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Understanding ecological impacts in rivers in England and Wales and identifying their possible causes: Part 1 - the Effect and Probable Cause (EPC) method

Science Report – SC030189/SR5

SCHO1008BORM-E-P

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Author(s):

D. De Zwart, E. Pemberton, L. Posthuma, A. Veal, C. Wells

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RIVM, Dutch National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

Environment Agency's Project Manager:
Emma Pemberton,
Environment Agency Science Group, Red Kite House. Howbery Park. Wallingford OX10 8BD

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Executive summary

Water quality is subject to a variety of pressures, including changes in physical habitat, point-source discharges, urban diffuse sources of contaminants, water abstraction, inputs of nutrients, and trace organics from domestic, agriculture and industrial sources. Understanding the impacts of these stressors on aquatic life is important in developing River Basin Management Plans (RBMP) as prescribed under the Water Framework Directive (WFD). In order to determine appropriate Programmes of Measures (PoM) within each RBMP we must identify those pressures on river basins that pose the greatest threat to water quality. Targeting remedial action toward those activities or emissions whose impact is large will result in the greatest environmental benefit, and efforts on those activities with a less significant impact will be minimised. To do this we need to be confident of (i) the presence of local impacts, (ii) the pressures that may be contributing to those impacts and (iii) the relative contribution of pressures from different sources in causing the impacts (where there are multiple sources). A source apportionment technique is required to help identify impacts and the causes of biological deterioration within catchments in order to inform appropriate PoM. The outputs of such a technique should relate to the concept of Good Ecological Status of the WFD. Further, results should be easy to communicate.

A technique developed by the Dutch National Institute for Public Health and the Environment (RIVM) in collaboration with partners in the U.S. uses ecological and ecotoxicological principles to identify local impacts on ecosystems and their probable causes, using biological and non-biological monitoring data. The technique allows the relative importance of different stressors to be presented visually as geographically plotted pie charts, with pie sizes denoting the magnitude of local ecological impacts, and slice sizes the relative importance of the likely contributing causes. These are known as Effect and Probable Cause (EPC) pie diagrams. The EPC approach consists of (1) RIVPACS-type modelling to quantify local impacts and to identify expected but missing species, (2) ecotoxicological analyses to quantify a single summary value for the probable toxic pressures of local mixtures of contaminants, (3) Generalised Linear Modelling (GLM), to relate variation in pressure variables to variation in individual species' abundances, and (4) the derivation and mapping of EPCs.

This report describes the application of this eco-epidemiological technique using paired stressor- and biological data for rivers in England and Wales over a 10-year period. We investigate its potential for use by policy makers and river basin planning managers as a tool for water quality management, especially in relation to developing PoM under the WFD.

Paired chemical and biological monitoring data were supplied by the Environment Agency for the period 1996–2004. Stressor monitoring data included: habitat characteristics, classical water chemistry, nutrients, industrial chemicals (primarily metals) and pesticides. Biological monitoring data covering 76 aquatic macroinvertebrate taxa (BMWP-taxa, family level) were also supplied. RIVPACS (River Invertebrate Prediction and Classification System) modelling performed by the Environment Agency yielded both the impact magnitudes for the sites (represented as pie sizes in the EPCs) and the identities of species that were expected but missing.

Bioavailability modelling was used to calculate dissolved (available) concentrations per compound from total concentrations. Species Sensitivity Distributions (SSD) modelling was used to convert available concentrations into the local toxic pressure per compound (single-substance Potentially Affected Fraction (ssPAF) of species). Thereupon, the ssPAF values were aggregated with mixture toxicity models to represent the overall toxic pressures (multi-substance PAF (msPAF)) of the locally occurring mixtures. Using this approach offers a significant advantage because of the

enormous improvement in statistical sensitivity to distinguish signal variability (caused by exposure to an array of stressors) from natural variability.

Generalised Linear Modelling was used to describe the variability of species abundance as functions of the stressor variables considered, including the summary parameter of msPAF to represent overall toxic pressure.

An impact-attribution algorithm was used to finally determine the relative contributions of different stressors (slice sizes) to the overall impact (pie size) for each site, which includes quantification of the unexplained variance in species abundances.

The process of data collection and analysis as well as the results were of interest in this scoping study, in order to consider the feasibility of the Environment Agency using this technique for future river basin management.

The results show that:

- The compilation of data for eco-epidemiological analyses is a major step that is of critical importance for the success of the EPC (and any other) method. This could be simplified if monitoring and data collection is targeted to meet the requirements of eco-epidemiological techniques.
- Sufficient data was available for England and Wales to allow the EPC analysis to be completed. Both site-specific and national information on ecological effects and their probable causes is presented in the format of some selected examples; more comprehensive analyses and output presentations are possible.
- The current analyses should be considered as preliminary results, to show that the method is operational. Further refinements in data analysis are possible, should more data and funding become available.
- The results suggest that classical water chemistry parameters and habitat characteristics play a significant role in shaping local macrofauna assemblages, as compared to the set of chosen reference sites.
- At a local scale, species loss can be attributed to a lesser or greater extent to mixture exposures, confirmed by a major association between (mixture) toxic pressure and family abundance data in the majority of taxa. The GLM analyses identified highly significant associations between family abundance data and acute toxic pressure for more than 50 per cent of the families.
- The observed loss of families attributed to mixture exposure in field conditions significantly covaries with the acute toxic pressure, so that the local value of acute toxic pressure ($msPAF_{EC50}$) seems to imply an upper limit estimate of family loss due to mixture exposure.
- There are significant correlations between family abundance and toxic pressure, such that various opportunist families and sensitive families can be recognised.
- The results are a product of the data available. They do not represent all sites at which the Environment Agency monitors biology and do not represent all pressures that may be impacting on biology at a site. Many sites were excluded from the analysis because of insufficient data. Further consideration should be given to how the data gaps can be filled and how data collection could be targeted to ensure the most appropriate data are collected for use in eco-epidemiological techniques such as this. Whether EPCs can be presented for sites with incomplete data coverage is a subject for future investigation.

- Despite only limited data, diagnostic results have been obtained from the analysis, and these can be considered useful for addressing practical regulatory problems under the WFD. Further consideration of the outputs by local staff is now required to 'ground truth' the outputs and identify additional data sources that may be available.

Acknowledgement

The Environment Agency and RIVM acknowledge Dr. Mark Crane, WCA Environment, for establishing the link between the Environment Agency and RIVM which has led to this collaboration.

The Director-General of RIVM is acknowledged for funding research in the field of eco-epidemiology, in the framework of RIVM's Strategic Research Program, under project nrs. S/860703 (2003-2006) and S/607001 (2007-2010), within the research spearheads "Quantitative Risk Assessment (QRA)" and "Environmental Quality and Health (EQH)". Based on this funding, the EPC technique was developed and first deployed on a (bio)monitoring database for Ohio. Further works (other geographies, other taxa, other stressor combinations) can be executed to further develop and implement the method, meant to support the implementation of, amongst others, the EU WFD.

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1 Introduction

1.1 Problem definition

Water quality is subject to a variety of pressures, including changes in physical habitat, point-source discharges, urban diffuse sources of contaminants, water abstraction, inputs of nutrients, and trace organics from domestic, agriculture and industrial sources. These stressors impact on and alter ecosystems. Each stressor will contribute to the overall net impact, although the significance of each individual pressure will vary between locations.

Prevention and minimisation of adverse alterations to ecosystems are major goals of environmental management. The Water Framework Directive (WFD) requires that waterbodies meet Good Ecological and Chemical Status by 2015. Understanding the impacts of stressors, their sources and their relative contribution to the overall state of the ecosystem is important in developing River Basin Management Plans (RBMPs) as prescribed under the WFD. In order to determine appropriate Programmes of Measures (PoMs) within each RBMP, we must identify those pressures on river basins that pose the greatest threat to water quality. Targeting remedial action toward those activities or emissions will result in the greatest environmental benefit, and efforts on those activities whose impact is small can be minimised.

To do this we need to be confident of (i) the presence of local impacts, (ii) the pressures that may be contributing to those impacts and (iii) the relative contribution of pressures from different sources in causing the magnitude of impacts (where there are multiple sources). Tools are required to help identify impacts and causes of biological deterioration within catchments and thus inform appropriate PoM.

1.2 Eco-epidemiology supports management decisions

Eco-epidemiology is concerned with the identification of local impacts and their probable causes. Methods to quantify the magnitude of local degradation in aquatic communities are well developed (for example, Karr, 1981, Moss *et al*., 1987). The diagnosis of probable causes has typically relied on expert judgment, application of multivariate statistics, and weight-of-evidence methods. Such methods require significant expertise to use and interpret, and their results are often difficult to communicate. Further, mixtures of potentially toxic compounds also need to be considered, but are often not a part of such assessments. Considering low-level exposures to mixtures of toxicants in the field is too complicated for experts to judge directly. Modelling could help to solve this problem.

Recently, the Dutch National Institute for Public Health and the Environment (RIVM), in collaboration with scientists in the U.S., has designed a method that:

- Quantifies local ecological impacts in river ecosystems.
- Considers the issue of mixtures of potentially toxic chemicals.
- Assigns those local impacts to probable causes.
- Enables easy communication of all results to river basin managers.

The method is described by De Zwart (2006), using chemical and biological monitoring data from Ohio (U.S.) surface waters. Abundance data for 96 species of fish from

approximately 700 sites were used to develop the method. De Zwart *et al*. (2006a) use an eco-epidemiological method to produce 'Effect and Probable Cause (EPC) pie charts' for each sample site. The EPC charts are mapped using a Geographical Information System (GIS). For each of the sites, the size of the pie chart represents the magnitude of local ecological impact. The slice sizes represent the relative importance of different stressors in causing a local impact. The EPC-approach combines ecological, ecotoxicological, and exposure modelling to provide statistical estimates of the probable effects of different natural and anthropogenic stressors on assemblages of biological species, including mixtures of toxic compounds.

1.3 Approach and aims

This report describes the application of this eco-epidemiological technique to paired chemical and biological data for rivers in England and Wales collected over the past 10 years. The dataset used for the work described herein does not represent all potential stressors to which aquatic organisms may be subjected, but was considered sufficient for the purposes of this scoping study.

This project was carried out as a proof of concept study to investigate the potential of an eco-epidemiological technique as a water quality management tool for use by policy makers and river basin planning managers, especially in relation to developing PoM under the WFD.

The work is a collaboration between the Environment Agency and RIVM. The objectives of this project were to explore the use of the EPC approach to readily available data for England and Wales, with specific focus on:

- The approach to express toxicant concentrations in terms of toxic pressure for separate chemicals and mixtures.
- Appraisal of the strengths and limitations of the approach.
- Consideration of the approach in relation to other diagnostic systems developed by the Environment Agency.
- Making recommendations as to the wider application of the approach.

2 Methods and Approaches

2.1 Schematic outline

In the following sections we describe the detailed preparation of the data set, intermediate analyses, and the simplification of these analyses to produce mapped EPC pie diagrams. A schematic outline of these data analysis steps is presented in Figure 2.1 and further description is provided in the following sections.

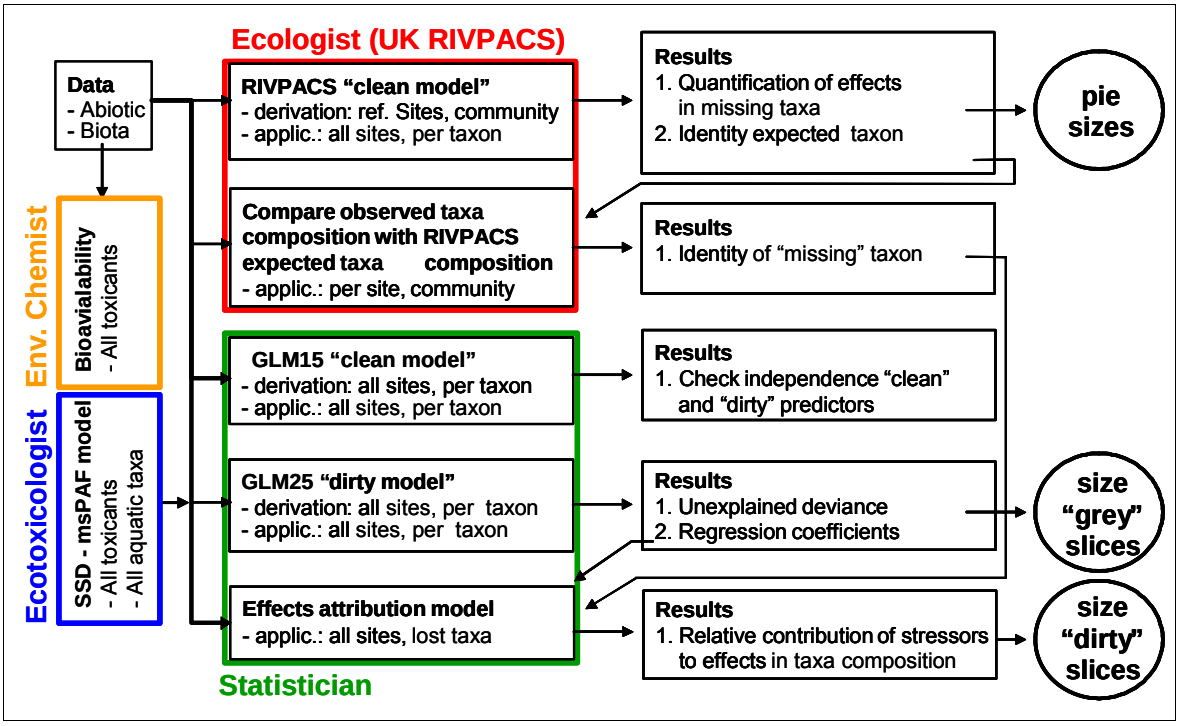

Figure 2.1 Outline of (a) the conceptual model of the Effect and Probable Cause (EPC) pie diagram method, and (b) the analytical steps to derive EPC-pie diagrams from (bio)monitoring data

2.2 Raw data

The EPC-analysis can only be performed when a sufficient number of sampling sites have complete and consistent data coverage. This is termed a 'square data block'. For this reason, the entire available dataset is first screened to extract a subset of data, the square dataset. We compiled large sets of raw and modelled data, and screened them for coverage. The number of sampling sites required can increase substantially with an increasing numbers of potential stressors. The use of the ecotoxicological modelling, such as the Potentially Affect Fraction (PAF) concept used in this work, is extremely useful in lowering the number of stressor variables and so increasing the statistical power of the diagnostic analyses. The data was provided by the Environment Agency and is described further in Sections 2.2.1 and 2.2.2.

2.2.1 Biotic data

The biotic data consists of aquatic macroinvertebrate taxon composition and abundance data recorded at Biological Monitoring Working Party (BMWP) level for over 5,000 sites. The data were collected according to highly standardised protocols in the period between 1993 and 2004. Sampling locations were sampled every three years in spring and autumn as part of the Environment Agency's General Quality Assessment (GQA) monitoring protocol. The taxa are listed in Appendix 1.

2.2.2 Abiotic data

Site-specific river habitat and chemical monitoring data collected between 1995 and 2004 were provided by the Environment Agency. Data included:

- Sixteen habitat characteristics: Lat (latitude), Long (longitude), Alk (alkalinity-hardness), DisS (Distance to Source), DCat (Discharge category), Width (width), Depth (depth), BolCob (boulders and cobbles), PebGrav (pebbles and gravel), Sand (sand), Silt (silt), Phi (average grain size), Slope (slope), Alt (altitude), MAT (mean annual temperature) and AATR (annual average temperature range).
- Five classical water chemistry characteristics: $CaCO_3$-Hardness, Cl^- (chloride), TSS (total suspended solids), pH, and BOD (biological oxygen demand).
- Three nutrients: phosphate (PO_4^-), ammonia (NH_4^+), nitrate (NO_3^-).
- Eight Industrial chemicals: lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn), ammonia (NH_3), and nitrite (NO_2^-).
- 109 pesticides (see Appendix 2 for list).

Dissolved oxygen was accidentally omitted during the data analyses, but is highly correlated with BOD so its omission was deemed unimportant. The list of variables and their physico-chemical and ecotoxicological properties is given in Appendix 3. A summary of variable names, abbreviations, units and type is given in Table 2.2.

Additional variables pertained to land use data (such as "Urban", "Forest", "Crop" and "Cattle"). These data were not used in the analyses due to large data gaps. Including these data would result in significant loss of complete data blocks.

#	Code	Meaning	Units	Type
1	LAT	Latitude	dec. degrees	Habitat
2	LONG	Longitude	dec. degrees	Habitat
3	ALK	Alkalinity	mg/L CaCO3	Habitat
4	DisS	Distance to Source	km	Habitat
5	DCat	Discharge Category	Category 1-10	Habitat
6	Width	Width	m	Habitat
7	Depth	Depth	m	Habitat
8	BolCob	Boulders and Cobbles	percent	Habitat
9	PebGrav	Pebbles and Gravel	percent	Habitat
10	Sand	Sand	percent	Habitat
11	Silt	Silt	percent	Habitat
12	Phi	Average grain size	-	Habitat
13	Slope	Slope	percent	Habitat
14	Alt	Altitude	m	Habitat
15	MAT	Mean Annual Temperature	oC	Habitat
16	AATR	Annual Average Temp. Range	oC	Habitat
17	CaCO3	CaCO3-Hardness	mg/L CaCO3	Classical water chemistry
18	Cl-	Chloride	mg/L	Classical water chemistry
19	TSS	Total Suspended Solids	mg/L	Classical water chemistry
20	pH	Acidity	-	Classical water chemistry
	BOD	Biological Oxygen Demand	mg O2 /L	Skipped from analyses
21	PO4-	Orthophosphate	mg/L	Nutrients
22	NH4+	Ammonium	mg/L	Nutrients
23	NO3-	Nitrate	mg/L	Nutrients
23a	Pb	Lead	mg/L	Industrial chemicals
23b	Ni	Nickel	mg/L	Industrial chemicals
23c	Cr	Chromium	mg/L	Industrial chemicals
23d	Cu	Copper	mg/L	Industrial chemicals
23e	Cd	Cadmium	mg/L	Industrial chemicals
23f	Zn	Zinc	mg/L	Industrial chemicals
23g	NH3	Ammonia	mg/L	Industrial chemicals
23h	NO2-	Nitrite	mg/L	Industrial chemicals
24	msPAF(i)	Industrial chemicals toxic pressure	fraction	Industrial chemicals
25(1-109)	msPAF(p)	Pesticides toxic pressure (109 compounds)	fraction	Pesticides

Table 2.2 Variables used in the assessments

2.2.3 Toxicant data

2.2.3.1 Industrial (measured) compounds

Surface water concentration data that met the square dataset requirements were available for eight industrial compounds: the metals cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn), and lead (Pb), and for nitrite (NO_2^-) and ammonia (NH_3). Measured total concentrations were converted to bioavailable concentrations as described in Section 2.4.3. The bioavailable fractions were used to calculate local toxic pressures per compound, producing Potentially Affected Fraction (PAF) values for each compound and subsequently multi-substance PAF values for all 'industrial'[1] compounds at each sampling site as per Section 2.3.1. These values are coded as (ms)PAF_I, to show that they are based on industrial chemicals.

2.2.3.2 Modelled compounds: pesticide data

Measured concentrations of pesticides in surface water were not consistently available for each sampling point, and the number of measured data points for which pesticide monitoring data was available consistently was too low to be considered representative

[1] For the purposes of this report, industrial compounds are cadmium, chromium, copper, nickel, zinc, lead, nitrite ion and ammonia.

or meaningful for EPC analyses. In addition, the pesticides for which monitoring data were available were generally of little relevance, typically being compounds no longer approved for use in the UK. As pesticides were of particular interest for this trial, the pesticides dataset was supplemented using modelled data. The POPPIE model (Prediction of Pesticide Pollution In the Environment) was used to predict the surface water concentrations of pesticides at each sampling point.

POPPIE uses a surface water model (SWATCATCH; Hollis *et al*., 1996) to assess risk from diffuse agricultural inputs of pesticides. The surface water model incorporates a number of datasets in a Geographic Information System (GIS). Model input data included: pesticide usage data, cropping data per region, 903 surface water catchments, soil types and properties, pesticide physicochemical properties (adsorption coefficient Koc, half-life, solubility and Henry's constant), and MORECS (Met Office Rainfall and Evaporation Calculation System) and HER (hydrologically effective rainfall) data. POPPIE predicts the monthly mean surface water concentration of each pesticide at the outlet of each catchment.

A single predicted pesticide value (annual average of the monthly values) was derived per catchment studied. Because POPPIE generates modelled outputs representing the catchment outflow, the values may not realistically represent the pesticide exposure at the sampling locations. In addition, point source inputs of agricultural pesticides and inputs from non-agricultural uses of pesticides such as use in parks, on railways and roads and industrial discharges are not taken into account in POPPIE predictions. The modelled values are therefore likely to underestimate the true pesticide load at some sites within a catchment. This is especially true in urban areas where POPPIE predictions will underestimate environmental concentrations of pesticides. POPPIE has been validated against measured environmental concentrations and further discussion can be found in the Environment Agency's discussion of the model (2001). However, whilst the shortcomings of the modelled data were recognised, it was agreed that the POPPIE predictions represented the most available national scale pesticide modelled data available within the timescales of this scoping study.

2.3 Toxic pressure modelling

2.3.1 Calculating toxic pressures: motives and principles

The more variables that are included in an analysis the lower the power of the analysis to detect significant relationships. Therefore, instead of using individual compound concentrations of toxicant in the analysis, we calculated summary indicators of risk of mixtures of chemical compounds that are present and may influence local macrofauna assemblages. The use of this summary statistic, instead of raw concentration data per compound, minimises the number of predictors in the assessment and thus increases its statistical power. For this study, these calculations are based on the bioavailable fractions of the measured industrial chemicals and the modelled pesticide concentrations.

The summary statistics are calculated per compound first, and then per compound group (defined by having the same Toxic Mode of Action, for example organic phosphate insecticides, photosynthesis inhibitors), and finally for the whole mixture of compounds and compound subgroups with similar and dissimilar Toxic Modes of Action. The parameter used to quantify potential influences is called 'toxic pressure', and is expressed as the fraction of species that can locally occur and that is probably

affected at a level higher than 50 per cent. This fraction is abbreviated as PAF: Potentially Affected Fraction. For mixtures, it is called the multi-substance PAF (msPAF).

The analyses to calculate PAF and msPAF consist of (a) data compilation and collecting compound property information, (b) assessing the available fraction, acknowledging local exposure conditions, (c) determination of Species Sensitivity Distributions (SSD) for all compounds, and (d) determining toxic pressures per compound and for mixtures.

This section describes these analyses, resulting in two novel parameters for further modelling, namely: $msPAF_I$ and $msPAF_P$ for industrial chemicals and pesticides, respectively. The summary variables were used in the EPC analyses, and in simple product-moment correlation analyses, looking into covariation between toxic pressure and taxon abundance.

2.3.2 Compound property data (physico-chemical and ecotoxicity)

Two types of data were collected for each chemical compound. Data on physico-chemical properties were compiled to enable calculation of the dissolved, bioavailable concentrations from total concentrations, according to formulae given below. Data on the ecotoxicity of the compounds were compiled to enable derivation of SSD. The data originated from various sources including the RIVM e-toxBase (Wintersen *et al*., 2004).

2.3.3 Availability assessment of toxicant concentrations

The bioavailability of compounds is dependent on local exposure conditions, and is influenced by factors such as dissolved organic carbon, pH and water hardness. Existing methods to estimate exposure concentrations from total concentrations were used to determine local bioavailable concentrations for the selected compounds.

Three groups of compounds were distinguished:

- Metals. The toxicity of heavy metals to biota is strongly associated with the dissolved fraction in ionized form (Sorensen, 1991). This, in turn, depends strongly on water hardness. We estimated the bioavailable fractions of the metals using hardness-based availability correction formulae (Ohio Environmental Protection Agency (EPA), 1996), as summarised in Equation 1.

Equation 1. MeBioavailable = MeTotal * 12.522 * Hardness-0.7852 (for H > 25 mg/L CaCO3)

Where: MeBioavailable is the metal fraction calculated to be available for uptake, MeTotal is the total measured metal concentration, and H is hardness.

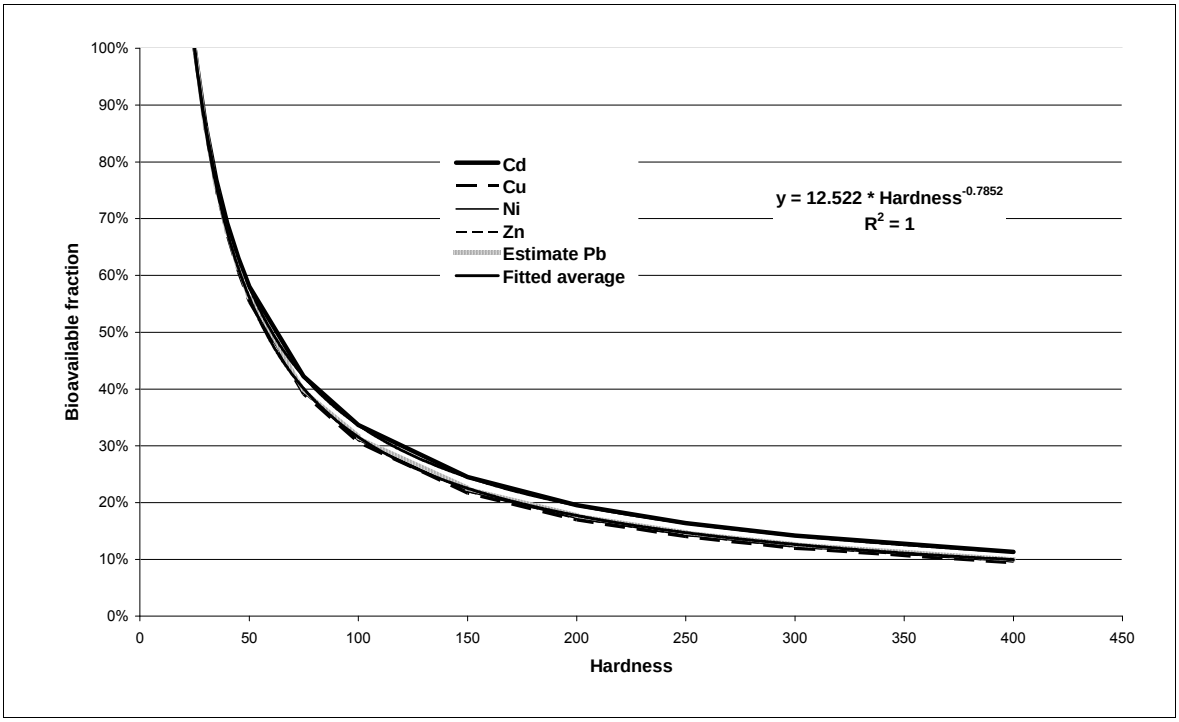

Figure 2.2 Relationship between bioavailable fraction of heavy metals and water hardness. Adopted for all heavy metals (including chromium).

- Organic chemicals, including the pesticides. The toxicity of organic chemicals is strongly influenced by the Dissolved Organic Carbon concentration. We estimated the bioavailable fractions of organic compounds (De Zwart *et al*., in press).

Equation 2. $\mathbf{Organic_{Bioavailable} = Organic_{Total} / (1 + fDOC * K_{OC})}$

Where: fDOC is the w/w/ fraction of dissolved organic carbon in the water and Koc is the partitioning coefficient of a substance between water and organic carbon.

- The rest of the compounds. For the other compounds, the concentrations as measured were considered fully available for uptake.

2.3.4 Derivation of multi-substance toxic pressure (msPAF)

We used Species Sensitivity Distributions (SSDs) (Posthuma *et al*., 2002) to estimate toxic pressure for each compound. An SSD is defined by a log-normal function in which μ specifies the median log transformed toxicity (EC_{50}) and σ the standard deviation of log transformed EC_{50}'s (variability across taxa). These parameters are listed for all compounds in Appendix 3.

Based on these SSDs, an extensive data set of single-substance PAF values per site and per year has been generated. These output data are not shown here. The data were used to calculate multi-substance PAF-values for industrial chemicals and pesticides, as well as for overall toxic pressure.

2.3.5 Derivation of multi-substance toxic pressures (msPAF)

Using the local toxic pressures of each of the substances studied (single-substance PAF), we calculated the local toxic pressures for industrial chemicals and pesticides separately, and for the overall mixture.

To obtain all msPAF-values, we applied the method described in De Zwart and Posthuma (2006). First, we determined the primary Toxic Mode of Action (TMoA) of each substance. Substances were then grouped into subgroups with similar TMoAs (such as photosynthesis inhibitors, insecticides, and so on). The TMoA assignments are summarised in Appendix 3. For each subgroup, we calculated the local toxic pressure of the mixture. We aggregated these TMoA-specific toxic pressure values per site to give the overall toxic pressure per site induced by the total local mixture.

2.3.6 Use of multi-substance toxic pressures (msPAF) in impact modelling

The multi-substance toxic pressure data were now treated as summary parameters for ecotoxicity in the EPC analyses, and replaced the original total concentrations of each of the separate compounds in the project dataset.

By reducing the number of parameters in this way the statistical power of the eco-epidemiological analysis is enhanced, as fewer sampling sites are required to recognise signal in the natural variability. “Simpson’s rule of thumb”, also known as the “curse of dimensionality” (Bellman, 1961), implies that to minimise error in regression analysis, an absolute minimum of 10 observations are needed for each predictor variable (Vaughan and Ormerod, 2003). Safer interpretations of this rule of thumb mention a requirement of 20, 50 or even 100 observations (in this case, sampling sites) per predictor. Reducing the number of input parameters is therefore beneficial to the analysis.

Summarising data on individual compounds into a single parameter may seem to imply loss of information on local impacts per compound. This is counteracted by the ability to disaggregate the net toxic pressure of mixtures into the TMoA-specific toxic pressures for subgroups of compounds, and finally to single-substance toxic pressures when needed.

2.4 Combining abiotic and biotic data

The two sets of data (biotic and abiotic) are not directly suited for EPC analysis. Selection of variables and sites are needed, to identify those combinations of sites and variables that would result in an optimal “square” data set. This is an optimisation process with various possible outcomes.

Within the process, we decided which abiotic variables were of primary interest for the assessment, and also had sufficient data coverage to be used. This optimisation process for abiotic variables resulted in 954 sites with data coverage (mostly multiple-year data), with mostly multiple-measurements per site due to repeated sampling rounds. Overall, the combined database contains more than 17.10^6 records.

Biotic data were supplied by the Environment Agency for the selected sites optimised for abiotic variable coverage. Biological data (macroinvertebrate abundance) were collected once in three years, in both spring and autumn, resulting in multiple samples per site (site/year combinations). To match the abiotic data to the biotic data we

calculated three-year moving averages in the abiotic data per variable per site. This links the biotic information to the abiotic characteristics of the previous three years.

From the abiotic moving-average data set, a data set with full data representation was derived. Combining this data with the biological data resulted in 307 sampling sites with sufficient abiotic and biotic data.

In summary, the optimised data set of biotic and abiotic data consisted of data for 76 taxa, 307 sites, 1,042 site-year combinations and 25 abiotic parameters (including two summary parameters for toxic pressure of industrial chemicals and pesticides). A summary of variable names, abbreviations, units and type is given in Table 2.2.

2.5 Ecological and statistical analyses

2.5.1 Quantifying biological condition and impairment: RIVPACS modelling

The EPC concept defines magnitude of local impact (the size of the EPC pie chart) by quantification of the deviation from expected conditions. This is done by comparing those taxa observed in the field with those expected to be present in an unimpaired site (reference conditions). In identifying reference conditions it needs be acknowledged that different types of surface waters are populated with characteristic faunal assemblages. Based on data from these best available sites, the conditions of other sites can be qualified as 'deviating' from the expectation for the water body type. Pie sizes produced in the catchment pressure identification are based on this quantified deviation[2].

The Environment Agency uses RIVPACS (the River Invertebrate Prediction and Classification System) to predict the taxa that would be expected at a site if it were unimpaired. RIVPACS models consider the presence or absence of species for a set of reference sites, and use data on the physical characteristics of a new site to predict the presence or absence of species at that site.

2.5.1.1 RIVPACS Modelling

The RIVPACS reference sites were chosen as being the best available sites of their type across the UK at the time of sampling. At each site, three-minute macroinvertebrate kick samples were collected in spring, summer and autumn of one year, and the biology data held as abundance category at the lowest taxonomic unit. A suite of environmental parameters were also recorded at each site in a standard manner. These environmental parameters represent the optimum correlates with biological community. These are:

- Altitude (m above sea level).
- Latitude.
- Longitude.
- Distance from source (km).
- Slope (km).
- Mean air temperature.

[2] Note that *any* set of reference sites can be chosen. For example, if the most impacted sites were selected as reference sites, the catchment analyses would result in similar looking results, but with the size of the pie now indicating deviation from bad condition.

- Air temperature range.
- Discharge category (1-10).
- Stream width (cm).
- Stream depth (cm).
- Substrate.
- Alkalinity (mg l^{-1} $CaCo^{3}$).

The RIVPACS model was based on these site-stressor-family data combinations for reference sites. By comparing the environmental parameters of a new site with the RIVPACS model, we are able to predict the presence and abundance of each taxon at non-reference sites – that is, make a prediction of the taxa assuming the site was at 'reference condition'. This becomes the 'expected' list of fauna, with each taxa having a probability of capture and a probability of abundance category.

This is done by calculating the probability of membership of each of the end groups within the model based on the environmental parameters of a new site. To predict the presence and abundance of a taxa RIVPACS multiplies the probability of group membership with the percentage of reference sites in that group that have that particular taxon. For example, Site 111 has a 60 per cent chance of being in Group 1, 5 per cent of being in Group 2,and so on. Taxon x is found in 33 per cent of Group 1 samples, therefore RIVPACS would multiply 0.6 by 33 per cent, so there is a 20 per cent contribution of Group 1 to the likelihood of capture of Taxon x. This is repeated for each group, and the contributions summed to get the 'expected' likelihood of capture for Taxon x at Site 111.

2.5.1.2 Use of RIVPACS to calculate pie size

The Environment Agency used RPBATCH: RIVPACS III+ Release 3.3 to produce: (1) the probability of capture (Pc) of each BMWP family, and (2) the expected abundance of each family at each of the sites for which optimised abiotic data were available.

These Pc values are summarised over species per site, by calculating the observed-over-expected ratio (O/E_{site}). A high O/E indicates favourable conditions; that is, similarity to the reference conditions of the water type. In RIVPACS the O/E-scale runs from 0 to approximately 1.6.

To calculate the radius of the EPC pie diagrams, impairment must be expressed on an absolute scale of 0 to 1. However, by applying the RIVPACS O/E method, O can theoretically exceed E because of sampling or prediction error. This could result in pie sizes (1-O/E) that are negative. Furthermore, this way of scaling may also result in a positive pie size that would imply impairment even when all species that are expected are actually observed. To address this issue we considered any species with $Pc \geq 0.5$ as expected to occur and counted these species as an alternative way of estimating E and calculating pie sizes. This alteration resolves both problems of negative pie size and the potential problem of implying impairment when no species were missing.

The expected fauna produced through this process was then compared with abundances of the actual fauna collected; that is, the 'observed' fauna. By doing this we could quantify the magnitude of local impacts at our 1,042 site-year combinations. These data result in the pie sizes of the EPC diagrams. We further used the RIVPACS model to make lists of missing families per site; that is, species that are expected to be present but that were absent. The lists of taxa obtained in this way is required for the effect-to-cause attribution step within the whole analysis process (see below).

2.5.2 Identifying likely causes of impairment: GLM modelling

2.5.2.1 Construction of GLM models

We used Generalized Linear Models (GLM) (McCullagh and Nelder, 1989) to quantify the associations between macroinvertebrate family abundance and the environmental variables.

We built two sets of models. We used one set of models (GLM15) to predict family abundance from the same predictors as used in the RIVPACS model. The form of these models was:

GLM15: $\ln(O_i)$ = intercept + Lat + Lat^2 + Long + Long^2 + DisS + DisS^2 + DCat + DCat^2 + Width + Width^2 + Depth + Depth^2 + BolCob + BolCob^2 + PebGrav + PebGrav^2 + Sand + Sand^2 + Silt + Silt^2 + Phi + Phi^2 + Slope + Slope^2 + Alt + Alt^2 + MAT + MAT^2 + AATR + AATR^2

where O_i = Observed abundance of families i, and the other parameters are the predictors also used in RIVPACS.

We then constructed another set of models (GLM25) to describe family responses to both the gradients of the natural and the 10 other variables. These models took the form:

GLM25: $\ln(O_i)$ = intercept + Lat + Lat^2 + Long + Long^2 + DisS + DisS^2 + DCat + DCat^2 + Width + Width^2 + Depth + Depth^2 + BolCob + BolCob^2 + PebGrav + PebGrav^2 + Sand + Sand^2 + Silt + Silt^2 + Phi + Phi^2 + Slope + Slope^2 + Alt + Alt^2 + MAT + MAT^2 + AATR + AATR^2 + BOD + BOD^2 + CaCO3 + CaCO3^2 + pH + pH^2 + TSS + TSS^2 + Cl + Cl^2 + NH4 + NH4^2 + NO3 + NO3^2 + PO4 + PO4^2 + ImsPAF + ImsPAF^2 + PmsPAF + PmsPAF^2

Note that the GLM15 and GLM25 both use the RIVPACS variables. The formula contains linear and quadratic terms to allow for optimum-responses (like for pH).

We added both linear and quadratic forms of the probable stress variables to the models by a stepwise procedure. The stepwise procedure used the Bayesian Information Criterion (BIC) (Schwarz, 1978) to restrict the addition of terms to those that had a significant contribution to the overall model ($p < 0.05$), based on type I evaluation of sums of squares. Calculations were conducted with S-Plus 2000, Professional Release 3 (MathSoft, Inc., Cambridge, MA, USA). Predictor variables that were not selected by this procedure received a regression coefficient of zero.

We created both full (GLM15 and GLM25) and null models for each family. Null models were of the form GLM0 = ln(abundance) = a (constant), where a is the mean abundance of the family across all sites.

GLM output consisted of regression coefficients, degrees of freedom, and deviance residuals for both the full (DEV_{full}) and null models (DEV_{null}). We used explained deviance (ED) as a measure of the explanatory capacity of each model, where $ED = (DEV_{null} – DEV_{full})/DEV_{null}$.

The objective of the GLM-modelling was to isolate the likely effects of different stressor variables on macrofauna abundance. As in the RIVPACS models, we needed to distinguish between the effects of stressor variables on macrofauna and effects associated with natural factors. However, direct regression of the differences between observed abundances and those expected from the GLM15 models (that is, [ln(Oi) – ln(Ei)]) on the 10 stressor variables resulted in significant convergence problems. To avoid this problem, we fitted GLM25 models directly to the ln(Oi) data. This approach is

only valid when the natural and other stressor variables are not substantially correlated, otherwise the values of the regression coefficients would not be independent of one another. The latter was tested specifically.

2.5.2.2 Identification of likely causes of local impairment to calculate pie slices

We used statistically significant associations between family abundance and stressor variables (from GLMs), and the magnitudes of impacts and the identities of missing families (from RIVPACS), to identify likely causes of biological impairment. While we recognise that such associations do not necessarily imply causation, we use the term 'cause' in this restricted sense in the remainder of the paper.

We linked the abundances of individual families and the stressors occurring at individual sites as follows:

- Predicted abundance.

We applied the calibrated GLM25 regression models to predict the abundance of family i at any site (Ei,GLM25) as a function of both the naturally occurring and stressor conditions occurring at a site.

- Unexplained variance (unknown causes).

We calculated the unexplained variance in family abundance at each site as the departure from a linear association between observed (Oi) and expected (Ei,GLM25) abundances over all families. We expressed unexplained variation as $(1\text{-}r^2)$, and included this value as one of the slices in the EPC pie diagrams.

- Identity of missing families.

The RIVPACS model output allowed us to identify those families that were expected at $Pc \geq 0.5$ but not observed at the sampling sites.

- Associations with different stressor variables.

If a family was missing at a site as a possible consequence of unfavourable levels of some or all stressors we measured, the contribution of those stressor variables in the GLM25 model prediction should be negative. For example, if family i is missing at site x because of toxic stress by mixtures of pesticides, the value of $(y_{1,i} \bullet msPAF_P + y_{2,i} \bullet msPAF_P^2)$ should be negative. The relative potential influence of each stressor variable is simply that stressor's negative contribution divided by the sum of all negative stressor contributions for missing families. These proportions along with the unexplained variance were used to size the pie slices in the EPC graphs.

- Aggregation over sites.

We aggregated site-based estimates to derive insight regarding the overall regional importance of different stressors. We calculated regional values as simple averages of the percentage of variation in abundances associated with different measured factors observed at individual sites. These percentages were used along with per cent unexplained variation to construct a regional summary EPC pie graph.

We recognise that variables used in this study are in part composite, and need not be purely of natural origin, or purely of an added stress variety. Although the above procedure attributes variance to the different variables, we acknowledge that the attribution need not reflect man-induced changes only. For example, the local value of the variable pH can be determined by both natural causes (such as humic acids) and man-made causes.

We noted that the RIVPACS model also identifies families that are not expected at a site, but were nonetheless observed. The attribution model can be adjusted to also identify the likely causes for such increases by evaluating just the positive contributions of individual stressor variables in the GLM25 models for unexpected families observed. However, because of length and complexity limitations, we do not present these complementary assessments in this paper.

2.6 GIS-mapping of EPC pie diagrams

Completion of the analyses described above yielded information on the pie sizes (from comparing observed assemblages to RIVPACS predictions) and slice sizes (from GLM and RIVPACS modelling and the cause-attribution method). These results were plotted as pie charts known as 'Effect and Probable Cause' diagrams using GIS-based maps. All analyses were performed by RIVM without prior knowledge of local issues at the site, in a double blind procedure.

3 Results

3.1 Raw data: overview and stressor covariation

Data selection is described in the previous chapter. A summary overview in Table 3.1 presents the statistical properties of the variables (percentile values). Consideration of the range of values for all variables (including $msPAF_I$, and to a lesser extent $msPAF_P$), suggests that there was sufficient data for the EPC analyses to distinguish a signal for each of the variables where they shaped the composition of macrofauna assemblages.

	Used for RIVPACS and GLM15														
	Lat	Long	DisS	DCat	Width	Depth	BolCob	PebGrav	Sand	Silt	Phi	Slope	Alt	MAT	AATR
5th	50,68	-3,53	4,2	1,0	2,3	13,3	0	4,0	0	0	-6,5	0,2	5	9,11	10,74
25th	52,35	-2,75	14,1	3,0	5,8	22,9	9	21,0	5	1	-5,07	0,6	10	9,48	12,06
50th	53,13	-1,67	35	5,0	12,4	35,6	31	36,0	12	9	-2,49	1,6	46	9,81	12,76
75th	53,85	-1,25	53,1	6,0	20,1	53,8	50	47,0	18	26	0,86	4,2	70	10,1	13,06
95th	54,75	0,17	112,9	8,0	34,2	204,6	72	64,9	39	80	6,516	11,1	135	10,8	13,28
99th	54,98	1,13	139,8	9,0	55,1	235,0	86	69,0	54	100	8	25	215	11,3	13,5

	Used for GLM25									
	BOD	CaCO3	pH	TSS	Cl	NH4	NO3	PO4	ImsPAF	PmsPAF
5th	1,07	37,0	7,12	3,22	10,53	26	0,58	0,02	0,3%	0,00%
25th	1,47	128,4	7,62	7,7	25,07	54	2,83	0,07	0,5%	0,05%
50th	1,95	246,4	7,89	13,7	47,86	117	5,44	0,33	0,9%	0,3%
75th	2,44	312,5	8,03	18,4	71,92	210	8,35	0,74	1,4%	0,5%
95th	4,87	434,5	8,21	33,5	148,1	993	12,39	2,10	4,9%	1,6%
99th	6,72	557,2	8,40	52,3	299,2	2145	15,41	3,40	29,3%	3,0%

Overall msPAF
0,4%
0,7%
1,4%
2,0%
5,4%
29,3%

Table 3.1 Summary of variables and their statistical properties used in the analysis of ecological impacts and probable causes in rivers in England and Wales (1,042 data points). Note that the variable "alkalinity" was used additionally in RIVPACS as predictor.

We determined product-moment correlations between abiotic variables based on the available data for 1,042 site/year combinations. The correlation analysis is necessary to avoid statistical flaws in the impact attribution process that can occur when two or more variables are highly correlated. The results are shown in Table 3.2. Variables that apparently covary (positively or negatively) represent sets of logical associations, such as the variable "Latitude" being negatively associated with the variable "Mean Annual Temperature". It was decided to keep all variables in the assessment, with the exception of alkalinity, which was highly correlated with $CaCO_3$-hardness (data not shown in Table 3.2).

The data set remaining after preliminary data analyses consisted of 76 macrofauna taxa, 307 sampling sites with 1,042 site-year combinations and 25 abiotic variables. The final EPC analysis considers 10 variables as potential stressors to which impacts can be attributed.

	Lat	Long	DisS	DCat	Width	Depth	BolCob	PebGrav	Sand	Silt	Phi	Slope	Alt	MAT	AATR	BOD	CaCO3	pH	TSS	Cl-	NH4+	NO3-	PO4-	lmsPAF
Lat	1																							
Long	-0,09	1																						
DisS	0,02	0,21	1																					
DCat	0,21	0,03	0,82	1																				
Width	0,23	0,11	0,72	0,83	1																			
Depth	-0,14	0,33	0,64	0,49	0,45	1																		
BolCob	0,49	-0,44	-0,27	0,02	0,09	-0,46	1																	
PebGrav	-0,04	-0,26	-0,19	-0,17	-0,18	-0,49	0,00	1																
Sand	-0,22	0,26	0,13	0,00	-0,06	0,18	-0,55	-0,07	1															
Silt	-0,32	0,45	0,32	0,10	0,07	0,67	-0,66	-0,64	0,08	1														
Phi	-0,41	0,50	0,33	0,07	0,02	0,66	-0,86	-0,49	0,38	0,94	1													
Slope	0,07	-0,22	-0,44	-0,50	-0,36	-0,34	0,30	0,09	-0,19	-0,25	-0,30	1												
Alt	0,15	-0,08	-0,39	-0,37	-0,28	-0,39	0,31	0,15	-0,09	-0,34	-0,35	0,41	1											
MAT	-0,93	-0,10	-0,07	-0,24	-0,26	0,09	-0,37	0,09	0,14	0,22	0,29	0,00	-0,14	1										
AATR	0,06	0,83	0,29	0,10	0,13	0,24	-0,38	-0,11	0,24	0,31	0,38	-0,23	0,11	-0,22	1									
BOD	0,11	0,22	0,06	0,09	0,13	0,08	-0,04	-0,10	0,11	0,04	0,06	-0,18	-0,08	-0,10	0,28	1								
CaCO3	-0,21	0,39	0,07	-0,16	-0,15	0,21	-0,51	-0,08	0,30	0,38	0,47	-0,22	-0,14	0,15	0,49	0,21	1							
pH	-0,03	0,38	0,31	0,16	0,09	0,21	-0,32	0,01	0,14	0,22	0,28	-0,15	-0,09	-0,10	0,47	-0,05	0,48	1						
TSS	-0,21	0,08	0,18	0,07	0,00	0,11	-0,22	-0,06	0,03	0,23	0,23	-0,14	-0,15	0,19	0,10	0,18	0,17	0,07	1					
Cl-	-0,11	0,16	0,04	-0,04	-0,01	0,12	-0,19	-0,10	0,14	0,18	0,21	-0,14	-0,19	0,15	0,17	0,43	0,52	0,05	0,18	1				
NH4+	0,07	0,11	-0,03	0,00	0,03	0,01	-0,02	-0,13	0,07	0,06	0,07	-0,11	-0,12	-0,04	0,13	0,64	0,22	-0,14	0,14	0,41	1			
NO3-	-0,22	0,34	0,12	-0,07	-0,10	0,20	-0,47	-0,04	0,31	0,31	0,41	-0,27	-0,12	0,17	0,48	0,27	0,73	0,35	0,16	0,43	0,14	1		
PO4-	-0,10	0,25	0,06	-0,05	-0,03	0,06	-0,22	0,01	0,17	0,12	0,18	-0,20	-0,05	0,10	0,38	0,49	0,38	0,07	0,12	0,41	0,34	0,69	1	
lmsPAF	-0,30	-0,41	-0,14	-0,13	-0,12	-0,06	0,10	0,04	-0,07	-0,09	-0,10	0,11	-0,09	0,42	-0,51	-0,05	-0,05	-0,50	0,07	0,17	0,11	-0,12	-0,05	1
PmsPAF	-0,08	0,50	0,39	0,31	0,34	0,53	-0,30	-0,28	0,15	0,39	0,40	-0,30	-0,32	-0,03	0,39	0,21	0,26	0,20	0,09	0,22	0,13	0,26	0,12	-0,08

Table 3.2 Degree of association (r) for the 25 selected variables (n =1,042 site/year combinations). Values larger than (plus or minus) 0.5 are coloured orange, values larger than (plus or minus) 0.8 are coloured red. To obtain correlation coefficients: calculate r^2 from the data shown.

3.2 RIVPACS results and the derivation of pie sizes

3.2.1 Grouping of water bodies

Twinspan analysis performed on the biology data of the 614 reference sites alone produced 35 'end groups' or community types in the England and Wales model (Figure 3.1). Sites with similar species composition are grouped together at lower dissimilarity distances than sites with different species compositions. Using Multiple Discriminant Analysis, the grouping of sites was then explained by a set of environmental parameters. This forms the fundamental model of RIVPACS, allowing the prediction of taxa capture at a new site.

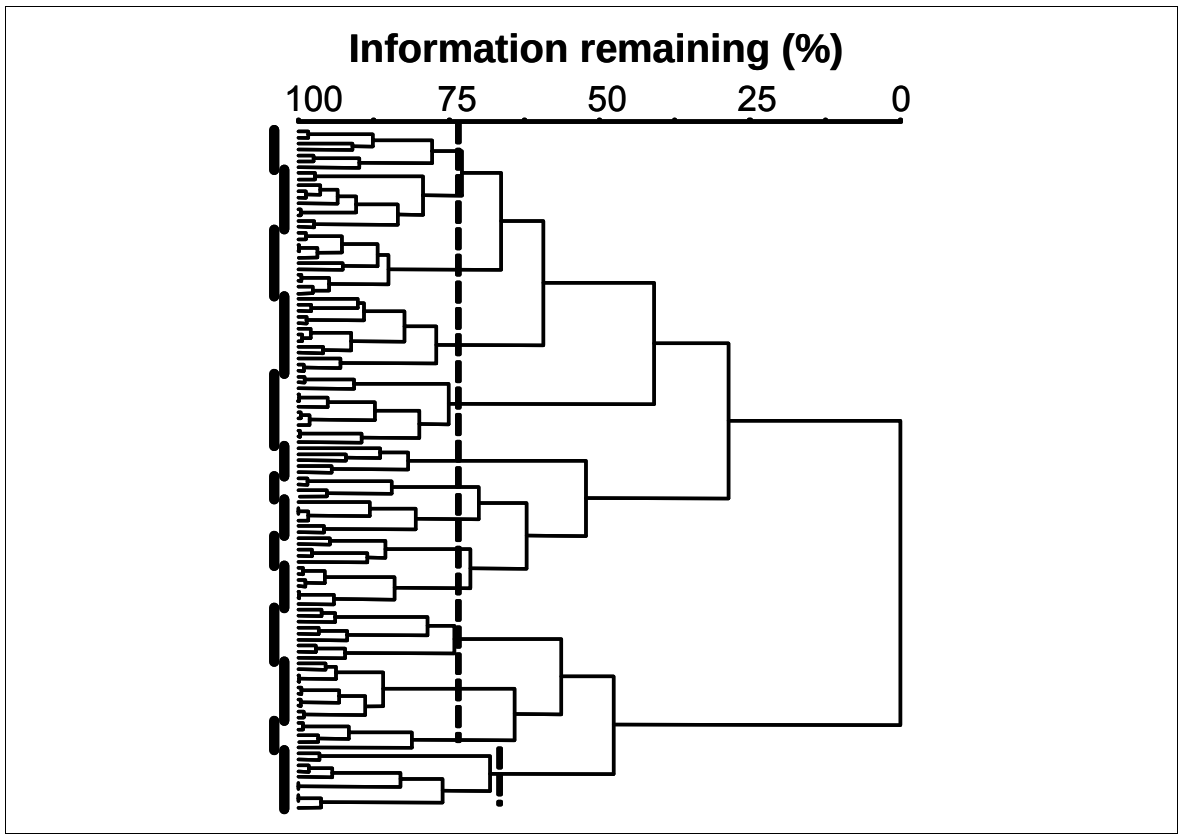

Figure 3.1 Example of a TWINSPAN presentation of RIVPACS-grouping of reference site

The site grouping shows that there are various subgroups of water body types, characterised by specific “reference fauna assemblages”. This categorisation is needed to quantify ecological impacts (pie sizes), by calculating the fraction of families expected (for the water body type, with a probability of capture Pc higher than 0.5) but missing.

3.2.2 Use of RIVPACS results in EPC analyses

A comparison of observed to expected (O/E) families is used to generate the radius of the EPC diagrams. To obtain radius values between 0 and 1, the RIVPACS O/E was adapted, but this had no influence on the interpretation of RIVPACS-based O/E values as compared to adapted O/E-values. The relationship between the original RIVPACS output and the adapted output (scaled 0 -1) are highly correlated (r^2=0.987, Figure 3.2).

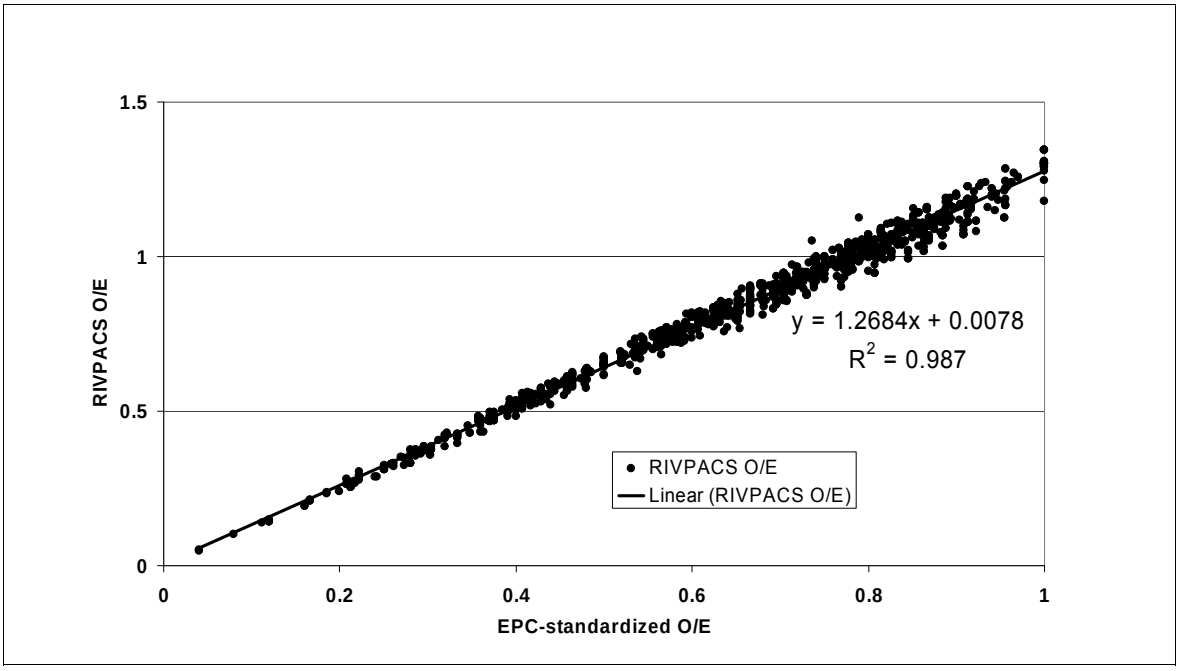

Figure 3.2 Relationship between the O/E values as used to map pie sizes on maps (ranging between 0 and 1) and the original RIVPACS-output.

The RIVPACS model output was then used to quantify the magnitude of local impacts (1-O/E, using adapted O/E) at all study sites. The radius of the pie chart represents magnitude of impact. .

The distribution of RIVPACS-based impact magnitude estimates (due to all stressors) over sites is shown in Figure 3.3. In approximately 5 per cent of the sites there was almost no family loss compared to the expected family composition, whilst in half the sites there was approximately 30 per cent or less. Ten percent of the sites were characterised by a family loss greater than 90 per cent.

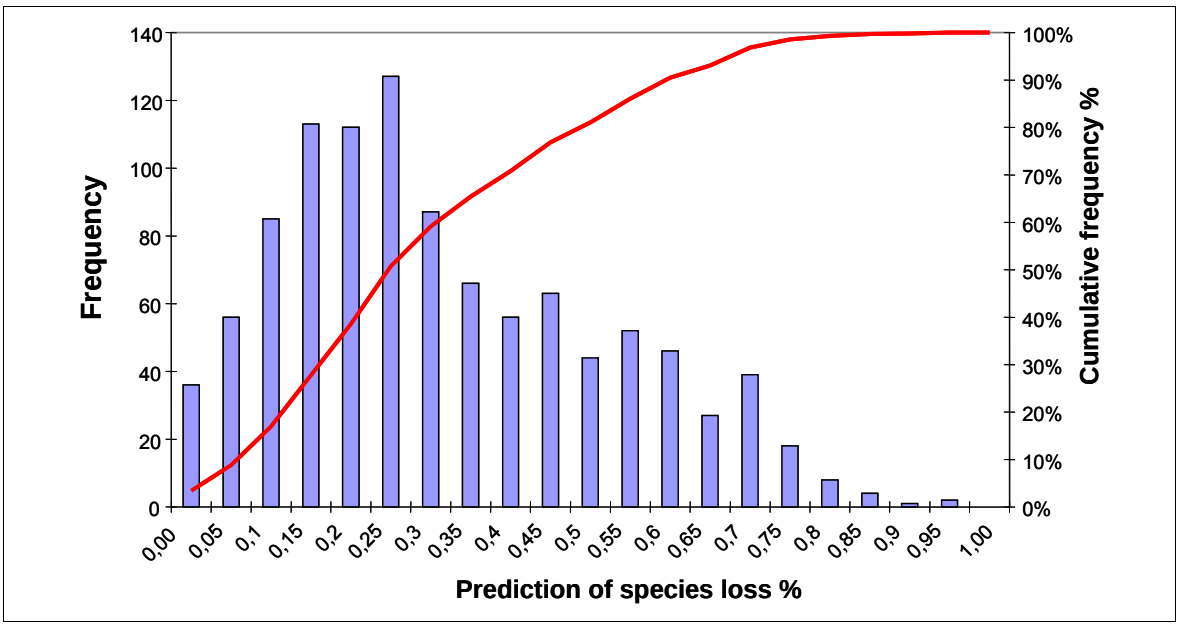

Figure 3.3 Distribution of impact magnitudes (expressed as per cent families lost as compared to reference conditions) over the sampling sites.

In addition to using RIVPACS to determine the size of the pies in the EPC diagrams, we also extracted the identity of species expected but missing. This intermediate result is necessary for source attribution and to determine the slices of each pie chart, and is described below.

3.3 Predicted toxic pressure variation

The raw chemical compound data (total measured or modelled concentrations) were recalculated into toxic pressures per compound, per group of compounds with similar Modes of Action, and for the total mixtures of industrial chemicals ($msPAF_I$), pesticides ($msPAF_P$) and all compounds ($msPAF_{All\ compounds}$) per site. The toxic pressure data for industrial chemicals and pesticides were used as summary parameters for toxic pressure for the EPC analyses.

3.3.1 Variation of multi-substance toxic pressure (msPAF)

Acute toxic pressure caused by the exposure of an assemblage to a mixture of compounds is defined as the fraction of species that may occur at a site and that is likely to be exposed at a level such that their EC_{50} is exceeded during short-term exposure. The variation in the acute toxic pressure for industrial chemicals and pesticides across the data set is summarised in Table 3.3 and Figure 3. Note that the field data are on macrofauna families rather than species. However, Species Sensitivity Distributions (SSDs) are based on species, not families. This does not influence the EPC analysis, since the toxic pressure is a proxy variable that summarises expected influences of toxicant mixtures on species and families.

The data suggest that in 95 per cent of the sites, exposure to the defined mixture of industrial chemicals ($msPAF_I$) leads to less than 5 per cent of the species being seriously affected (exposed higher than their EC_{50}). Similarly, concentrations at approximately one per cent of the sampling sites would induce serious effects in nearly 30 per cent of the species. This variance in expected mixture risks also pertains to other taxonomic levels, such as family level data, since toxic pressure is the parameter that summarises the local environmental condition.

The toxic pressure of pesticides ($msPAF_P$) calculated from monthly mean surface water concentration of each pesticide at the outlet of each catchment appears to be lower, with only one per cent of the sites having greater than three per cent affected species. In reality, we would expect higher pesticide concentrations than those predicted by POPPIE upstream and in areas close to application, directly after use, and so this result may simply reflect the underestimation of the pesticide load as derived by POPPIE, with overall risk from pesticides therefore also underestimated.

Percentiles	msPAF(I)	msPAF(P)	msPAF (I & P)
5th	0,3%	0,00%	0,4%
25th	0,5%	0,05%	0,7%
50th	0,8%	0,2%	1,4%
75th	1,4%	0,5%	2,0%
95th	4,9%	1,6%	5,4%
99th	29,3%	3,0%	29,3%

Table 3.3 Variation of $msPAF_I$ (industrial chemicals), $msPAF_P$ (pesticides) and the overall msPAF (for I and P) in the data set for England and Wales.

When whole mixtures are considered (msPAF(I&P)), sites strongly dominated by industrial chemicals (high $msPAF_I$, for example, 99th percentile) have lower toxic pressure from pesticides, since the total toxic pressure does not increase. In sites with lower exposure to industrial chemicals (95th percentile and lower), pesticide contributions increase the total pressure. The highest value of $msPAF_{All\ compounds}$, was approximately 70 per cent.

Two points must be borne in mind when considering the outputs of this assessment. If the EC_{50} is known for a large set of species, it is possible to estimate the fractions of taxa that could be seriously affected by toxic mixture exposure as per the current analyses. However, a similar analysis based on different endpoints, for example SSD_{EC40}, SSD_{EC10} or SSD_{NOEC}-data, would also result in curves similar to Figure 3.4, but shifted left as the ecotoxicity endpoint becomes more sensitive. In other words, higher fractions of species than shown in the EC_{50} graph will be exposed beyond their no observed effect concentration (NOEC) at the ambient concentrations in the data set. This implies that there may be larger variability of toxic pressures experienced by the species in their habitats than is suggested by the graphs of fEC_{50} exceedences. In addition, the SSD model typically uses species-level toxicity data as input parameters, and so the output also pertains to a potentially affected fraction of species. The monitoring data used in the analysis, however, are family-level data rather than species-level. By definition, the parameter “loss of families” is less sensitive than species loss. Therefore it is to be expected that that msPAF values based on species data overestimate the loss of families.

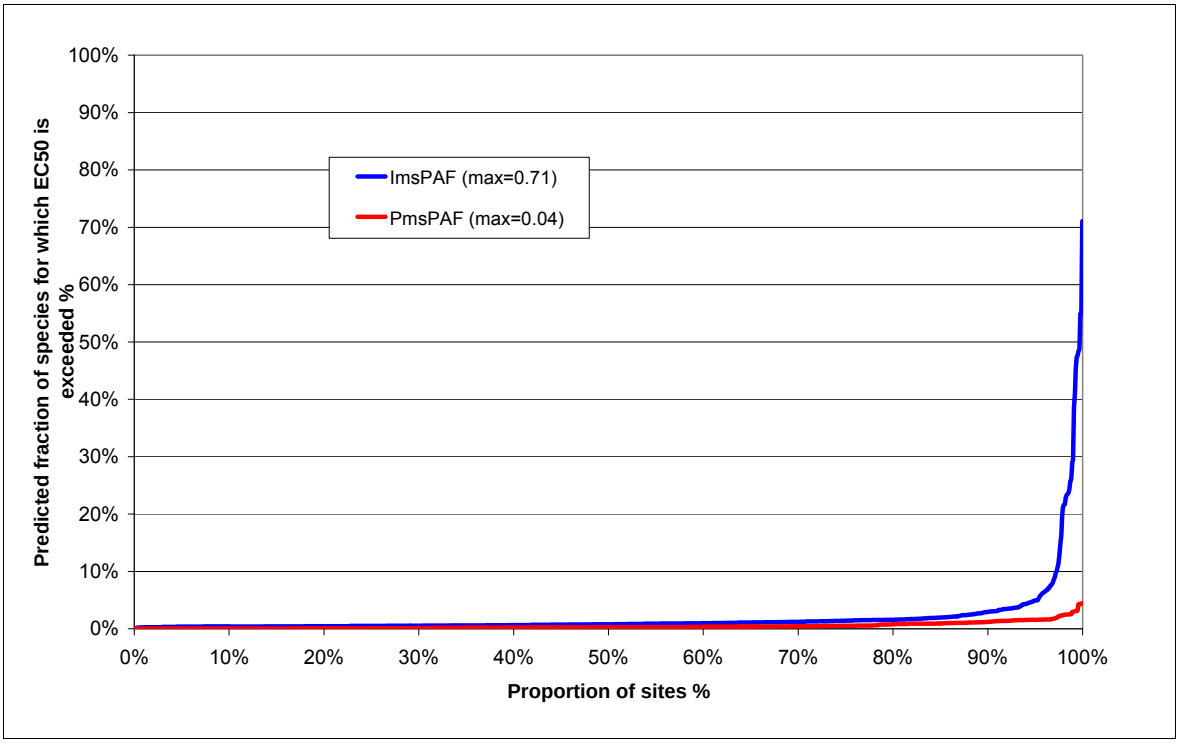

Figure 3.4 Cumulative distribution of the variation of $msPAF_I$ (industrial chemicals, blue upper line) and $msPAF_P$ (pesticides, red lower line) in the data set for England and Wales.

3.4 Variation of stressor variables

The variation of the other stressor variables was analysed in a similar way to the toxic pressure analysis and is depicted in Figure 3.5. Data variability and the use of

variables in the different models (RIVPACS, GLM15 and GLM25) are shown in Table 3.1.

Variability of each of the parameters across the whole data set is crucial for the GLM analysis. In eco-epidemiological analysis, which is based on the analysis of gradients, all stressor variables should be independent of each other (see covariation analyses, Table 3.2) and would exhibit an equal distribution of observed values across the whole range of variability. Hypothetically, a diagonal line for all variables in Figure 3.5 would therefore be optimal. When these conditions are fulfilled, the data structure would imply that all variables have an equal chance of being identified as a stressor variable in the GLM- and EPC analyses. The position of the curves for the different stressors in Figure 3.5 found for the current dataset, however, shows deviance from the optimal data structure.

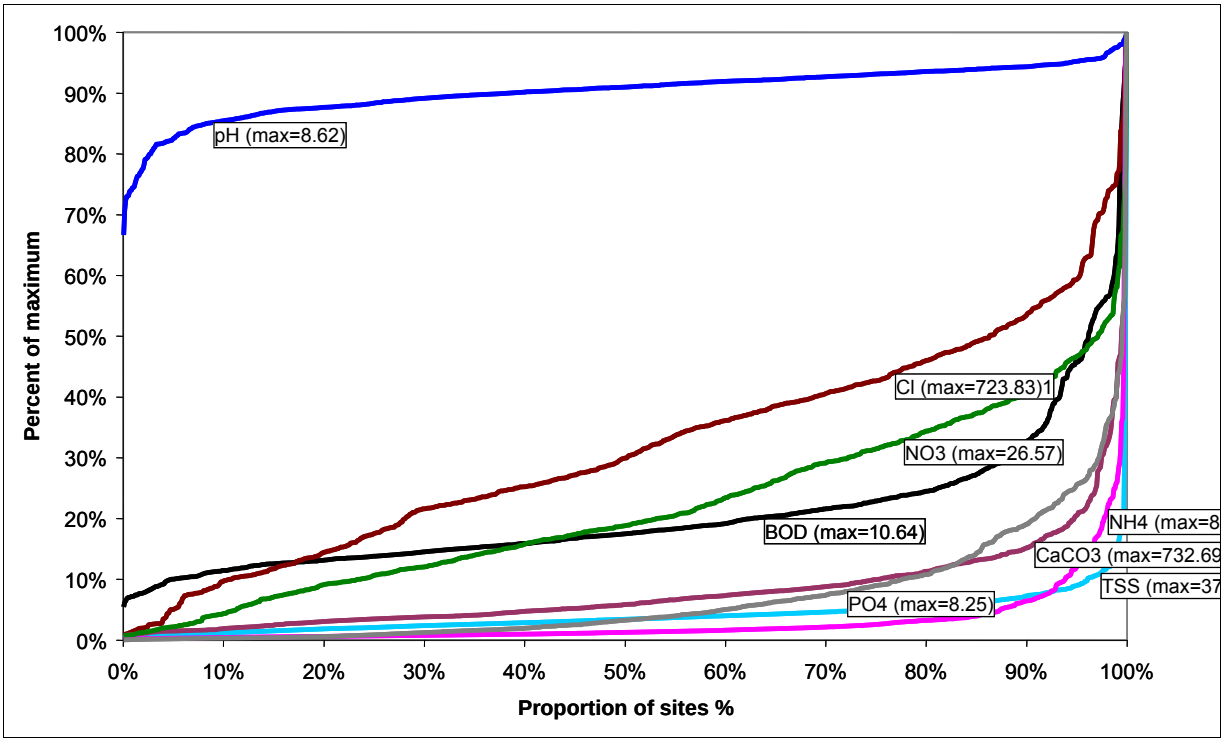

Figure 3.5 Cumulative distribution of the variation of stressor variables in the data set for England and Wales. The identity of the variables is given in the text boxes, of which the upper left corner identifies the associated PDF. All values were scaled on a (Y) axis between 0 and 100%.

3.5 GLM modelling and the derivation of slice sizes

3.5.1 Comparison of GLM15 and GLM25 models

We were able to produce GLM15 and GLM25 models for 75 of the 76 taxa assessed by the RIVPACS model. We could not construct a GLM model for the family *Hirudinidae*, due to convergence problems in the model fit iteration procedure.

The GLM25 models were compared to separately derived GLM15 models (the latter based on the RIVPACS variables) to investigate the presence of bias in the EPC analyses. Bias would occur when regression coefficients of the GLM25 differ from those of the GLM15 models for similar descriptors. The regression coefficients for the 15 natural predictors in both sets of models were generally correlated. This is shown in

Appendix 4 for all 15 sets (15 GLM coefficients) of the GLM15-to-GLM25 comparisons. Figure 3.6 shows one correlation in detail. In view of these findings, we are confident that the use of GLM25 models in the EPC analyses reflects realistic responses of families to the different stressors.

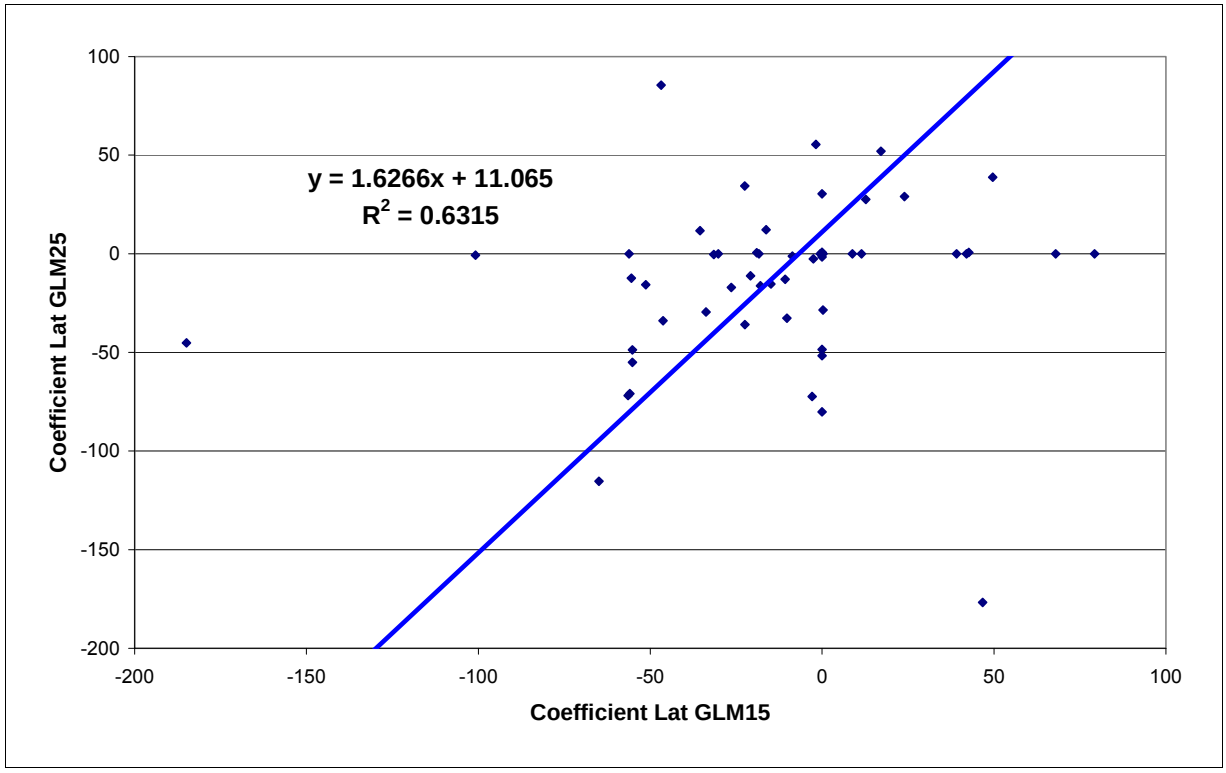

Figure 3.6 Analysis of the role of stressor coefficients in GLM15 and GLM25 models. In ideal cases the estimated coefficients in the GLM15 and GLM25 models should be the same (and thus correlated). The example shows the analysis for the variable Latitude. P < 0.01.

The difference in the amount of variation associated with GLM15 and GLM25 models indicated that, on average, stressor variables influenced family abundances by about 15 per cent above that associated with the 15 natural variables (Figure 3.7).

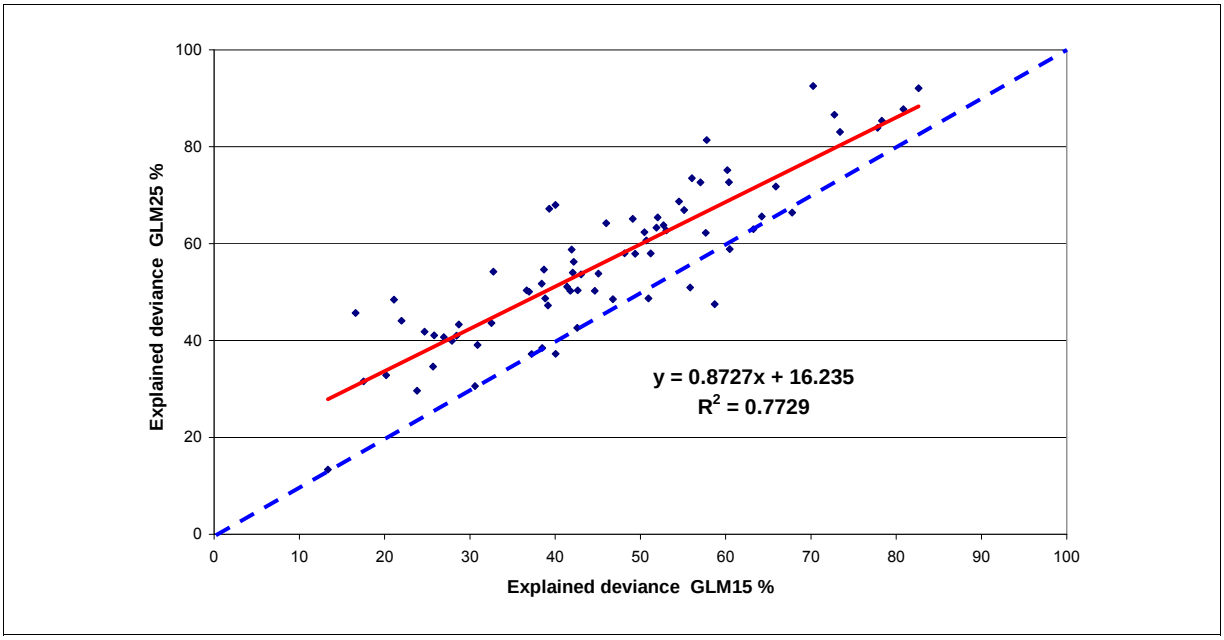

Figure 3.7 Explained deviance of GLM15 and GLM25 models.

3.5.2 Explanatory capacity of the GLM25 models over families

The explanatory capacity of the GLM25 models differed between families (Figure 3.8). Over 30 per cent of the deviance was explained in around 95 per cent of families, and over 75 per cent deviance in 20 per cent of the families.

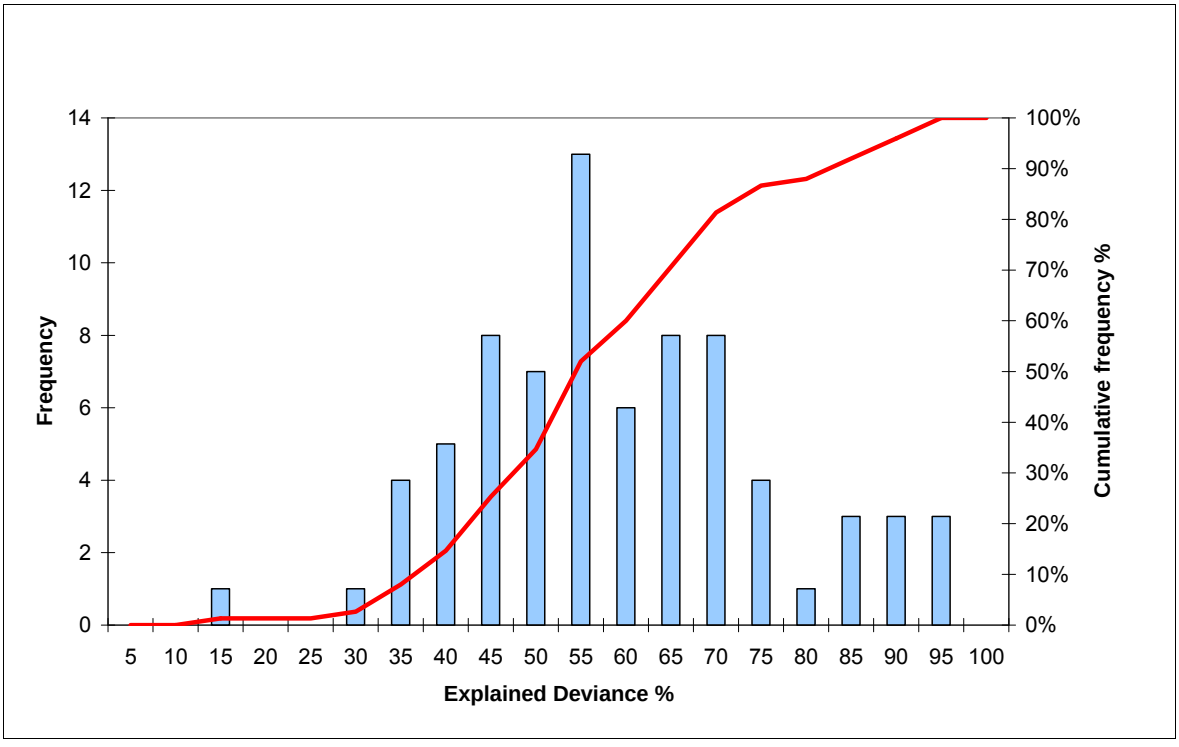

Figure 3.8 Explanatory power of the GLM25 models.

As an example of the explanatory power of the GLM25-formulae, Figure 3.9 shows the predicted and observed abundances of the set of families that occur at a selected sampling site. Abundances are predicted by filling out the GLM25-formula using site

measured data. The graph is plotted on a log-log scale. The association between predicted and observed abundance is high and significant.

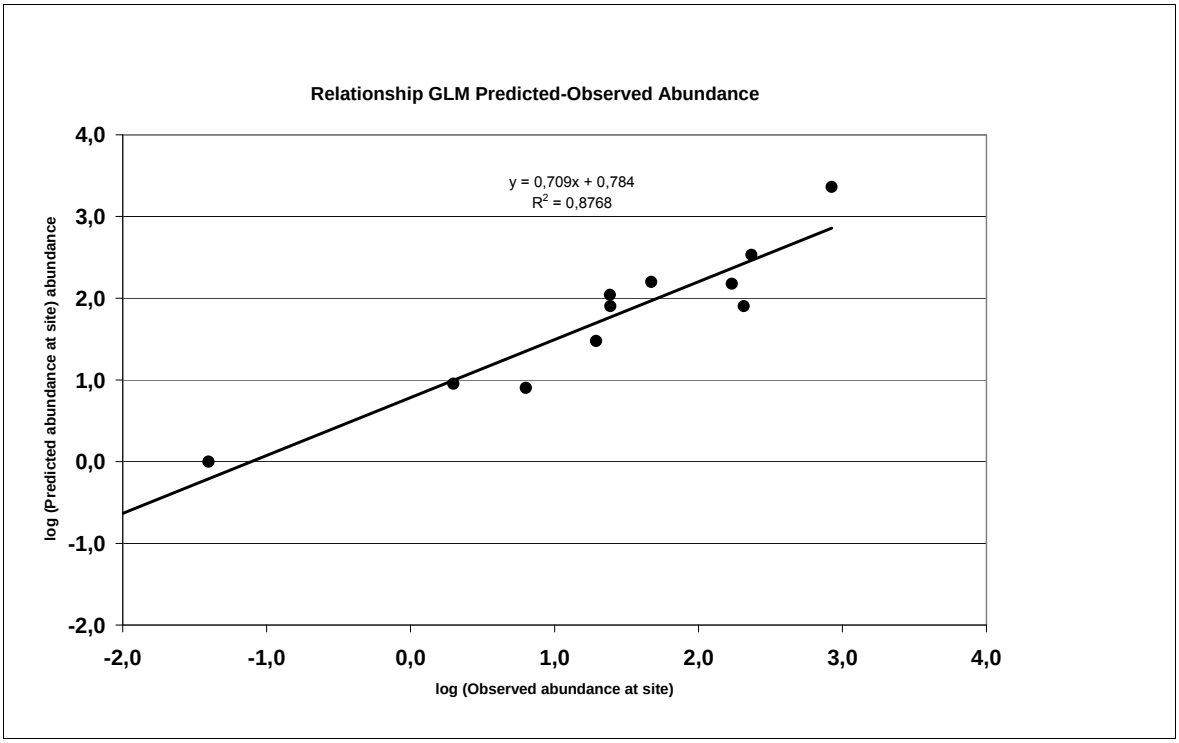

Figure 3.9 Example of predicted and observed abundances of all the families at a selected sampling site.

The significance of adding a variable to the model in the GLM25 analyses (linear and quadratic terms together) is detailed in Table 3.4. The toxic pressure of industrial chemicals is a highly significant descriptor for more than 70 per cent of the families. The toxic pressure of pesticides is a highly significant term in more than half of the families, despite the fact that it represents the toxicity-normalised modelled pesticide concentrations of catchment annual average concentration. These values are higher than the product-moment correlations between family abundance and toxic pressure, which implies that the latter are apparently low due to multiple-confounding factors (see also Figure 3.3 and associated text).

Regression term	Category	Percent of species with term	Significance of regression terms		
			p < 0.001	p = 0.01	p = 0.05
LongpDEV	Natural	73%	100%	0%	0%
DCatpDEV	Natural	72%	98%	0%	2%
AATRpDEV	Natural	72%	96%	2%	2%
NO3pDEV	Nutrient	71%	96%	4%	0%
ImsPAFpDEV	Toxic pressure	71%	96%	4%	0%
SiltpDEV	Natural	69%	98%	0%	2%
DisSpDEV	Natural	67%	100%	0%	0%
SlopepDEV	Natural	67%	98%	2%	0%
pHpDEV	Water chemistry	67%	96%	4%	0%
LatpDEV	Natural	65%	100%	0%	0%
AltpDEV	Natural	64%	100%	0%	0%
CaCO3pDEV	Natural	64%	98%	0%	2%
TSSpDEV	Water chemistry	64%	100%	0%	0%
DepthpDEV	Natural	63%	100%	0%	0%
BolCobpDEV	Natural	63%	100%	0%	0%
NH4pDEV	Nutrient	63%	98%	2%	0%
PebGravpDEV	Natural	61%	98%	2%	0%
PhipDEV	Natural	61%	98%	0%	2%
MATpDEV	Natural	61%	98%	2%	0%
ClpDEV	Water chemistry	61%	98%	2%	0%
WidthpDEV	Natural	60%	98%	2%	0%
PO4pDEV	Nutrient	57%	100%	0%	0%
PmsPAFpDEV	Toxic pressure	56%	100%	0%	0%
SandpDEV	Natural	55%	95%	5%	0%

Table 3.4 Percentages of families for which the GLM25 analysis revealed a significant effect of addition of a variable to the GLM25 model (and the degree of significance).

3.6 EPC pie diagrams

3.6.1 Exploration of EPC results and general strengths and limitations

Effect and Probable Cause (EPC) charts were produced for national data and then broken down by Environment Agency Region. EPC charts are geographically referenced representations of the data analysis. The size of each individual chart on the map represents the magnitude of local impact. The sizes of the pie 'slices' represent the relative importance of different stressors in causing that impact.

The EPC pie diagrams produced in this study were obtained by 'double blind' analysis. In other words, RIVM undertook the data analysis with anonymised site and species/family data.

In all the EPC charts presented below there is a large fraction of unexplained variance in the composition of macrofauna assemblages. As well as model error and natural variability, this can also be caused by other stressors known to affect aquatic ecosystems, such as alteration in stream flow, pesticides peak concentrations (rather than catchment-wide annual average concentrations), industrial discharges, input of cooling water, and other human activities. These factors were not included in our analyses due to lack of available data at the time the study was undertaken. We anticipate that the 'unexplained' area can be reduced by further investigation of the use of additional modelled data to refine the analysis and represent other significant catchment pressures.

It is important to note that the EPC diagrams are simply another representation of the input data, and that conclusions on RBMP and PoM should not be drawn from these diagrams alone, unless the data used are fully representative of the specific catchments. For example, a stressor can only be identified as relevant if there is data for it to be included in the analysis. There are many potential toxic stressors that have not been included in this analysis but are likely to be present in the catchments.

3.6.2 Site-specific visual presentation of EPC pie diagrams

Using the impact attribution process described earlier, we derived pie slice sizes per site. The results for one sampling year for England and Wales (2004) are shown in Figure 3.10, together with the pie sizes derived from RIVPACS modeling.

Sites that are highly impaired by loss of families occurred across the country and in both urban and rural areas. The slice sizes indicate that water chemistry stressors were most often associated with family loss, followed by region-dependent secondary and lower-order (subgroups of) stressors (see also Section 3.6.3). Heavy metal impacts caused impairment predominantly in Southwest England, in Devon and Cornwall, but also at several sites in the North West. The sites identified by the method to be impacted by heavy metals are sites well-known for metal mining and smelting activities. Since the method has been operated ‘double blind’, this suggests that the method itself is apparently sensitive to describe stressor effects where they are to be expected based on expert judgement. Further comparisons between expert judgement and method outputs are needed to generalise this supposition.

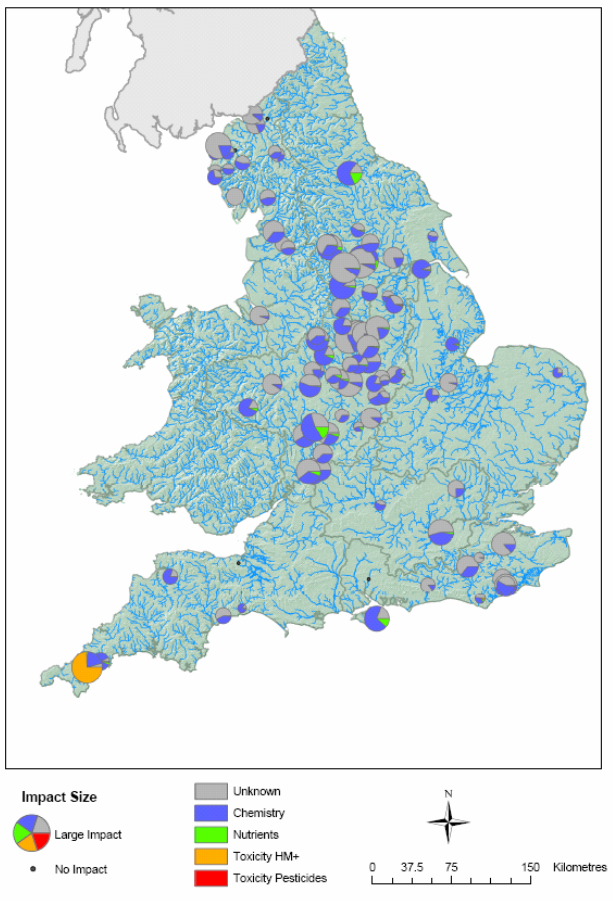

Figure 3.10 **EPC pie diagram for England and Wales, sampling year 2004.**

The EPC charts are GIS-based and so can be produced at a range of scales and times depending on needs. Examples of further results broken down by region are presented in Figure 3.11. Note that by combining different sampling years into one map, the number of pies shown on the maps will increase considerably and may reduce clarity.

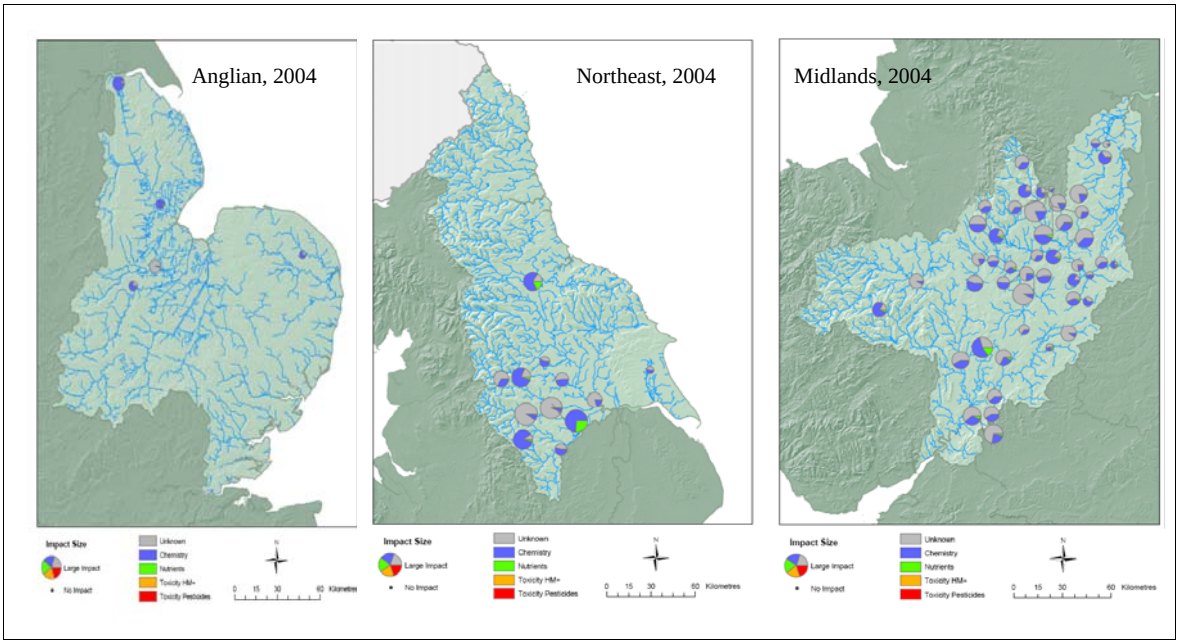

Figure 3.11 **Mapping of EPC pie diagrams (selected regions 2004).**

3.6.3 Aggregation and disaggregation of EPC information

Using the EPC data, various aggregations and disaggregations of data can be made, to present differences between regions, catchments or years, depending on the assessment endpoint. As an example, we aggregated all EPC data for all years for England and Wales. This is illustrated in Figure 3.12.

The analysis shows that, on average, 35 per cent of the invertebrate taxa were missing relative to reference site expectations (Figure 3.12).

Slightly more than 50 per cent of biological effects were attributable to unknown factors and model error. The remaining 50 per cent of effects were related to (in order of reducing importance, on average) alteration in water chemistry, nutrient status, toxicity of industrial compounds (mainly heavy metals) and pesticides. In comparative assessments, similar graphs for two areas of interest, catchments for example, can be of help in identifying areas that are affected more than others, and the most significant stressors.

In all EPC diagrams, colours represent various subgroups of stressor variables; such as habitat-related stressors, water-chemistry-related stressors and so on. Each of these slices can be disaggregated to the original stressor variables if additional detail is required. For example, the "nutrients" slice is green, but is composed of three variables, which could be represented diagrammatically as three subslices of varying shades of green to represent the relative significance of PO_4^-, NH_4^+ and NO_3^- individually. Similarly, the toxic pressure slices can be disaggregated using the underlying single substance toxic pressure data. The contribution of individual compounds to the toxic pressure slices can be different in different regions. Such information is clearly useful for catchment managers when considering remediation measures.

Overall, less than 2 per cent of the biological degradation for England and Wales was attributable to the combined toxicity of all compounds. By averaging the data across the whole of England and Wales, the strong relationship between the toxic compounds and biological degradation found in the GLM analyses is masked. The training set of site data contained relatively few sites where acute toxic pressures were high. The

averaged stressor attribution graph therefore reflects the deviation from the optimal data set, where sites would be evenly distributed between impacted and unimpacted as discussed earlier. Next to model error and natural variability, the high proportion of unexplained effects represents other stressors not included in these analyses, such as alteration in stream flow, pesticide peak discharges, other industrial discharges, input of cooling water, and habitat alterations which were not included in our current analysis because of lack of readily available data at the national scale.

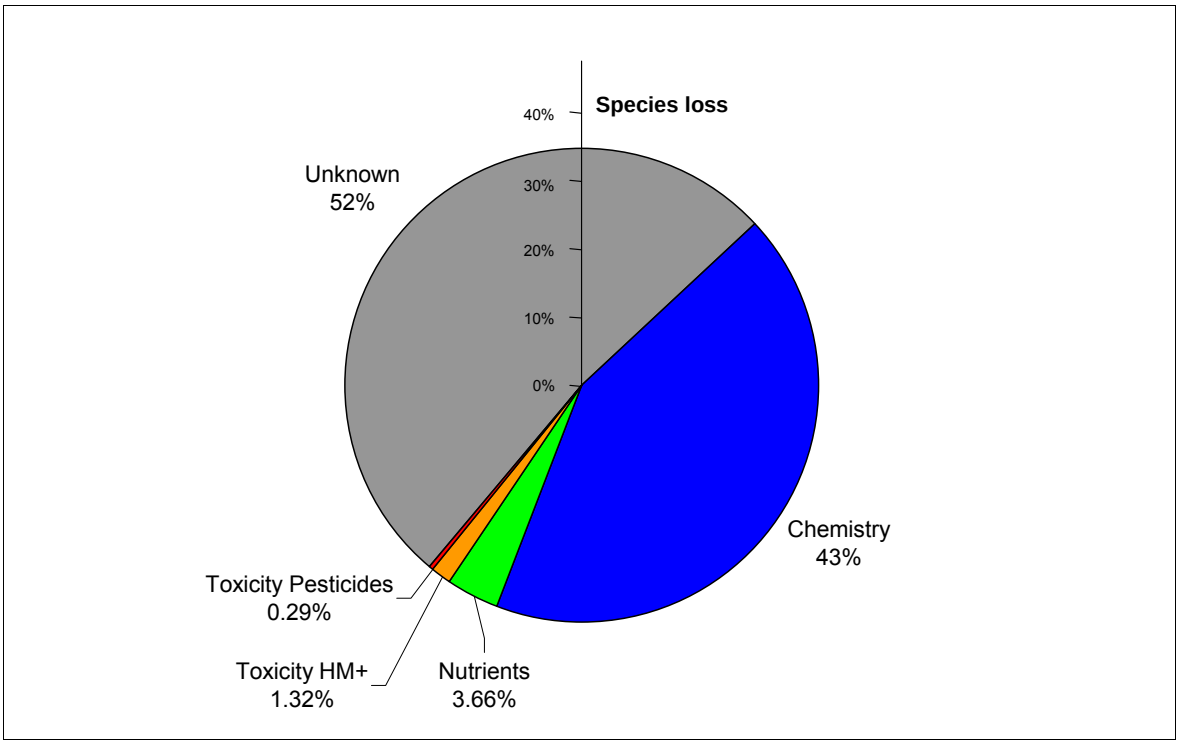

Figure 3.12 Averaged EPC pie diagram for the dataset for England and Wales. Note that the graph can be biased by differences in representation of stressors in the dataset.

3.6.4 Observed family loss attributed to toxic mixtures and predicted acute toxic pressure

Family loss can be analysed, summarising associations between observed effects and any of the predictors, providing more details than the GLM significance analysis of Table 3.4. Theoretically, as soon as the family loss itself becomes fully explained by one predictor alone, the shape of the data cloud is expected to resemble a diagonal linear distribution as shown below in Figure 3.13.

In this section we focus on the relevance of the model-derived parameter 'toxic pressure'. The variable $msPAF_{All\ compounds}$ (acute toxic pressure) is assumed to predict the degree of toxic stress. Toxic pressure is expressed as the fraction of species that are exposed at a concentration level that exceeds the laboratory test effect criterion, in this instance the acute EC_{50}. The EC_{50} was chosen since it implies significant effects on endpoints, such as mortality, growth, and reproduction. The calculated msPAF may not result in actual species (and family) loss for two reasons: differences between the species used in laboratory toxicity data and the families occurring in the field, and the fact that any exceedance of an acute EC_{50} in a particular species is not necessarily related to family loss. To investigate whether the toxic pressure concept holds true we considered the attribution of family loss to toxic pressure.

We investigated the relationship between the predicted acute toxic pressure for the mixtures of all compounds (industrial compounds plus pesticides, Y) and the extent of family loss attributed to toxic mixtures from the EPC analyses (X). The latter is expressed as the fraction of families lost, and is calculated by multiplying pie size by slice size. We demonstrate that the observed family loss in the field that we attribute to mixture exposure (X) is described by the formula:

Equation 3: $\mathbf{Log(msPAF_{EC50}) = 0.53\ Log\ (Species\ loss_{Toxic\ exposure}) - 0.21}$ **(P<0.001)**

The relationship is shown in Figure 3.13.

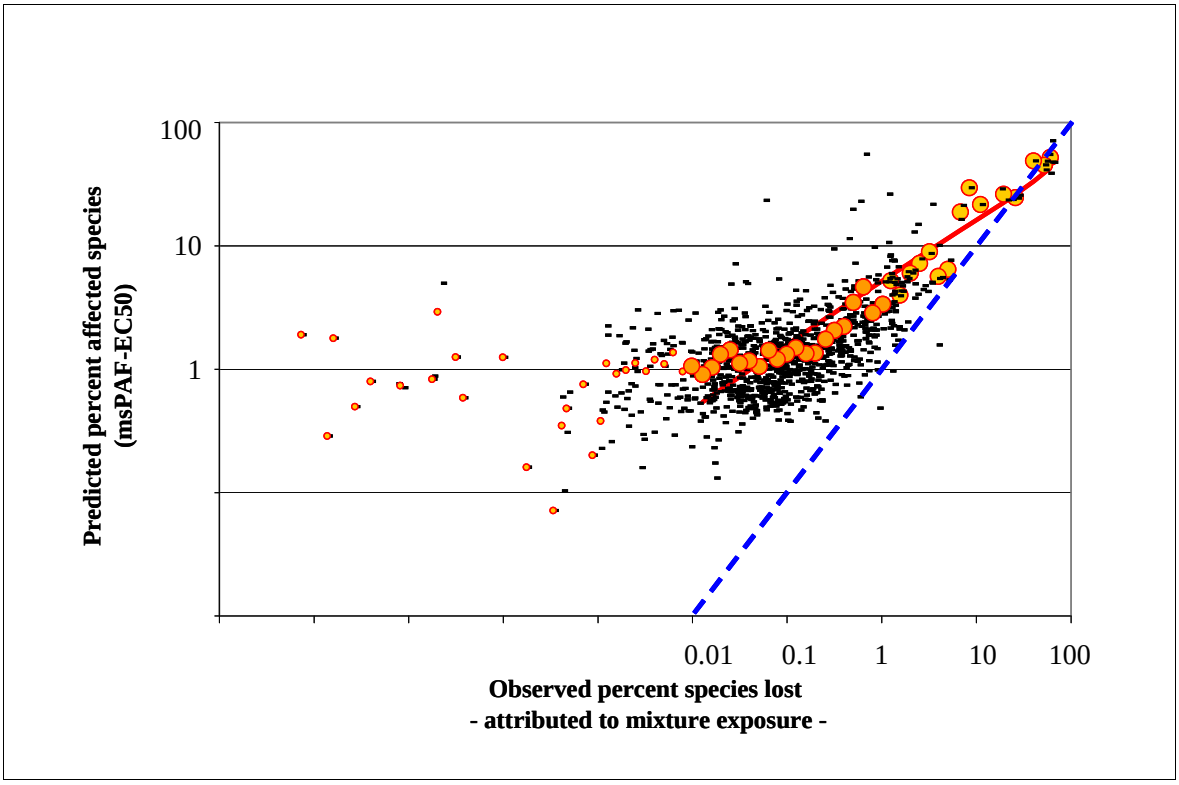

Figure 3.13 Association between (X), the loss of families attributed to mixture exposures in field conditions, and (Y), the predicted $msPAF_{All\ compounds}$. The red line is the relationship summarized in Equation 3. The blue dotted line is the expectation when Families $loss_{Mixture\ exposure}$ is fully explained by $msPAF_{EC50}$.

The graph illustrates the original data as black markers (n=1,007). For 39 site/year combinations there was no family loss according to the RIVPACS-model, and thus no attribution information. The graph also presents the pattern of values after the creation of bins for the X-variable, and the associated averages of the Y-values per bin (coloured dots). Further, large markers are used to represent bins where both the X and the Y variable could have an ecotoxicological meaning, that is, a predicted loss of <1 per cent of families (Y=1) is meaningless when the data set contains 76 taxa. With 75 taxa studied, the array of sites with such low impacts are characterised by the loss of less than a single family (these sites are presented with small markers). Thus, as a taxa loss of less than 1 per cent is a meaningless numerical result given the study on 76 taxa only, the data below Y=1, and thus below X=0.01, were not used in the derivation of the mathematical relationship. If family loss were only attributable to toxic pressures at all sites we would expect the blue dotted line. The red curved line represents the above formula. Note that it curves slightly upwards in the upper right, as a consequence of the probit-probit model being non-linear on the log-log scale. In the

centre of the graph, the bins merge many sites into a weighted average, while in both tails bins (dots) may represent low numbers of data points, or even single data points. The latter is shown by a single black marker in the centre of the bin-marker.

The data plotted in the graph suggest that the acute toxic pressure ($msPAF_{EC50}$) almost fully explains the loss of taxa for sites where a high loss of taxa was observed (for example, losses between 10 and approximately 70 per cent, upper right). This compares well with the pie chart for a site located in Cornwall (Lands End), where a large impact (family loss) is indeed explained by a large contribution of toxic pressure, see Figure 3.110). Figure 3.134 suggests that smaller observed losses are predicted to an increasingly lesser extent by toxic pressure only, that is: at low-impact sites other variables appear to be of increasing importance in causing family loss.

The observations summarised in the graph, and those of the GLM model analyses in Table 3.4 suggest that the acute toxic pressure relates to family loss and changes in family abundance in field conditions, but also that family loss at lower toxic pressures is increasingly caused by other stressors. Further, it shows that family losses of more than approximately 10 per cent do not occur at acute toxic pressures that are lower than 10 per cent; that is, there are no observations below the diagonal line. The *predicted* fraction of families lost due to acute toxic pressure might thus be interpreted as an *upper bound of family loss due to mixture exposure* in field conditions, in this data set. In other words: maximum family loss attributed to mixtures = $msPAF_{All\ compounds}$.

3.6.5 Alternative family responses: sensitive species and opportunists

The EPC method has been presented for family loss and for some of the stressors thought to be contributing to that process. However, the presence of certain stressors might also lead to an increase in opportunist families. EPC graphs could therefore be produced that show abundance *increases* at higher stressor exposures. Whilst these graphs have not been produced here, the product-moment correlations between abundances of families and the local $msPAF_{All\ compounds}$ have been calculated resulting in 75 correlation coefficients (one for each taxon), using raw data for 1,042 sampling site-year combinations. Positive and negative significant correlations for various families were discovered, but non-significant associations were found for the majority of families (Figure 3.14). The majority of families (85 per cent) show a slight but mostly non-significant negative trend in abundance at increased exposures (when not considering confounding factors, as in the GLM analysis). Fifteen per cent of the families show opportunistic trends (not all significant), with large and significant increases in abundance in a few families (identity not shown). It can be concluded that the change of macrofauna communities in response to mixture exposure probably consists of increases in abundance for some families (opportunists), in addition to family loss for others (sensitives).

Note that these product-moment correlations are simple raw-data statistics, in which no ecological or GLM-like statistical modeling is involved. GLM statistics and attribution statistics revealed a much higher relevance of toxic pressure in shaping macroinvertebrate assemblages than these correlations (see Table 3.4).

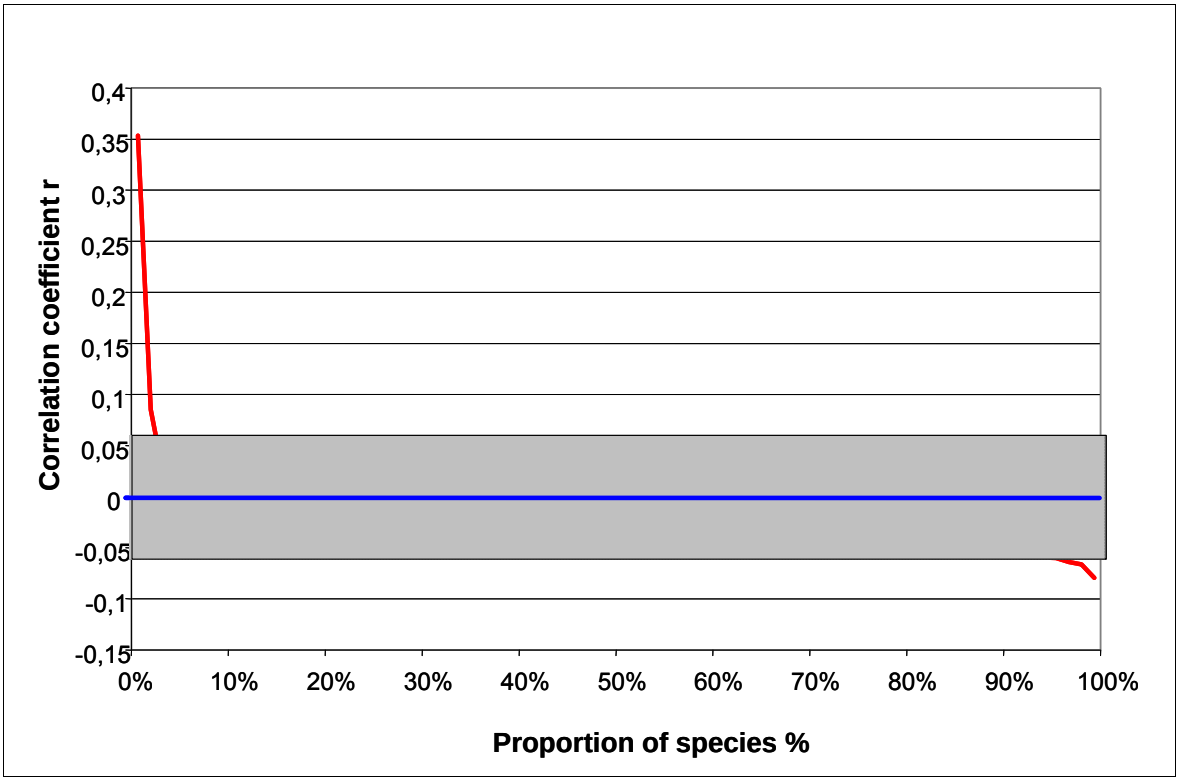

Figure 3.14 Correlations between $msPAF_{EC50,All\ compounds}$ and abundance of 75 families. The 75 correlation coefficients are sorted from left to right, going from positive association between toxic pressure and abundance (opportunists) to negative values (sensitives). The box identifies the non-significance range, given n= 1,043 observations.

4 Discussion

4.1 Exploring diagnostic features of the EPC approach

Within this report we have demonstrated that it has been possible to re-shape a large data set on measured and modelled stressors and the abundance of 75 macroinvertebrate taxa for rivers in England and Wales, into diagnostic, geographically referenced Effect and Probable Cause (EPC) pie diagrams. These EPC diagrams present:

- The magnitudes of local ecological impacts of stressor combinations on the local macroinvertebrate assemblages (at the family level).
- The relative contributions of various causal factors to those impacts.

The process has involved a series of modelling steps, each of which is well established in literature and practice:

- RIVPACS, a standard analysis model that is used to quantify magnitudes of local ecological impacts, and commonly used by the Environment Agency for this purpose.
- Toxic pressure modelling, based on Species Sensitivity Distribution (SSD) modelling and mixture impact assessment methodologies developed by the Dutch RIVM and the US EPA and RIVM, respectively, to quantify (on an ecological scale between 0 and 1 or 0 and 100 per cent) the fraction (or percentage) of species that is probably acutely affected by compound (mixture) exposure, and used by various regulatory agencies to derive environmental quality criteria and local probabilities of compound (mixture) impacts.
- Generalised Linear Models, a standardised statistical analysis approach to describe non-linear relationships between response variables and multiple (possibly) causal variables.
- An impact-to-cause attribution step, to merge information from all other steps.

The data analysis steps were preceded by significant data preparation steps, to obtain a data set that is “a square data block”, with limited or known covariation across variables, representing the study area and addressing the parameters of interest to the maximum extent possible.

The combination of these models to derive EPCs was developed recently, and published in a Special Issue on water quality assessment, of a US-based International Ecological Journal, *Ecological Applications* (De Zwart *et al*., 2006). This report describes the second application of the EPC method to analyse a (bio)monitoring data set.

The results of the catchment pressure analysis for England and Wales using the EPC method shows that the data were sufficient to complete the process and produce geographically referenced EPC diagrams. It was possible to calculate local toxic pressures per compound and for mixtures.

4.1.1 Exploring technical feasibility

Toxic pressures were calculated for more than 100 single compounds, subsequently for subgroups of compounds with similar Toxic Modes of Action, and finally the mixtures of industrial chemicals and pesticides that occurred at each of the sites. In addition, the latter data can be aggregated per site to generate a toxic pressure value for both compound groups together, or disaggregated per site based on the database of compound-specific toxic pressure values. It is concluded that there were no major technical problems in calculating toxic pressure for a large set of compounds.

Based on the preliminary status of the current analyses, it should be noted that various improvements can be made in the process of deriving single-substance and multi-substance toxic pressures per site.

First, bioavailability assessments can be improved for various compounds. For example, the exposure of species to copper is not only dependent on water hardness, but also on organic matter content and composition. A speciation model could be used in this case to refine the bioavailability assessment. More sophisticated approaches are also available for other compounds, as model concept or as measurement techniques. However, applying such improved techniques in the context of diagnostic analyses require monitoring data on bioavailability-modifying parameters or data on measured dissolved concentrations. These are usually not available.

Second, the set of available ecotoxicity data for deriving the respective SSDs is limited for several compounds compared to others. This implies that the analyses contain a larger degree of uncertainty in toxic pressure calculation for such compounds. This issue may ask for specific consideration in the whole analysis. In the current analysis, all toxic pressures are expressed as median (ms)PAF estimates (and confidence bounds of these estimates are not considered), while in further analyses focus may be on the 'better' SSD for example, or on developing methods to address uncertainty throughout the analyses.

4.1.2 Exploring strengths and limitations of toxic pressures

SSD have, up until recently, been used mostly for the derivation of Environmental Quality Standards in various countries around the globe (Posthuma *et al*., 2002). For that usage of SSD, standard operation procedures have been adopted by regulatory bodies requiring relatively large numbers of input data (for example: more than 10 data entries for multiple taxa), all being NOECs or similar no- or low-effect toxicity test endpoints. In the current analysis, an alternative use of SSD has been applied, that is, calculating the local toxic pressure of single compounds and/or mixtures based on measured or modelled ambient concentrations. This assessment provides novel insights into local toxicant stress when environmental concentrations of chemicals do not exceed the regulatory quality criteria but may still be contributing to mixture effects.

When smaller- or larger scale exceedences of criteria occur, water management authorities can simply take measures to fulfil the requirements set by the WFD and reduce the magnitude and frequency of such exceedences using appropriate measures, for example, by stricter waste water effluent permits. This should result in a reduction of frequencies and magnitudes of the observed concentration exceedences, and eventually in improved chemical water quality.

When the condition of Good Ecological Status would however not be reached after such measures, and various chemicals are still present at concentrations higher then the natural background or the criterion value, the water management authorities need to identify catchment pressures, and derive additional PoM to reach Good Ecological Status. Expert judgement on the causes of deviation from Good Ecological Status,

however, usually falls short when mixtures of toxic chemicals are present at relatively low concentrations. No single ecotoxicologist is able to attribute deviation from Good Ecological Status to individual chemicals in such conditions. The EPC method and the mixture toxic pressure assessment method (ms-PAF) have been designed to help solve that problem. According to this method, concentrations of all measured chemicals are recalculated into ecotoxicity-relevant units (Potentially Affected Fractions of species), which can be aggregated over compounds to a single summary value for expected ecotoxicity per site. This novel site parameter is used instead of expert judgement in the catchment pressure analysis, and can be seen as an approximation of the role of toxicants in shaping the composition of local species assemblages. This approximation can be final and used in a diagnostic system, like in this report, or it can help to focus next steps in a multi-step diagnostic system. For example, it could focus on the type of bioassays to be executed, or on the species groups of most interest for field inventories.

In the catchment pressure analysis, EC_{50} data were used to construct the SSD instead of NOEC. There is typically an increase in the number of available EC_{50} ecotoxicity data as compared to the commonly used NOEC subset, and higher sensitivity of the assessments for moderate to highly contaminated systems. When using a NOEC in such systems, the results of toxic pressure analyses would be numerically similar (and all >99 per cent), due to 'reading off' in the asymptotic upper tail of the SSD. Standard procedures have been adopted on deriving SSD in the context of regulatory risk assessments (especially the derivation of quality criteria). For the inverse application of SSD in the current report, the SSD based on EC_{50} have not been derived using those criteria, since that would imply both using NOEC and other data criteria, which would make the possibility of deriving useful msPAF-values considerably reduced. Instead, SSD were derived on the basis of the compiled RIVM-experience on deriving and using SSD, given the available data. In case of future standardised use in environmental assessments, it is worthwhile to derive and adopt standard sets of SSD-parameters for compounds and effect levels of interest.

Comparison of mixture toxic pressure data between pesticides and industrial compounds revealed large differences in magnitudes of values, associated with the origin of the data. Industrial toxic pressures were generally higher than those for pesticides. In the Ohio-study (De Zwart *et al*., 2006), we generally used 90^{th} percentile values from the concentration data, to acknowledge the fact that species usually decline due to peak exposures, in addition to baseline concentrations or stress. In the pesticide assessments for this report, the base data were catchment yearly averages of model-predicted concentrations, suggesting that a different set of pesticide concentration input data may result in higher pesticide toxic pressures. In comparison to an assessment of pesticide toxic pressure in the Netherlands, the concentration data from the POPPIE-model are extremely low, as are the estimated acute toxic pressures (see De Zwart, 2006).

Overall toxic pressures ($msPAF_{All\ compounds}$) were used in the EPC analyses, and various catchments showed a significant contribution of mixture exposure to the impact on local macroinvertebrate communities. The GIS maps of the EPC results showed that mixture exposures apparently resulted in ecological impacts, and identified specific compounds or compound groups suggested to be responsible. For example, in the Southwest, ecological impacts are likely to be related to metal exposure. Although not shown in the report, catchments or regions can be further investigated by looking in the database of single-substance and Mode-of-Action specific toxic pressures.

The overall toxic pressure can be disaggregated when needed. By selecting sites with apparent toxic impacts, it can be verified which groups of compounds are locally present in such a concentration that they contribute to the increase in total toxic pressure. In some cases this can be a single compound, in other cases it can relate to

a certain compound group and/or to certain area-specific activities such as pesticides in agricultural areas, and in some cases it relates to various compounds all of which contribute to the toxic pressure.

The toxic pressure calculations, in short, recalculate compound concentrations (expressed in mg/L) into dimensionless units (fractions of species probably seriously affected) that relate to potential toxicity (that is, risk). These dimensionless units can be cumulated over compounds according to the principles of mixture toxicology, by accounting for differences in Toxic Modes of Action. Compound concentrations, however, can only be cumulated on the basis of total mg/L, which does not make sense toxicologically.

The ecotoxicity-dimensionalised concentration data are expressed on a relative scale, 0–1, as a fraction of species probably affected. This does not mean that the toxic pressure ruler can yet be considered an *absolute* ruler of impact (species loss). However, it is at least an ecotoxicity ruler that is improved over the summation of mg/L- or mMol/L units, and that predicts impacts in a realistic array, with an ecological meaning (no more than 100 per cent of species can be affected). That meaning is (a) the minimum and maximum values are realistic, and are zero or one hundred per cent of the species being likely affected, and (b) the value predicts which fraction of the species present in the training set (of ecotoxicity data in the RIVM e-toxBase) would suffer from the local mixture when reared in samples from the local water systems. This means that toxic pressure is an environmental characteristic that represents what the environment provides on a realistic toxicity scale to the local species. Further, the concept has been validated with data from various studies, amongst which the previous EPC study, on fish species in Ohio (Posthuma and De Zwart, 2006). As in the current study, it was shown that the fraction of local impacts (species loss) attributed to mixture exposure was related to the predicted species loss (quantified by msPAF based on laboratory ecotoxicity data, Posthuma and De Zwart (2006)).

For the EPC analysis, it is not necessary that the msPAF scale is an absolute predictor of impact. In the GLM analysis and the EPC attribution process, each variable can be modified (for example by shifting the decimal point one or a few places), without such manipulations affecting the statistical association between stressor variable and statistical significance of impact. Hence, the relative scale of (ms)PAF is sufficient for the EPC analyses.

The toxic pressure calculations that were made as intermediate results in the EPC analyses showed that single-substance and multi-substance Potentially Affected Fraction values could be determined successfully for a large set of compounds. These calculations were made on the basis of ecotoxicity data that were obtained from the RIVM e-toxBase, pertaining to acute effects on exposed test species (that is, EC_{50}s). Eco-epidemiological analyses require that all stressor parameters are variable across the available (bio)monitoring data set. When variability in the raw data is absent or low in the ‘training set’, it is impossible for any statistical method to distinguish this from the natural variability that is also present. Although the training set for England and Wales contains a relatively large fraction of sampling points with relatively low toxic pressures (see Figure 3.3), it appeared that many taxa exhibited a strongly significant association between their abundance and mixture toxic pressures.

4.1.3 Toxic pressure and the diagnostic systems of the Environment Agency

Toxic pressure data for individual compounds mixtures were provided to the Environment Agency for optional further use. Based on the discussion of strengths and weaknesses above, we recommend repeating all data analysis steps again, using

additional data or improved data (for example using the 90th percentile measured pesticide data rather than yearly averaged catchment data) now that it is known that the whole process of generating EPCs has been executed successfully, as this will have a significant influence on the predicted values. Further, the data analyses could also involve assessment of exceedences of NOECs, EC10s and other effect levels. This will result in higher estimated values for toxic pressure compared to the EC_{50}-based assessments but it should be noted that this does not necessarily imply a different outcome of the EPC analyses. Input variables can be manipulated numerically (such as changing SSD_{EC50}-estimates to SSD_{NOEC}-estimates), but as long as the relative order of impact scaling between sites is similar, the output of the EPCs will be similar.

It can be concluded that there is no technical limitation to the use of toxic pressure data in any diagnostic system used by the Environment Agency or other organisation. For example, they could be incorporated into the Environment Agency's River Pollution Diagnostic System (RPDS) as summary parameters to quantify the toxic pressure of single compounds or mixtures The use of toxic pressure summary data is considered a highly relevant approach for any diagnostic system that is based on monitoring data, since it implies that many toxic compounds can be part of a diagnostic system without a crucial loss of statistical power in any form of analysis. This is a common situation that would occur when adding multiple chemicals individually.

4.1.4 Recommendations on using the toxic pressure approach

We recommend that the toxic pressure method is applied more widely in diagnosis of impacts. In summary, the rationale behind this recommendation is:

- The method re-calculates concentrations in dimensionless units that can be aggregated over compounds.
- The aggregation is ecologically meaningful, since:
    - The whole process acknowledges that SSD for different compounds are not straight lines with equal slopes (that is, it acknowledges different shapes of SSD).
    - It acknowledges different Toxic Modes of Action.
    - It acknowledges different compound availabilities between water bodies.
    - It does not predict impacts higher than 100 per cent of the species.
- The toxic pressure seems, based on logic and on the graphics of predicted-observed species (or families) loss relationships, to be at least an appropriate *relative* estimate of real impacts (species or families loss) in field conditions.
- The above-mentioned characteristics imply crucial strengths for eco-epidemiological analyses that aim to identify probable causes of impacts per sampling site, and that should involve not only classical parameters but also a suite of chemicals of regulatory interest.

We applied these recommendations in the current study, and used toxic pressure estimates in the diagnosis of impacts in rivers in England and Wales.

4.2 Exploring the diagnostic approach of EPCs

The data analyses undertaken in this scoping study have generated EPC pie diagrams, and a suite of further information supporting the interpretation of the outcomes. For example, Table 3.4, summarising the relevance of toxic pressures in shaping macrofauna assemblages next to the other variables (based on GLM analyses), suggests that toxic pressure is strongly correlated to family abundance for a major proportion of taxa.

However, it should be noted that the data presented in this report are the results of a *trial* of the approach, in order to investigate whether (a) the analyses needed can be made at all given the available data, and (b) whether the trial-output can withstand preliminary criticisms by experts. The results do not represent a complete analysis of all catchment pressures.

As already acknowledged in the first publication on the EPC approach (De Zwart *et al*., 2006), the method contains a series of analyses. Each step can be conceptually optimised, to address all issues of scientific concern. However, matching the idealised EPC method to (a) the regulatory problem definition, and (b) the available data asks for various decisions in data handling. Usually, (bio)monitoring data sets have not been, and cannot be, designed to be 'ideal' for post-hoc analyses. By the time of analysis, there may have been an evolution of thinking on the questions being posed, given changes in regulatory context, which are not reflected in the monitoring schemes. Because of these types of problems, cross-validation between the outcomes of different diagnostic methods would be beneficial, to compare EPC outcomes with independent expert judgment, with alternative eco-epidemiological analyses, or with leave-data-out-and-redo analyses. For example, the results of the EPC analysis of the Ohio data set were compared to the results of an independently developed diagnostic method, showing considerable similarity, but also dissimilarities (Kapo *et al*., in press). A comparison of these two methods is also made using the data set described here (Kapo et al., 2008).

Since eco-epidemiological analyses are usually made in the context of informing management decisions, a weight-of-evidence approach can be adopted to define PoM. Multiple lines of evidence that suggest that impacts are caused by some identifiable stress factors can be very valuable for taking measures, although eco-epidemiological methods are unlikely to ever provide full proof of the causation of observed impacts. Causation interpretation problems will remain, as they are integral part of the type of analysis (Brandon, 1990).

The current analyses were of an exploratory kind, and no cross-validation activities were undertaken. The results show that the series of modelling steps were successfully executed. It is beyond the scope of the current exploratory analysis to consider cross-validation in depth. However, the results obtained so far are promising enough to consider further actions (see below).

4.3 Exploring diagnosis results and river management

In a workshop (Bristol, May 25, 2007), the EPC method and its output (as presented in this report) were discussed with experts from various backgrounds, ranging from pesticide and water quality regulators to strategic research and policy planners to research scientists. The objective of the workshop was to discuss the EPC- approach in the broader practical context of the Water Framework Directive.

4.3.1 General workshop conclusions

The workshop participants concluded that the EPC approach, and other diagnostic approaches, do have many potentially relevant aspects for river basin management under the WFD. Such tools and their outputs could be part of a *Framework for Evidence-Based Decision Making*. Without such a framework, whilst such a diagnostic approach could deliver scientifically meaningful data, there is no clear link to the decision process and there is neither a trigger for problem definition (defining which assessment is to be made, commonly an extremely crucial step in any risk assessment) nor a clear way to adopt the assessment answers in practical decision making.

4.3.2 Specific workshop remarks

The workshop participants addressed an array of relevant issues. The evaluations focused on four major questions: (1) use: could the method be useful for practical decision making under the WFD; (2) technical: are there developments that would be required for the approach to be more useful, or to improve the approach; (3) choice and strategy: what would be the next steps and further programme of research and development, and (4) data: can we improve on data input (for any diagnostic method).

- Use and visual presentation of results
    - The method can be extremely useful for the purposes it was designed for (diagnosis); in particular, it could help prioritise which environmental stresses need to be tackled to improve ecological status.
    - The method addresses the issue of Good Ecological Status, and is complementary to the testing that looks at whether water quality criteria are met. It is true that GES cannot be met at a site when water quality criteria are not met, but the opposite need not be true, that is: when the quality criteria for regulated parameters are met, that certainly does not imply reaching GES.
    - The method can be useful in planning monitoring efforts, since the analyses strongly suggest that any post-monitoring data analysis requires a combination of problem-driven choices (what to measure) next to statistics-driven choices (sufficient number of sampling sites given the number of parameters measured).
    - The method can be useful for scenario analyses, that is, to answer “what if” questions. This alternative use of the model has not yet been trialled but is of interest.
    - Output can be interpreted with expert judgement and compared with local knowledge so as to use multiple lines of evidence for those parameters for which expert judgement and EPC type diagnosis can be combined (verification of one method by the other), and to increase trust in identified stress parameters (like toxicant mixtures at low exposure levels) for which expert judgement falls short. Evidently, when expert judgements and EPC results do show cross-verification for some parameters, this gives more confidence that the output for non-expert judged stressors can be trusted.
    - The presentation of the findings for “unknown cause of family loss” is considered useful, especially in the face of other methods that do not present such information. Presenting “unknown causes” puts the

observed ‘signal’ from other (identified) stressors in a quantitative context, avoiding the risk of focusing PoM on small contributors to overall stress.

- When needed, outputs can be aggregated to catchment or regionalised averages on impact magnitudes (family loss) and probable causes. In the case of presenting such averaged results (like in the example of the England-and-Wales overall averaged EPC, Figure 3.121), it must be clearly reported that despite low overall contributions of a stressor variable (such as msPAF) for a region, there may be a substantial or overarching local contribution at particular sites, for example, metal stress in Cornwall.
- When needed, output can be disaggregated. Based on available raw EPC data, the presentation of results can focus on individual stressors, individual catchment areas or regions, time-trends (such as before and after analyses), and so on. Which focus and format of presenting the results is chosen (GIS presentation of individual EPC, data tables with EPC-related data, averaged data) depends on the problem formulation of the assessment.
- Output can be very helpful in the phase of communicating investments in PoM to the public, since the output shows the issue of stressor significance in a clear and easy-to-understand way.

- Technical aspects
  - EPC results are strongly influenced by the impact quantification model, RIVPACS. This model requires identification of reference sites, and all results in the EPC analyses refer to the training data set of the references sites. In this study it must be acknowledged that the RIVPACS model has been defined from data collected more than a decade before the investigated set of non-reference data. In particular, the apparent major role of pH in shaping local assemblages can be hypothesised to be the result of generally improving pH conditions in the period between choosing references and judging other sites. This may be solved by re-definition of reference conditions.
  - The presentation of the current suite of exploratory results is limited to some typical examples and to sites that have a complete representation of all variables; it is worthwhile extending the presentation of results to those numerous sites where monitoring efforts have resulted in many, but not all, data. It needs be investigated whether this extrapolation of EPC results for sites with incomplete data is technically possible and scientifically appropriate, and whether an outcome would simply be that at those sites the “unexplained loss of families” just increases.
  - The POPPIE-based predicted pesticide concentration might be replaced by other more realistic modelled pesticide concentrations, to obtain data of higher site-specific ecological relevance. For example, in the earlier study (De Zwart *et al*., 2006), 90th percentile values for industrial toxicants were taken to drive toxic pressure, since these data represent peak concentrations per site, and these concentrations are expected to be of high relevance in shaping biotic communities.
  - For some assessors familiar with the standardised ways of using SSD for deriving quality criteria, it must be emphasised that the current problem definition leads to SSD that are appropriately tailored to the new problem. Thus instead of regulatory adopted criteria being used for

deriving environmental quality criteria, the inverse use of SSD requires tailoring the SSD to the problem: at high ambient exposure, it is for example more logical, useful and meaningful to use EC_{50}s rather than the traditionally used NOECs.

- Choices and strategy
    - The EPC method should be compared more thoroughly than was possible in this trial to other diagnostic findings, ranging from expert judgements to alternative diagnostic systems (such as the diagnostic system developed based on artificial neural networks and field monitoring data by Paisley et al., (2008).
    - The EPC approach should be subjected to a sensitivity analysis, to quantify statistical confidence intervals for the relationships found.
    - Attention should focus on developing appropriate presentation formats for results for different problem definitions.
    - Attention should also focus on linking magnitudes and causes of local impacts to a next step: the possibility to "manage" the different stressors taking away the cause of impact, and to predict whether recovery can take place (or what limitations there are for recovery). Knowing the cause of impacts and reducing them does not necessarily imply recovery, for example when families have been exterminated and there is no refugium from which the local site can be recolonised.
    - As a major choice-and-strategy conclusion, the strategy of research and development of the method should be accompanied by the design of a *Framework for Evidence-Based Decision Making.*
- Data
    - The trial of the EPC analysis emphasised that collection and compilation of appropriate monitoring data is crucial. Ideally the use of data should be considered at the design stage of any monitoring programme to ensure it is fit for purpose.
    - It is clear that the exploratory data analysis focused on available data, but that some stressor types are missing or are poorly represented in the data set.
    - Further investigation as to whether data collection should encompass compilation of data on biotic taxa other than macroinvertebrates is required, and whether those other groups would be of specific interest for other stressors, for example, macrophyte data for nutrient inputs.

4.4 General conclusions

We have integrated different assessment tools to identify both the magnitude and likely causes of biological impairment for a (bio)monitoring data set for England and Wales, given the variability in taxon composition and taxon abundances that occurs naturally. The method combines ecological, ecotoxicological, and exposure modelling to provide both a measure of impact and statistical estimates of the probable effects of different potential stressors on local stream macroinvertebrate assemblages. In short, the method reshapes tabular (bio)monitoring data into easy-to-understand GIS diagrams on impact magnitudes (family loss) and probable causation.

The assessments were made as an intensive trial of two methods: (1) the method to introduce analysis of the data using toxic pressure as a summary parameter for toxic stress, and (2) the EPC method itself. Although a set of statistical analyses was required, a fair proportion of variance was accounted for, and the end product could be presented as simple Effect and Probable Cause pie-charts that facilitate both interpretation and communication of results and (finally) decision-making. The end product involves the identification of pressures in surface waters including the parameter 'toxic pressure'. The analysis involved measured or modelled concentration data for more than 100 compounds, which is unique compared to other eco-epidemiological assays. The calculation of such a summary parameter has recently become a realistic option due to the definition of the summary variable itself, the multi-substance Potentially Affected Fraction, in combination with the availability of a suite of ecotoxicity data (currently > 188,000 data available in the e-toxBase (Wintersen *et al.,* 2004)). The trial of both methods was successful given the available data set, but various improvements in data analysis can be suggested.

The regulatory driver for the development of the method was the delivery of scientific support for decision-making. Specifically, the EU WFD not only considers the 'hard' assessment that exceedences of environmental quality criteria should not occur for a chosen set of compounds, but also the complementary issue of Good Ecological Status. When sites are impacted, water authorities should be informed of the magnitudes of impacts (deviation from the ecological status needed) and probable causes, to formulate appropriately targeted management plans and programmes of measures.

A secondary driver for the work was scientific interest in the meaning of toxic pressure as a summary parameter. The data analyses suggest that toxic pressure is strongly associated with the abundance of a majority of families, and that it also determines the local composition of the assemblage of families. The analyses have also shown that toxic pressure is a dominant predictor of family loss from local assemblages when it is high (acute toxic pressure exceeding 10 per cent), and that other variables become the more dominant causes of species loss when species loss is lower than 10 per cent.

Constraints imposed by statistical power limited our ability to address interactions among variables. This implies that attribution of effects to likely causes can in some cases be conservative.

Despite the latter constraint, the results of the study showed that:

- The compilation of data for eco-epidemiological analyses is a major step that is of critical importance for the success of the EPC (and *any* other) method. This could be simplified if monitoring and data collection is targeted to meet the requirements of eco-epidemiological techniques.
- Sufficient data was available for England and Wales to allow the EPC analysis. Both site-specific and national information on ecological effects and their probable causes is presented in the format of some selected examples; more comprehensive analyses and output presentations are possible.
- The current analyses should be considered as preliminary results, to show that the method is operational. Further refinements in data analysis are possible, should more data and funding become available.
- The results suggest that classical water chemistry parameters and habitat characteristics play a significant role in shaping local macrofauna assemblages, as compared to the set of chosen reference sites.
- At a local scale, species loss can be attributed to a lesser or larger extent to mixture exposures, confirmed by a major association between mixture toxic

pressure and family abundance data in a majority taxa. The GLM analyses identified highly significant associations between family abundance data and acute toxic pressure for more than 50 per cent of the families.

- The observed loss of families attributed to mixture exposure in field conditions significantly covaries with the acute toxic pressure, so that the local value of acute toxic pressure ($msPAF_{EC50}$) seems to imply an upper limit estimate of family loss due to mixture exposure.
- There are significant product-moment correlations between family abundance and toxic pressure, so that various opportunist families and more sensitive families can be recognised.
- The results are a product of the available data. They do not represent all sites at which the Environment Agency monitors biology and do not represent all pressures that may impact biology at a site. Many sites were excluded from the analysis due to insufficient data. Further consideration should be given as to how the data gaps can be filled and how data collection could be targeted to ensure the most appropriate data are collected for use in eco-epidemiological analyses such as this. It needs be investigated whether EPCs can be presented for sites with incomplete data coverage.
- Despite only limited data, diagnostic results have been obtained from the analysis, and these can be considered useful for addressing practical regulatory problems under the WFD. Further consideration of the outputs by local staff is now required to 'ground truth' the outputs and identify additional data sources that may be available.

A major characteristic of this study is the linking of different types of models, all of which have been individually applied regularly in the past for many purposes. Applying these models in concert yielded results that matched expectations for some selected sites, such as metal problems being identified as influential stressors in the Lands End area. Since our analyses were blind to both previous assessments of impairment and (expert) inferences regarding the probable causes of impairment at specific sites, the match of our results with some other data suggests that this approach provides a means of assessing the likely causes of biological impairment in the freshwater ecosystems of England and Wales.

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List of abbreviations

BMWP	Biological Monitoring Working Party
Ei	Expected, calculated expectation for a species i being present, used in RIVPACS
EC_{50}	50-per cent effect concentration
EPC-diagram	Effect and Probable Cause pie diagram (De Zwart *et al*., 2006)
EQC	Environmental Quality Criteria
GES	Good Ecological Status
GIS	Geographic Information System
GLM	Generalized Linear Models
GQA	General Quality Assessment
msPAF	Multi-substance PAF (Posthuma *et al*., 2002b, De Zwart and Posthuma, 2006)
NOEC	No Observed Effect Concentration
Oi	Observed, recording for a species i being present, used in RIVPACS
PAF	Potentially Affected Fraction of species (Posthuma *et al*., 2002a)
PAF^{EC50}	Potentially Affected Fraction exposed beyond their EC_{50}
PAF^{NOEC}	Potentially Affected Fraction exposed beyond their NOEC
Pc	Probability of Capture (in RIVPACS)
PoM	Programme of Measures
POPPIE	Prediction of Pesticide Pollution in the Environment
RBMP	River Basin Management Plan
RIVPACS	River Invertebrate Prediction and Classification System
SSD	Species Sensitivity Distribution (Posthuma et al. 2002a)
ssPAF	Single-substance PAF
TMoA	Toxic Mode of Action (of a chemical)
WFD	Water Framework Directive

Appendix 1

List of taxa as used in the EPC analyses

TaxonObs = Name of taxon as observed in the sampling data set
TaxonCode = Code of taxon in the analyses
TaxonPc = Name of taxon in the RIVPACS analyses made by Environment Agency

A total of 82 taxa were scored in the samplings. Of these, 76 taxa were analysed in the RIVPACS analyses. There were convergence problems in the GLM analyses and the EPC analyses therefore pertain to 75 taxa, for which there are data in the third column. For the taxon “Hirudinidae” there were no significant predictors in the GLM analyses.

TaxonObs	TaxonCode	TaxonPc
Aeshnidae	Sp1	Aeshnidae
Calopterygidae	Sp10	Calopterygidae
Capniidae	Sp11	Capniidae
Chironomidae	Sp12	Chironomini
Chloroperlidae	Sp13	Chloroperlidae
Coenagriidae	Sp14	Coenagriidae
Cordulegasteridae	Sp15	Cordulegasteridae
Corduliidae	Sp16	
Corixidae	Sp17	Corixidae
Corophiidae	Sp18	Corophiidae
Dendrocoelidae	Sp19	Dendrocoelidae
Ancylidae	Sp2	Ancylidae(incl.Acroloxidae)
Dryopidae	Sp20	Dryopidae
Dytiscidae	Sp21	Dytiscidae(incl.Noteridae)
Elmidae	Sp22	Elmidae
Ephemerellidae	Sp23	Ephemerellidae
Ephemeridae	Sp24	Ephemeridae
Erpobdellidae	Sp25	Erpobdellidae
Gammaridae	Sp26	Gammaridae(incl.Crangonyctidae&Niphargidae)
Gerridae	Sp27	Gerridae
Glossiphoniidae	Sp28	Glossiphoniidae
Goeridae	Sp29	Goeridae
Aphelocheiridae	Sp3	Aphelocheiridae
Gomphidae	Sp30	Gomphidae
Gyrinidae	Sp31	Gyrinidae
Haliplidae	Sp32	Haliplidae
Heptageniidae	Sp33	Heptageniidae
Hirudinidae	Sp34	Hirudinidae
Hydrobiidae	Sp35	Hydrobiidae(incl.Bithyniidae)
Hydrometridae	Sp36	Hydrometridae
Hydrophilidae	Sp37	Hydrophilidae(incl.Hydraenidae)
Hydropsychidae	Sp38	Hydropsychidae
Hydroptilidae	Sp39	Hydroptilidae
Asellidae	Sp4	Asellidae
Hygrobiidae	Sp40	
Lepidostomatidae	Sp41	Lepidostomatidae
Leptoceridae	Sp42	Leptoceridae
Leptophlebiidae	Sp43	Leptophlebiidae
Lestidae	Sp44	

Leuctridae	Sp45	Leuctridae
Libellulidae	Sp46	Libellulidae
Limnephilidae	Sp47	Limnephilidae
Lymnaeidae	Sp48	Lymnaeidae
Mesoveliidae	Sp49	Mesovelidae
Astacidae	Sp5	Astacidae
Molannidae	Sp50	Molannidae
Naucoridae	Sp51	Naucoridae
Nemouridae	Sp52	Nemouridae
Nepidae	Sp53	Nepidae
Neritidae	Sp54	Neritidae
Notonectidae	Sp55	Notonectidae
Odontoceridae	Sp56	Odontoceridae
Oligochaeta	Sp57	
Perlidae	Sp58	Perlidae
Perlodidae	Sp59	Perlodidae
Baetidae	Sp6	Baetidae
Philopotamidae	Sp60	Philopotamidae
Phryganeidae	Sp61	Phyrganeidae
Physidae	Sp62	Physidae
Piscicolidae	Sp63	Piscicolidae
Planariidae	Sp64	Planariidae(incl.Dugesiidae)
Planorbidae	Sp65	Planorbidae
Platycnemididae	Sp66	Platycnemididae
Pleidae	Sp67	
Polycentropodidae	Sp68	Polycentropodidae
Potamanthidae	Sp69	Potamanthidae
Beraeidae	Sp7	Beraeidae
Psychomyiidae	Sp70	Psychomyiidae(incl.Ecnomidae)
Rhyacophilidae	Sp71	Rhyacophilidae(incl.Glossosomatidae)
Scirtidae	Sp72	Scirtidae(=Helodidae)
Sericostomatidae	Sp73	Sericostomatidae
Sialidae	Sp74	Sialidae
Simuliidae	Sp75	Simuliidae
Siphlonuridae	Sp76	Siphlonuridae
Sphaeriidae_Pea_mussels	Sp77	Sphaeriidae
Taeniopterygidae	Sp78	Taeniopterygidae
Tipulidae	Sp79	Tipulidae
Brachycentridae	Sp8	Brachycentridae
Unionidae	Sp80	Unionidae
Valvatidae	Sp81	Valvatidae
Viviparidae	Sp82	Viviparidae
Caenidae	Sp9	Caenidae

Appendix 2

List of pesticides as used in the EPC analyses.

Pesticide data were obtained from measurements (field samples) and POPPIE (pesticide fate model). Non-selected compounds did not have enough data coverage.

Selected			Non-selected
2 4-D	Ethofumesate	Paraquat	Anilazine
2 4-DB	Fenbuconazole	Penconazole	Azinphos-ethyl
Aldicarb	Fenitrothion	Pendimethalin	Azinphos-methyl
Amidosulfuron	Fenoxaprop-ethyl	Phenmedipham	B
Amitrole	Fenpropidin	Phorate	Benalaxyl
Asulam	Fenpropimorph	Prochloraz	Carbaryl
Atrazine	Fentin hydroxide	Prometryn	Carbetamide
Azoxystrobin	Flamprop-M-isopropyl	Propachlor	Chlorfenvinphos
Benazolin	Fluazifop-P-butyl	Propamocarb hydrochloride	Chlorpropham
Benomyl	Flusilazole	Propaquizafop	Conductivity
Bentazone	Flutriafol	Propiconazole	Coumaphos
Bromoxynil	Formaldehyde	Simazine	Deltamethrin
Bupirimate	Glufosinate-ammonium	Tebutam	Desmedipham
Captan	Glyphosate	Terbuthylazine	Diazinon
Carbendazim	HCH-Gamma	Terbutryn	Dichlorprop
Carbofuran	Imazaquin	Thiabendazole	Dichlorvos
Chloridazon	Isoproturon	Thifensulfuron-methyl	Dithianon
Chlormequat	Kresoxim methyl	Thiophanate-methyl	DOC
Chlormequat chloride	Lenacil	Thiram	Endosulphan beta
Chlorothalonil	Linuron	Tolclofos-methyl	Esfenvalerate
Chlorotoluron	Maleic hydrazide	Tralkoxydim	Fenoprop
Chlorpyrifos	Mancozeb	Triadimenol	Fenthion
Chlorthal-dimethyl	Maneb	Tri-allate	Fenuron
Clopyralid	MCPA	Triasulfuron	Fomesafen
Cyanazine	MCPB	Triazophos	Hexachlorobenzene
Cycloxydim	Mecoprop	Triclopyr	Ioxynil
Cymoxanil	Mecoprop-P	Tridemorph	Malathion
Cypermethrin	Mepiquat	Trifluralin	Mevinphos
Cyproconazole	Metalaxyl	Vinclozolin	Omethoate
Dazomet	Metaldehyde		Oxamyl
Demeton-S-methyl	Metamitron		Parathion-ethyl
Dicamba	Metazachlor		Pentanochlor
Dichlobenil	Methiocarb		Propazine
Dichlofluanid	Methyl bromide		Propham
Diclofop-methyl	Metoxuron		Quinalphos
Diflufenican	Metribuzin		Trichlorfon
Dimethoate	Metsulfuron-methyl		Triphenyltin compounds
Disulfoton	Monolinuron		
Diuron	Napropamide		
Endosulfan	Oxadixyl		

Appendix 3

Physico-chemical and toxicological properties of the abiotic data used in the EPC analyses.

The table below summarises the data used to (a) address biological availability of compounds; (b) calculate toxic pressures (per compound) based on the parameters of the log-normal Species Sensitivity Distribution model: μ (median value of a set of EC_{50} data) and σ (standard deviation of a set of EC_{50} data); and (c) calculate mixture toxic pressures, on the basis of assignment of Toxic Modes of Action. Note that the mixture toxic pressure calculation formulae do not require the name of the TMoA, but only the information that TMoAs of different compounds are similar or dissimilar.

ChemName	Availability Correction	Slice in EPC	TMoA (for msPAF)	LOGKow	Koc	mu (of SSD-EC50)	sigma (of SSD-EC50)
Cl-	None	C (Water chemistry)					
TSS	None	C (Water chemistry)					
pH	None	C (Water chemistry)					
BOD	None	C (Water chemistry)					
CaCO3-Hardness	None	C (Water chemistry)					
NO2-	None	I (Indutrial chemicals)	NO2			4.00	0.70
Pb	Metal formulae	I (Indutrial chemicals)	Pb			3.20	0.71
Ni	Metal formulae	I (Indutrial chemicals)	Ni			3.70	1.51
Cr	Metal formulae	I (Indutrial chemicals)	Cr			3.40	0.91
Cu	Metal formulae	I (Indutrial chemicals)	Cu			2.30	0.74
Cd	Metal formulae	I (Indutrial chemicals)	Cd			2.20	1.02
Zn	Metal formulae	I (Indutrial chemicals)	Zn			2.90	0.69
NH3	None	I (Indutrial chemicals)	NH3			3.84	0.85
NO3-	None	N (Nutrients)					
PO4-	None	N (Nutrients)					
NH4+	None	N (Nutrients)					
Formaldehyde	Organics-formulae	P (Pesticides)	aldehyde	0.35	1.4	4.67	0.43
Metaldehyde	Organics-formulae	P (Pesticides)	aldehyde	0.12	0.8	5.83	0.67
Cymoxanil	Organics-formulae	P (Pesticides)	aliphatic nitrogen	0.59	2.4	4.49	1.28
Napropamide	Organics-formulae	P (Pesticides)	amide	3.08	745.4	4.35	0.18
Tebutam	Organics-formulae	P (Pesticides)	amide	3.00	620.0	4.31	0.58
Diflufenican	Organics-formulae	P (Pesticides)	anilide	4.90	49248.4	4.73	0.29
Oxadixyl	Organics-formulae	P (Pesticides)	anilide	1.40	15.6	5.42	1.15
Chlorothalonil	Organics-formulae	P (Pesticides)	aromatic	2.64	270.6	2.14	0.65
Flamprop-M-isopropyl	Organics-formulae	P (Pesticides)	arylalanine	4.24	10774.4	3.76	0.35
Diclofop-methyl	Organics-formulae	P (Pesticides)	aryloxyphenoxypropionic	4.62	25845.9	2.79	0.45
Fenoxaprop-ethyl	Organics-formulae	P (Pesticides)	aryloxyphenoxypropionic	4.95	55257.6	3.72	1.22
Fluazifop-P-butyl	Organics-formulae	P (Pesticides)	aryloxyphenoxypropionic	4.50	19606.1	3.20	0.49
Propaquizafop	Organics-formulae	P (Pesticides)	aryloxyphenoxypropionic	4.60	24682.6	3.02	0.61
Benazolin	Organics-formulae	P (Pesticides)	Benazolin	1.34	13.6	5.10	0.57
Bentazone	Organics-formulae	P (Pesticides)	Bentazone	2.34	135.6	5.13	0.32
Thiabendazole	Organics-formulae	P (Pesticides)	benzimidazole	2.47	183.0	5.46	1.52
Ethofumesate	Organics-formulae	P (Pesticides)	benzofuranyl alkylsulfonate	2.70	310.7	4.79	1.01
Dicamba	Organics-formulae	P (Pesticides)	Benzoic acid	2.21	100.6	4.92	0.56
Asulam	Organics-formulae	P (Pesticides)	carbamate	-0.27	0.3	6.09	1.47
Benomyl	Organics-formulae	P (Pesticides)	carbamate	2.12	81.7	3.03	0.50
Carbendazim	Organics-formulae	P (Pesticides)	carbamate	1.43	16.7	2.53	0.35
Carbofuran	Organics-formulae	P (Pesticides)	carbamate	1.63	26.4	2.90	0.66
Methiocarb	Organics-formulae	P (Pesticides)	carbamate	2.92	515.7	2.59	0.49
Propamocarb hydrochloride	Organics-formulae	P (Pesticides)	carbamate	-2.60	0.0	5.54	0.08
Thiophanate-methyl	Organics-formulae	P (Pesticides)	carbamate	1.40	15.6	4.20	0.64
Phenmedipham	Organics-formulae	P (Pesticides)	carbanilate	3.47	1829.7	3.42	0.24
Metazachlor	Organics-formulae	P (Pesticides)	chloroacetanilide	2.38	148.7	1.76	1.18
Propachlor	Organics-formulae	P (Pesticides)	chloroacetanilide	2.80	391.2	3.06	1.18
Atrazine	Organics-formulae	P (Pesticides)	chlorotriazine	2.61	252.6	3.35	0.59
Cyanazine	Organics-formulae	P (Pesticides)	chlorotriazine	1.80	39.1	4.27	0.07
Simazine	Organics-formulae	P (Pesticides)	chlorotriazine	2.18	93.8	4.22	0.59
Terbuthylazine	Organics-formulae	P (Pesticides)	chlorotriazine	3.04	679.8	3.57	0.33
Cyproconazole	Organics-formulae	P (Pesticides)	conazole	2.90	492.5	4.54	0.94
Fenbuconazole	Organics-formulae	P (Pesticides)	conazole	3.23	1052.9	3.02	0.27
Flusilazole	Organics-formulae	P (Pesticides)	conazole	3.70	3107.4	3.47	0.37
Flutriafol	Organics-formulae	P (Pesticides)	conazole	2.29	120.9	4.56	0.42
Penconazole	Organics-formulae	P (Pesticides)	conazole	3.72	3253.8	3.60	0.25
Prochloraz	Organics-formulae	P (Pesticides)	conazole	4.10	7805.3	1.60	1.00
Propiconazole	Organics-formulae	P (Pesticides)	conazole	3.72	3253.8	3.43	1.18
Triadimenol	Organics-formulae	P (Pesticides)	conazole	2.90	492.5	4.76	1.39
Cycloxydim	Organics-formulae	P (Pesticides)	cyclohexene oxime	1.36	14.2	5.10	0.36
Tralkoxydim	Organics-formulae	P (Pesticides)	cyclohexene oxime	4.46	17881.0	4.44	0.75
Dazomet	Organics-formulae	P (Pesticides)	Dazomet	1.40	15.6	4.65	0.84
Captan	Organics-formulae	P (Pesticides)	dicarboximide	2.35	138.8	2.13	0.44
Vinclozolin	Organics-formulae	P (Pesticides)	dicarboximide	3.10	780.5	4.11	1.18
Dichlofluanid	Organics-formulae	P (Pesticides)	Dichlofluanid	3.70	3107.4	2.71	1.10
Pendimethalin	Organics-formulae	P (Pesticides)	dinitroaniline	5.18	93840.8	2.62	0.96
Trifluralin	Organics-formulae	P (Pesticides)	dinitroaniline	5.34	135641.2	2.50	0.96
Mancozeb	Organics-formulae	P (Pesticides)	dithiocarbamate	0.00	0.6	2.51	0.86
Maneb	Organics-formulae	P (Pesticides)	dithiocarbamate	0.62	2.6	2.78	0.61
Thiram	Organics-formulae	P (Pesticides)	dithiocarbamate	1.70	31.1	2.73	0.62
Fenpropidin	Organics-formulae	P (Pesticides)	Fenpropidin	2.59	241.2	2.00	1.30
Imazaquin	Organics-formulae	P (Pesticides)	imidazolinone	1.86	44.9	6.00	0.77
Methyl bromide	Organics-formulae	P (Pesticides)	Methyl bromide	1.19	9.6	3.39	1.31
Prometryn	Organics-formulae	P (Pesticides)	methylthiotriazine	2.99	605.9	3.28	1.17

Terbutryn	Organics-formulae	P (Pesticides)	methylthiotriazine	3.74	3407.2	2.74	0.59
Fenpropimorph	Organics-formulae	P (Pesticides)	morpholine	4.06	7118.6	3.98	0.84
Tridemorph	Organics-formulae	P (Pesticides)	morpholine	6.38	1487276.4	-0.23	0.13
Fentin hydroxide	Organics-formulae	P (Pesticides)	Multisite inhibitor	3.43	1668.8	1.30	0.53
Endosulfan	Organics-formulae	P (Pesticides)	Neurotoxicant: Cyclodiene-type	3.83	4191.7	0.85	1.11
HCH-Gamma	Organics-formulae	P (Pesticides)	Neurotoxicant: Cyclodiene-type	3.72	3253.8	2.12	1.11
Bromoxynil	Organics-formulae	P (Pesticides)	nitrile	2.99	605.9	3.98	0.16
Dichlobenil	Organics-formulae	P (Pesticides)	nitrile	2.74	340.7	4.26	0.44
Glufosinate-ammonium	Organics-formulae	P (Pesticides)	organophosphate	-4.81	0.0	5.51	1.25
Glyphosate	Organics-formulae	P (Pesticides)	organophosphate	-1.70	0.0	4.73	0.54
Tolclofos-methyl	Organics-formulae	P (Pesticides)	organophosphate	4.56	22510.8	3.48	0.39
Chlorpyrifos	Organics-formulae	P (Pesticides)	organothiophosphate	5.27	115449.4	1.78	1.43
Demeton-S-methyl	Organics-formulae	P (Pesticides)	organothiophosphate	-0.75	0.1	2.11	1.25
Dimethoate	Organics-formulae	P (Pesticides)	organothiophosphate	0.78	3.7	3.25	1.25
Disulfoton	Organics-formulae	P (Pesticides)	organothiophosphate	4.02	6492.2	3.07	0.78
Fenitrothion	Organics-formulae	P (Pesticides)	organothiophosphate	3.30	1237.1	2.22	1.25
Phorate	Organics-formulae	P (Pesticides)	organothiophosphate	3.35	1388.0	1.67	1.52
Triazophos	Organics-formulae	P (Pesticides)	organothiophosphate	3.34	1356.4	2.38	1.25
Aldicarb	Organics-formulae	P (Pesticides)	oxime carbamate	1.13	8.4	1.98	1.44
2 4-D	Organics-formulae	P (Pesticides)	phenoxyacid	2.81	400.3	4.78	0.77
2 4-DB	Organics-formulae	P (Pesticides)	phenoxyacid	3.53	2100.8	3.17	0.77
MCPA	Organics-formulae	P (Pesticides)	phenoxyacid	3.25	1102.5	3.19	0.77
MCPB	Organics-formulae	P (Pesticides)	phenoxyacid	3.50	1960.6	4.12	0.65
Mecoprop	Organics-formulae	P (Pesticides)	phenoxyacid	3.13	836.4	3.67	0.77
Mecoprop-P	Organics-formulae	P (Pesticides)	phenoxyacid	2.94	540.0	5.26	0.12
Chlorotoluron	Organics-formulae	P (Pesticides)	phenylurea	2.41	159.4	4.18	0.48
Diuron	Organics-formulae	P (Pesticides)	phenylurea	2.68	296.8	3.10	0.48
Isoproturon	Organics-formulae	P (Pesticides)	phenylurea	2.44	170.8	4.45	0.71
Linuron	Organics-formulae	P (Pesticides)	phenylurea	3.20	982.6	2.83	0.48
Metoxuron	Organics-formulae	P (Pesticides)	phenylurea	1.68	29.7	4.75	0.64
Monolinuron	Organics-formulae	P (Pesticides)	phenylurea	2.30	123.7	3.70	0.48
Chlorthal-dimethyl	Organics-formulae	P (Pesticides)	phthalic acid	4.28	11813.9	4.70	0.89
Clopyralid	Organics-formulae	P (Pesticides)	picolinic acid	1.06	7.1	5.04	0.90
Maleic hydrazide	Organics-formulae	P (Pesticides)	plant growth regulators	-0.84	0.1	5.20	0.88
Cypermethrin	Organics-formulae	P (Pesticides)	pyrethroid	6.60	2468264.5	0.14	1.40
Chloridazon	Organics-formulae	P (Pesticides)	pyridazinone	1.14	8.6	3.85	1.06
Triclopyr	Organics-formulae	P (Pesticides)	pyridine	2.74	340.7	3.81	0.66
Bupirimate	Organics-formulae	P (Pesticides)	pyrimidine	2.70	310.7	3.35	0.36
Chlormequat	Organics-formulae	P (Pesticides)	quaternary ammonium	-3.80	0.0	5.08	0.89
Chlormequat chloride	Organics-formulae	P (Pesticides)	quaternary ammonium	-3.80	0.0	5.37	0.71
Mepiquat	Organics-formulae	P (Pesticides)	quaternary ammonium	-2.82	0.0	4.71	1.63
Paraquat	Organics-formulae	P (Pesticides)	quaternary ammonium	-4.22	0.0	4.44	0.82
Azoxystrobin	Organics-formulae	P (Pesticides)	strobin	2.50	196.1	3.64	1.73
Kresoxim methyl	Organics-formulae	P (Pesticides)	strobin	3.40	1557.4	3.44	1.78
Amidosulfuron	Organics-formulae	P (Pesticides)	sulfonylurea	1.63	26.4	4.04	1.90
Metsulfuron-methyl	Organics-formulae	P (Pesticides)	sulfonylurea	2.20	98.3	4.36	2.47
Thifensulfuron-methyl	Organics-formulae	P (Pesticides)	sulfonylurea	1.56	22.5	6.20	0.88
Triasulfuron	Organics-formulae	P (Pesticides)	sulfonylurea	1.10	7.8	4.19	2.14
Tri-allate	Organics-formulae	P (Pesticides)	thiocarbamate	4.29	12089.0	3.10	0.39
Metamitron	Organics-formulae	P (Pesticides)	triazinone	0.83	4.2	4.67	0.88
Metribuzin	Organics-formulae	P (Pesticides)	triazinone	1.70	31.1	4.89	0.01
Amitrole	Organics-formulae	P (Pesticides)	triazole	-0.86	0.1	5.31	0.58
Lenacil	Organics-formulae	P (Pesticides)	uracil	2.31	126.6	3.67	1.56
Metalaxyl	Organics-formulae	P (Pesticides)	xylylalanine	1.59	24.1	5.08	0.84

Appendix 4

Challenging similarity of GLM15 and GLM25 models.

Bias may be introduced in EPC analyses when GLM15 and GLM25 would exhibit strongly different regression coefficients between models, for the same variable, only when those variables explain a large proportion of species abundance. The Table below is a summary-table of correlation coefficients between regression coefficients for GLM15 and GLM25 models, for the appropriate sets of species. Highlighted cells identify when regression coefficients are correlated (yellow for r ≥ 0.5, orange for ≥ 0.75 and red for ≥ 0.9). For some predictors, the GLM15 and GLM25 coefficients are correlated to a more limited extent, and for these the importance as an explanatory variable in GLM should be determined

	Lat15	*I(Lat^2)15*	*Long15*	*I(Long^2)15*	*DisS15*	*I(DisS^2)15*	*DCat15*	*I(DCat^2)15*	*Width15*	*I(Width^2)15*	*Depth15*	*I(Depth^2)15*	*BolCob15*	*I(BolCob^2)15*	*PebGrav15*	*I(PebGrav^2)15*	*Sand15*	*I(Sand^2)15*	*Silt15*	*I(Silt^2)15*	*Phi15*	*I(Phi^2)15*	*Slope15*	*I(Slope^2)15*	*Alt15*	*I(Alt^2)15*	*MAT15*	*I(MAT^2)15*	*AATR15*	*I(AATR^2)15*
Lat25	0.8																													
I(Lat^2)25		0.8																												
Long25			0.5																											
I(Long^2)25				0.7																										
DisS25					0.8																									
I(DisS^2)25						0.5																								
DCat25							0.3																							
I(DCat^2)25								0.1																						
Width25									0.8																					
I(Width^2)25										0.6																				
Depth25											0.8																			
I(Depth^2)25												0.6																		
BolCob25													0.5																	
I(BolCob^2)25														0.6																
PebGrav25															0.2															
I(PebGrav^2)25																0.0														
Sand25																	0.6													
I(Sand^2)25																		0.5												
Silt25																			0.6											
I(Silt^2)25																				0.3										
Phi25																					0.6									
I(Phi^2)25																						0.4								
Slope25																							0.6							
I(Slope^2)25																								0.8						
Alt25																									0.6					
I(Alt^2)25																										0.7				
MAT25																											0.9			
I(MAT^2)25																												0.9		
AATR25																													0.6	
I(AATR^2)25																														0.6

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Environment Agency
Rio House
Waterside Drive, Aztec West
Almondsbury, Bristol BS32 4UD
Tel: 0870 8506506
Email: enquiries@environment-agency.gov.uk
www.environment-agency.gov.uk