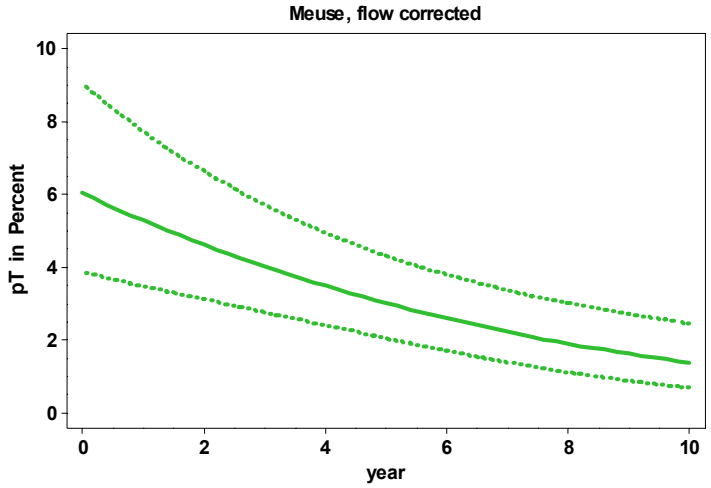


Figure B3 Bayesian posterior simulation of the decrease of pT over time and its uncertainty, calculated for the same case as in Figs. B1 and B2

Note the decrease of pT over time, as expected from Figure B1. The Bayesian simulation allows an assessment of the uncertainty of pT, also over time. Figure B3 shows the 5%, 50%, and 95% percentile curves of pT. The interpretation is that the 90% uncertainty interval of pT in the year 2000 is estimated as (4%, 9%) with a median value of 6%. The graph can be used to make statements, like: the 90% uncertainty interval of pT in the year 2000, i.e. at x = 0, is estimated as (4%, 9%) with a median value of 6%. In 2010, the upper 5% confidence limit of pT drops below 4%.

The procedure that we have followed is much like the calculation of the uncertainty of the fraction affected in SSDs (Aldenberg and Jaworska, 2000). Essentially, we have extended the fraction affected estimation of spaghetti plots to problems taking into account a so-called *covariate*, in this case time. The method could even be extended further by incorporating additional covariates through Bayesian multiple regression.

Appendix C Differences in toxicity patterns

Differences in the three most sensitive bioassays between the Meuse and Scheldt

Table C1 The average and standard deviation of ECf50 calculated from n ECf50 numbers (see Appendix A). Note the difference between the rivers Meuse and Scheldt

Sensitive bioassay	ECf50		
	Rhine *n* = 60	Meuse *n* = 60	Scheldt *n* = 18
Microtox®	213 (82)	137 (61)	86 (35)
PAM (algae)	182 (91)	149 (151)	40 (28)
Daphnia IQ	252 (155)	74 (97)	153 (122)

Long-term temporal variation in ECf50 Daphnia IQ

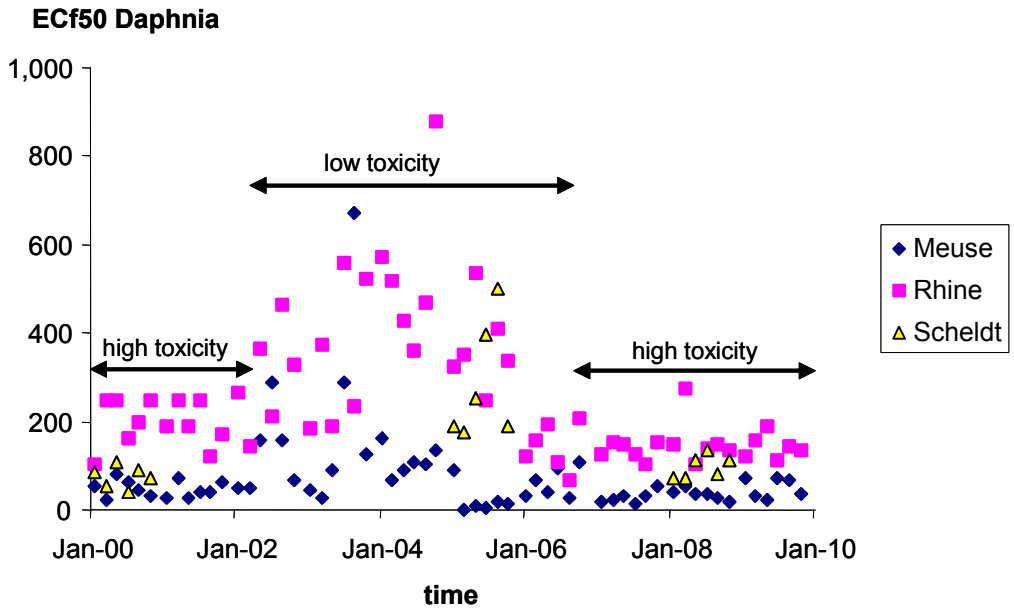


Figure C1 Relatively high ECf50s for Daphnia IQ between 2002 and 2006

Appendix D Influence of flow rate correction on ECf50

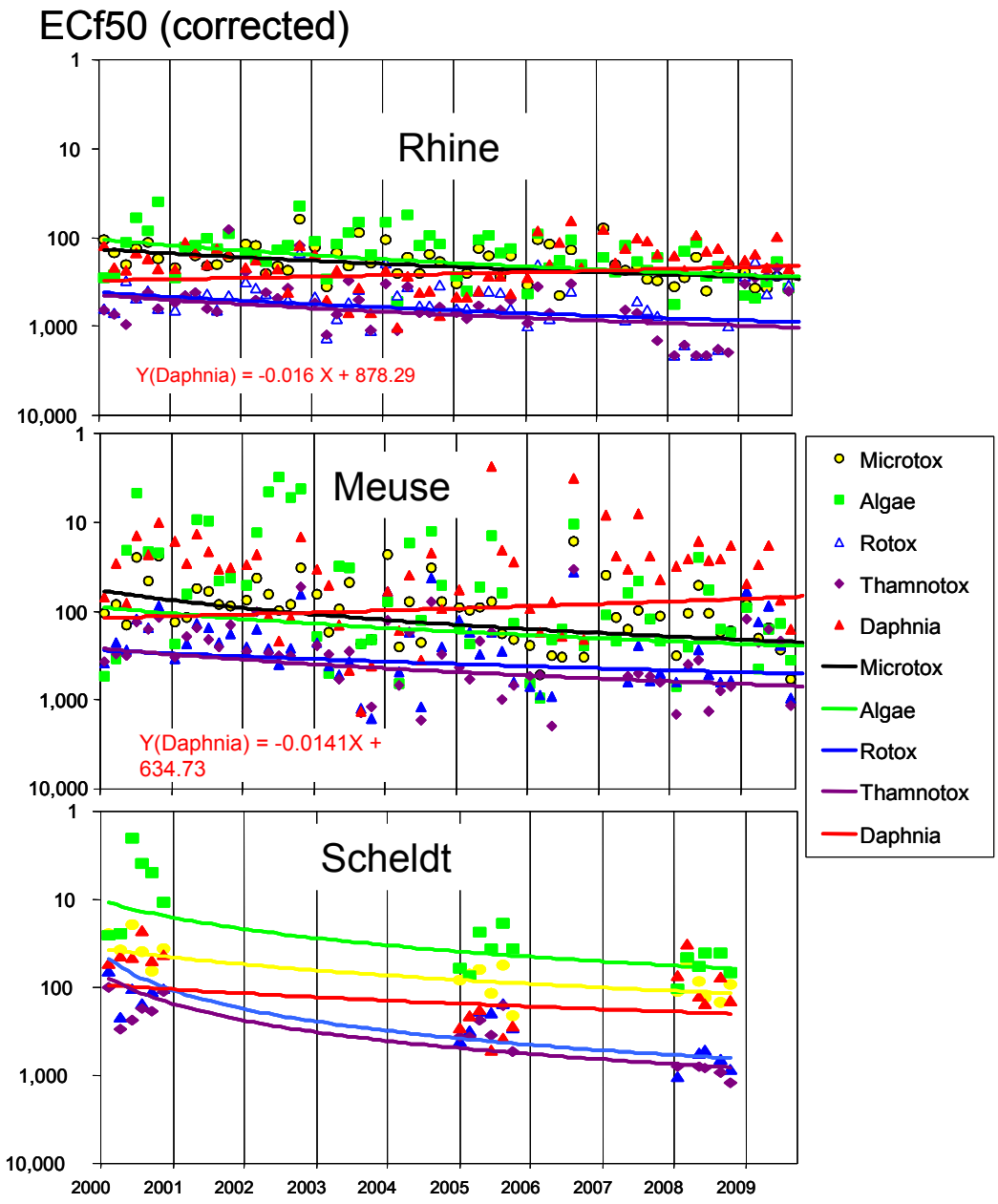


Figure D1 Concentration factors adjusted for flow rates at the day of sampling with a monthly median flow rate

Density of regression residuals for concentration factors of the river Meuse

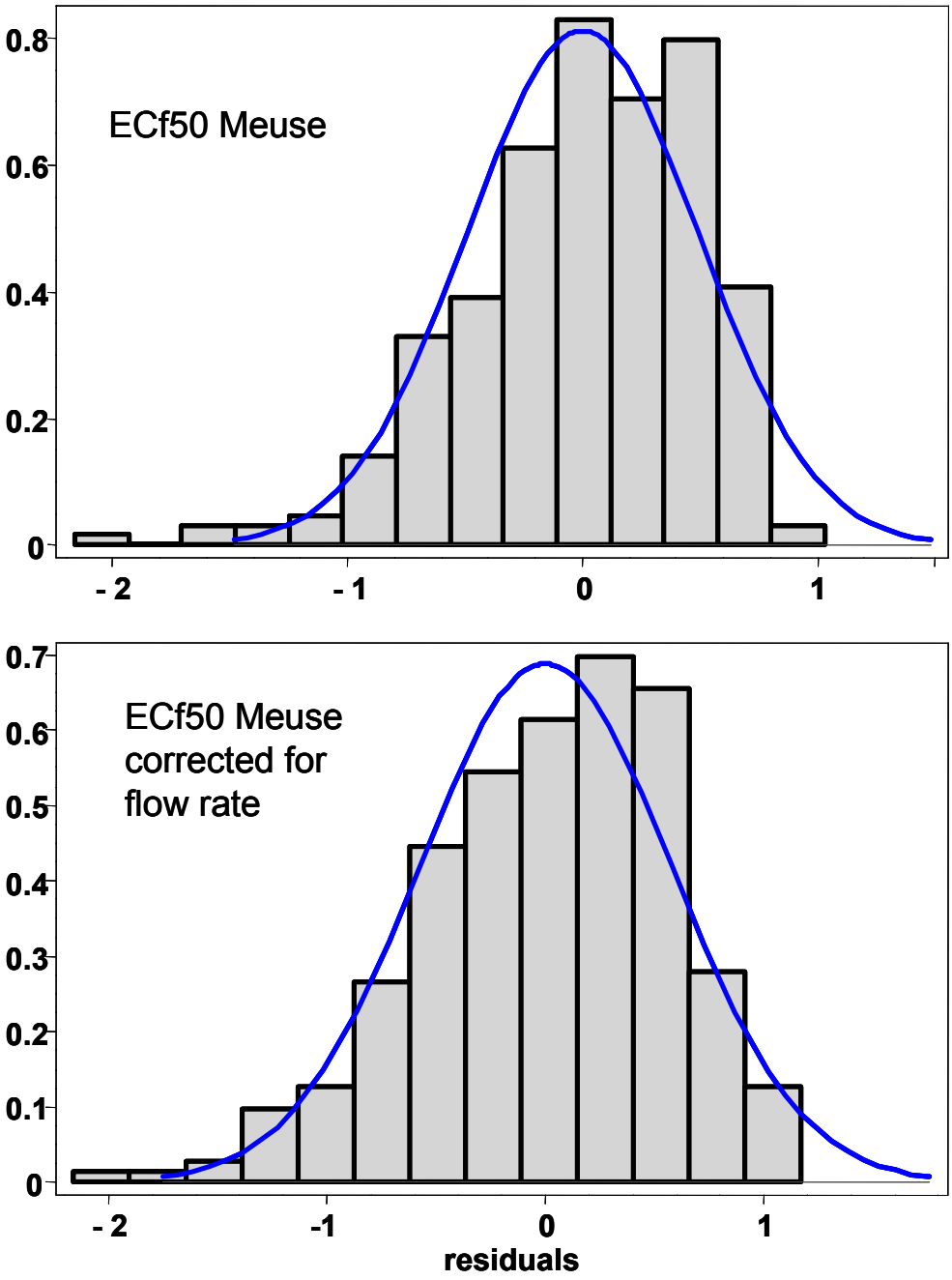


Figure D2 Concentration factors (river Meuse 2000-2009) are not log normally distributed; however, when adjusted for flow rates, the data are less skewed.

Appendix E Regression Analysis Results

Table E1 Statisical characteristics

		Estimate	*Error*	*p-value*	*Signif.**	*n*	*ECf50= 1000?*	*Subst*
Rhine (Lobith)	*Intercept*	2.430	0.038			280	Y	Y
	Slope	0.0088	0.0067	0.192	--			
	s-hat	0.332	0.014					
Wolderwijd	*Intercept*	2.570	0.172			55	Y	N
	Slope	0.0129	0.0248	0.606	--			
	s-hat	0.284	0.029					
Vrouwenzand	*Intercept*	2.442	0.083			80	Y	N
	Slope	0.0227	0.0178	0.204	--			
	s-hat	0.448	0.037					
Markermeer	*Intercept*	2.538	0.069			80	Y	N
	Slope	0.0296	0.0146	0.046	*			
	s-hat	0.369	0.030					
Ketelmeer	*Intercept*	2.466	0.059			85	Y	N
	Slope	0.0193	0.0103	0.065	.			
	s-hat	0.316	0.025					
Maassluis	*Intercept*	2.440	0.0644			85	Y	N
	Slope	-0.0287	0.0098	0.004	**			
	s-hat	0.344	0.027					
Amsterdam	*Intercept*	2.378	0.209			45	N	-
	Slope	-0.0059	0.0245	0.811	--			
	s-hat	0.248	0.028					
Meuse (Eijsden)	*Intercept*	2.075	0.056			280	Y	Y
	Slope	0.0182	0.0099	0.068	.			
	s-hat	0.491	0.021					
Meuse (Eijsden)	*Intercept*	1.901	0.066			280	Y	Y
flow corrected	*Slope*	0.0380	0.0117	0.001	**			
	s-hat	0.580	0.025					
Keizersveer	*Intercept*	2.290	0.079			80	N	-
	Slope	0.0177	0.0168	0.297	--			
	s-hat	0.424	0.035					
Bovensluis	*Intercept*	2.601	0.206			50	Y	N
	Slope	-0.0011	0.0299	0.970	--			
	s-hat	0.325	0.035					
Haringvlietsluis	*Intercept*	2.401	0.066			80	Y	N
	Slope	0.0285	0.0121	0.021	*			
	s-hat	0.351	0.029					
Steenbergen	*Intercept*	2.393	0.072			90	Y	N
	Slope	0.0252	0.0124	0.045	*			
	s-hat	0.391	0.030					

Scheldt (Schaar	*Intercept*	1.883	0.088			85	Y	N
van Ouden	*Slope*	0.0474	0.0155	0.003	**			
Doel)	*s-hat*	0.469	0.037					
Sas van Gent	*Intercept*	1.743	0.260			55	N	-
	Slope	0.0360	0.0317	0.261	--			
	s-hat	0.363	0.037					

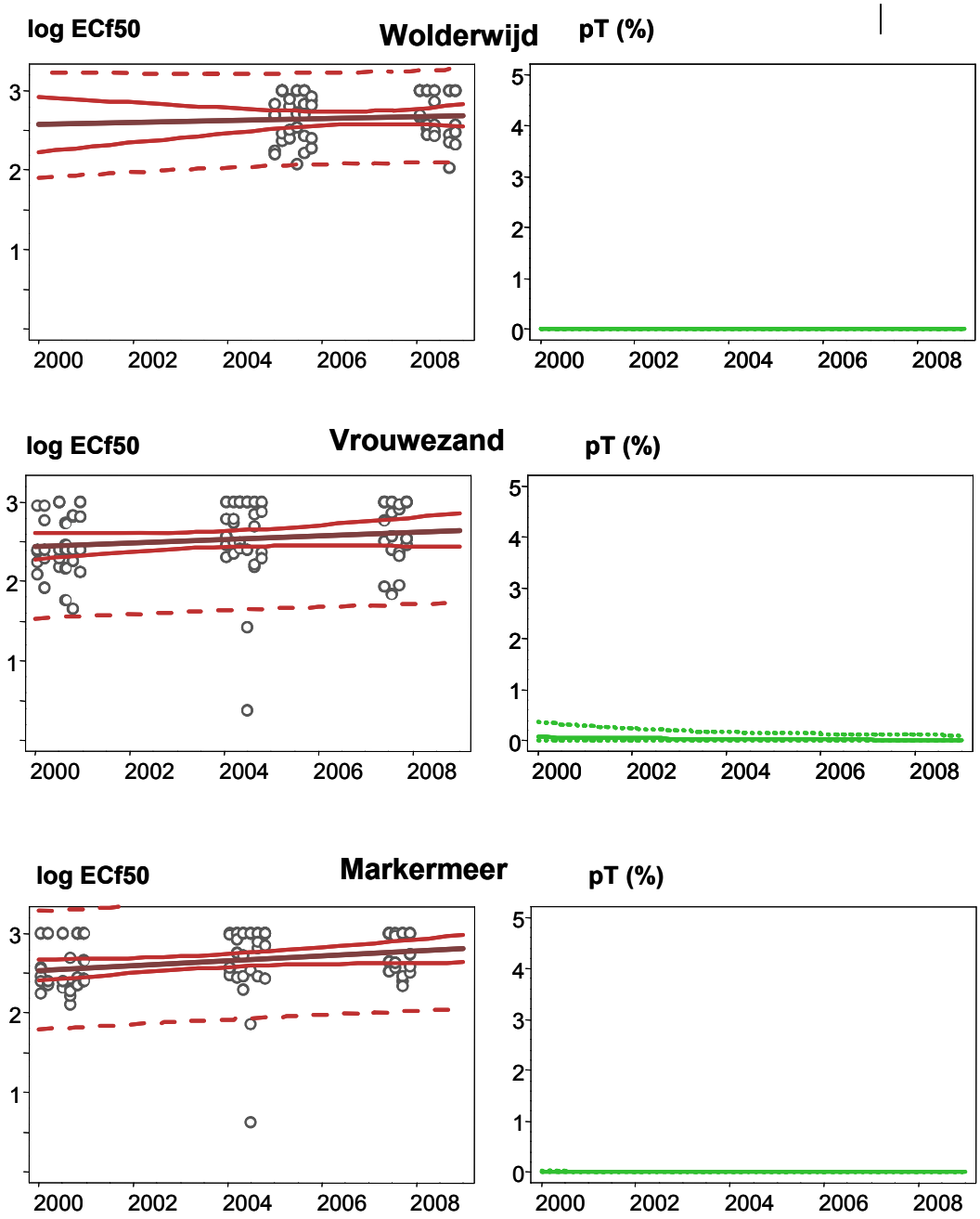
*The *Significance* column is modified from the indication system used in the statistical package R (R-Project, 2009). This is just a character expression of the *p*-value in the column immediately left to it. The meaning is given in Table E2.

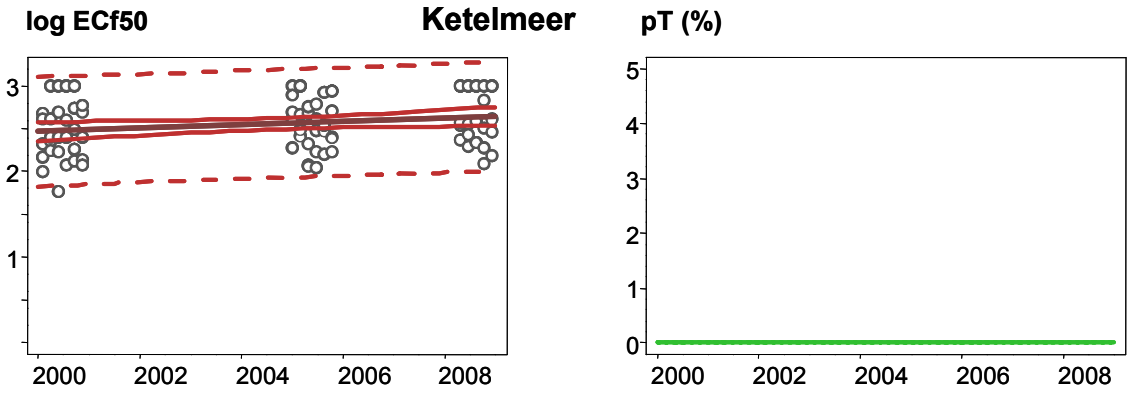
Table E2 Siginificance ranges

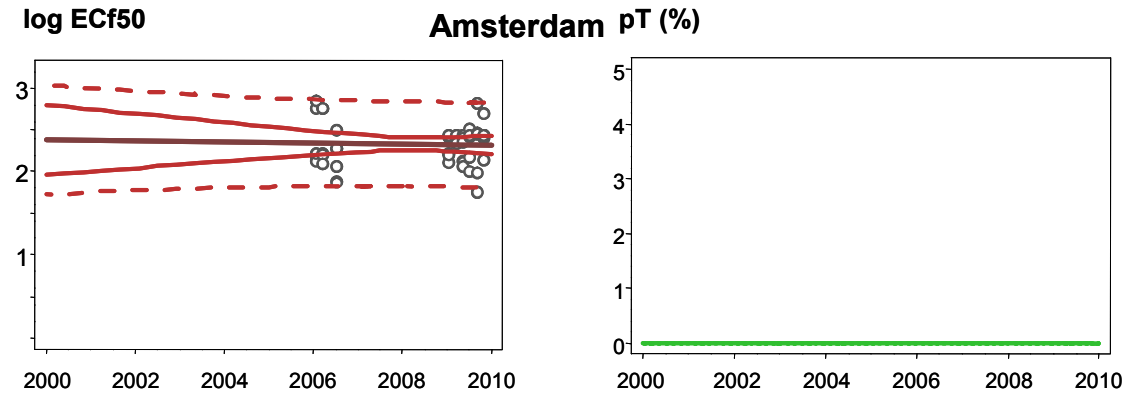
$0 \le p < 0.001$	***	extremely significant
$0.001 \le p < 0.01$	**	very significant
$0.01 \le p < 0.05$	*	significant
$0.05 \le p < 0.1$	.	not significant, but close
$0.1 \le p \le 1.0$	--	clearly not significant

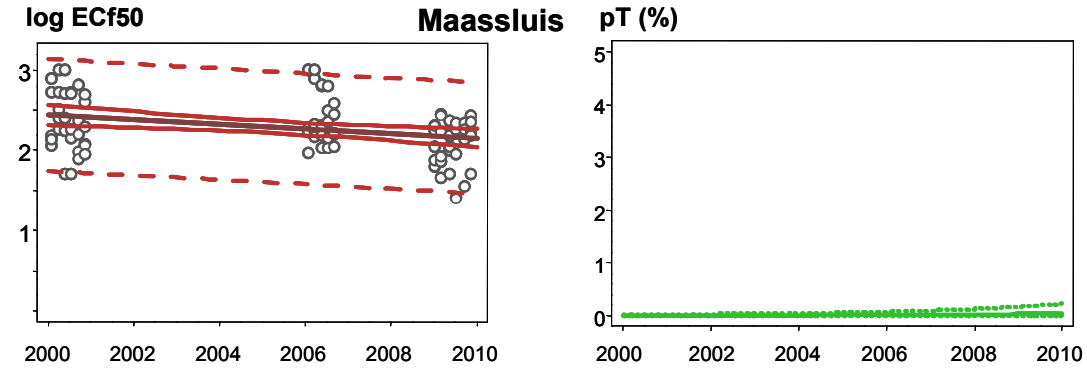
Rhine catchment

Figure E1 Trends in effect-concentration factors (left panel) and trend-pT (right panel) and uncertainty margins of locations of the Rhine basin



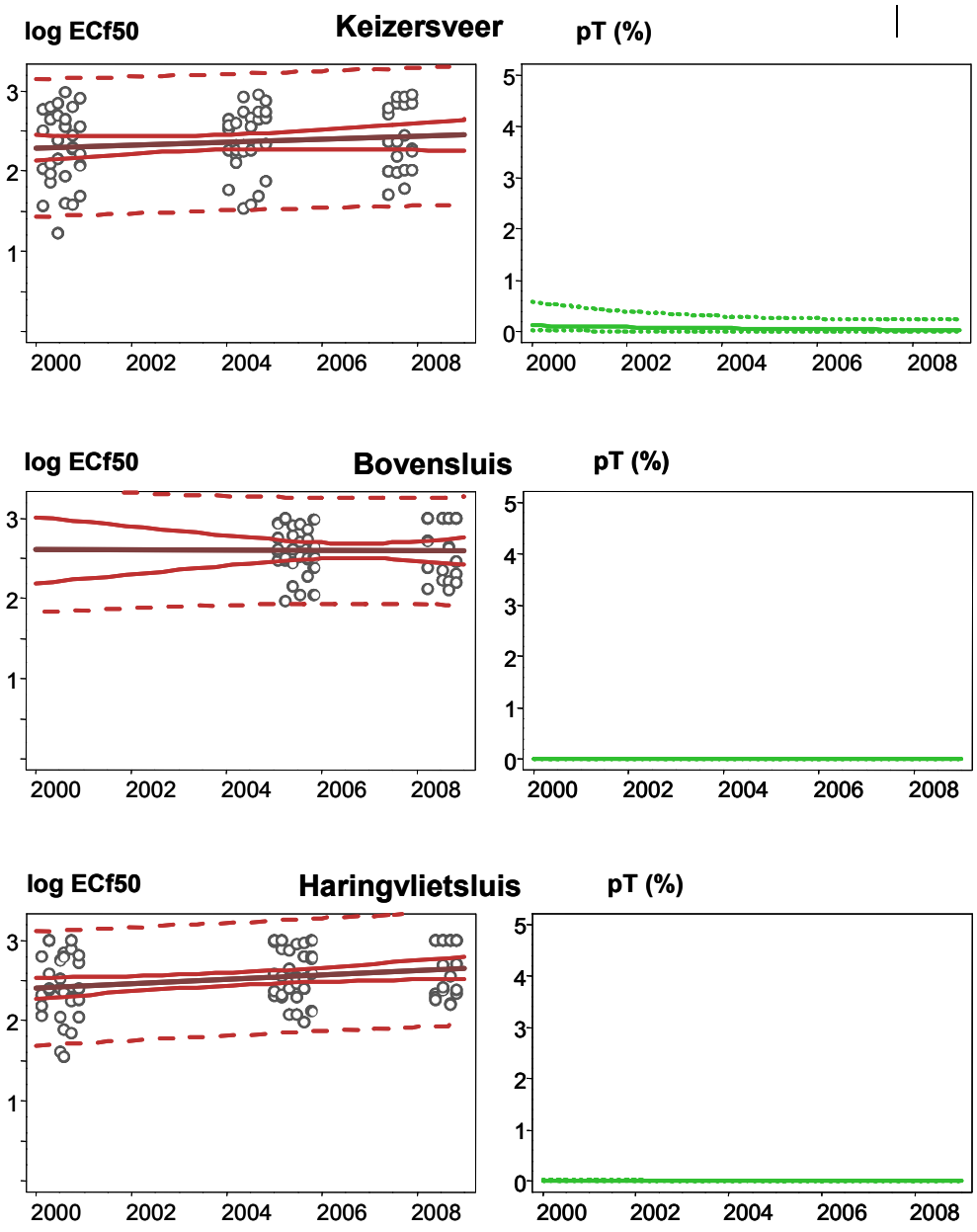

log ECf50
Ketelmeer
pT (%)
3
2
1
2000
2002
2004
2006
2008
5
4
3
2
1
0
2000
2002
2004
2006
2008

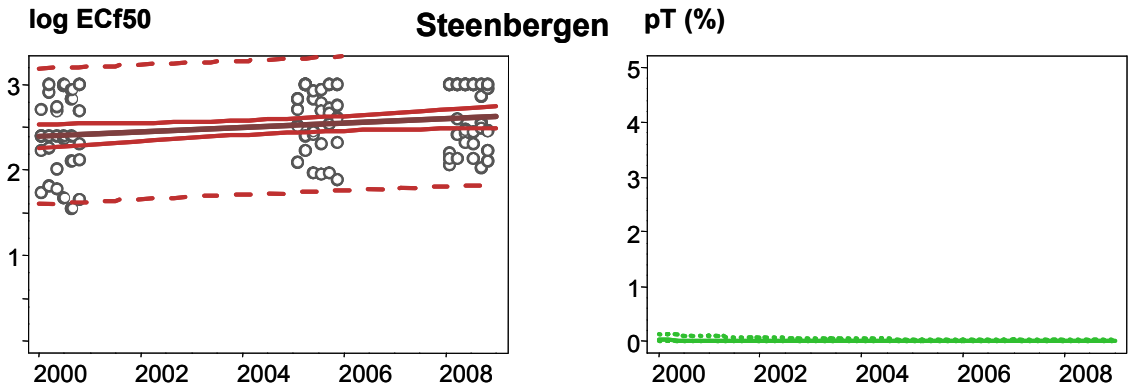

log ECf50
Amsterdam
pT (%)
3
2
1
2000
2002
2004
2006
2008
2010
5
4
3
2
1
0
2000
2002
2004
2006
2008
2010


log ECf50
Maassluis
pT (%)
3
2
1
2000
2002
2004
2006
2008
2010
5
4
3
2
1
0
2000
2002
2004
2006
2008
2010

Meuse catchment

Figure E2 Trends in effect-concentration factors (left panel) and trend-pT (right panel) and uncertainty margins of the Meuse basin




log ECf50
Steenbergen
pT (%)
3
2
1
2000
2002
2004
2006
2008
5
4
3
2
1
0
2000
2002
2004
2006
2008

Appendix F Principles of computing conventional pT (from Durand et al. 2009)

If sufficient toxicity data are available (4 or more test species), a risk analysis can be used based on a species sensitivity distribution (SSD) (Posthuma et al., 2002). The concentration factor at which no effect of chronic exposure (NOECf) is observed is depicted on the X-axis in Figure F1. However, the observation of NOECfs as chronic exposure is not feasible in bioassays. Therefore, NOECfs were estimated through dividing the results of acute bioassays (ECf50) by a factor of 10. This Acute-to-Chronic ratio (ACR) is based on a comparison of hundreds of tests for different species and substances (De Zwart and Sterkenburg, 2002; Raimondo et al., 2007). The potentially affected fraction of species due to chronic exposure to toxicants in the original sample, i.e., when the concentration factor equals one, is derived from the sensitivity distribution of NOECfs. For the hypothetical water sample in the example of Figure F1, toxic pressure (in terms of the potentially affected fraction of species), i.e., pT, is equal to 0.14 (or 14%). In Figure F1 the ecological risk in the original water sample (Struijs and De Zwart, 2003) is obtained through extrapolation to NOECf = 1.

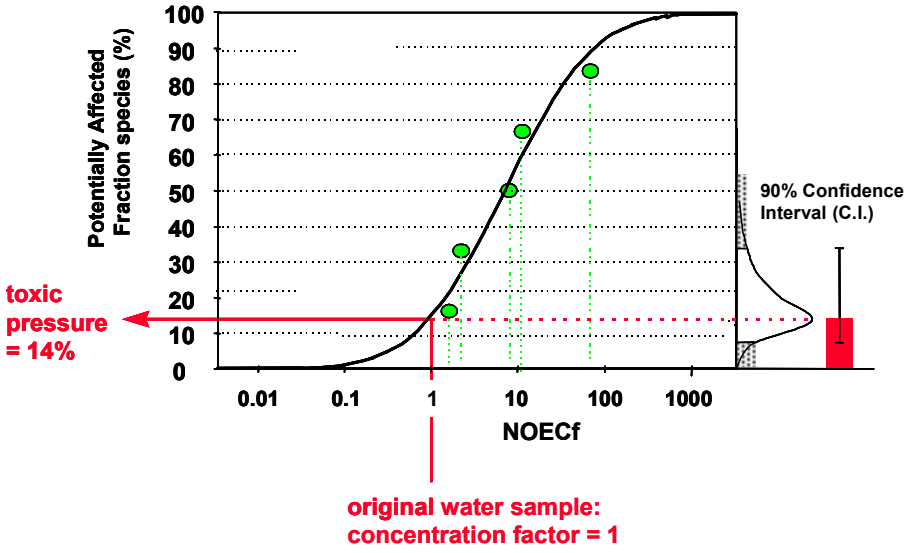


Figure F1 Example of a cumulative species sensitivity distribution curve for derived concentration factors of five bioassay tests (NOECf = ECf50/10).

De Zwart and Sterkenburg (2002) developed the method for calculating toxic pressure using the results from five toxicity tests. In the Netherlands, a minimum of four toxicity tests (from at least four taxonomic groups) is considered to be required to derive Environmental Risk Limits (Traas, 2001). To compare the toxic pressure for different locations, a minimum of four to five toxicity tests appears to be adequate (Durand et al., 2009), assuming that the same tests are performed for the different locations. The influence of the number of tests on uncertainty is evaluated by Durand et al. (2009).

The calculated pT value is useful to compare toxic pressure of different locations. However, we should be aware of the restrictions of the method: it is not suitable for metals in water. To judge the toxic pressure of a sample, Durand et al. (2009) propose two indication levels:

- indication of Chronic Effect (iCE), pT > 5 % for ECf50 = 1;
- indication of Negligible Effect (iNE), pT < 5 % for ECf50 = 10.

These indication levels have not been corrected for the limited recovery of substances in the concentrated sample. To take recovery into account as well, the toxicity results (ECf50 values) can be corrected for the limited recovery, e.g. by dividing the ECf50 values by the proposed safety factor of two from Durand et al. (2009). Taking this safety factor into account, the chance to neglect false-negative values is minimized.

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