Proportional contributions to organic chemical mixture effects in groundwater and surface water

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ABSTRACT

Semi-quantitative GC-MS and LC-MS measurements of organic chemicals in groundwater and surface waters were used to assess the overall magnitude and contribution of the most important substances to calculated mixture hazard. Here we use GC-MS and LC-MS measurements taken from two separate national monitoring programs for groundwater and surface water in England, in combination with chronic species sensitivity distribution (SSD) HC50 values published by Posthuma *et al.* (2019, *Environ. Toxicol. Chem,* 38, 905–917) to calculate individual substance hazard quotients and mixture effects using a concentration addition approach. The mixture analysis indicated that, as anticipated, there was an increased hazard from the presence of a cocktail of substances at sites compared to the hazard for any single chemical. The magnitude of the difference between the hazard attributed to the most important chemical and the overall mixture effect, however, was not large. Thus, the most toxic chemical contributed $\geq$ 20% of the calculated mixture effect in >99% of all measured groundwater and surface water samples. On the basis of this analysis, a 5 fold assessment factor placed on the risk identified for any single chemical would offer a high degree of in cases where implementation of a full mixture analysis was not possible. This finding is consistent with previous work that has assessed chemical mixture effects within field monitoring programs and as such provides essential underpinning for future policy and management decisions on how to effectively and proportionately manage mixture risks.

1. Introduction

The focus of (eco)toxicological research (and chemical management) remains predominantly on single substances. This does not take into account the fact that real exposures are overwhelmingly to mixtures that can vary in their nature, complexity and composition over space and time. The failure to routinely consider mixtures has been recognised as one of the key uncertainties in chemical and natural resource management (Van den Brink et al., 2018). Recognising this gap, there are increasing calls for risk assessments and regulation to routinely consider the health and environmental implications of mixture exposures within an evidence based framework (Backhaus and Faust, 2012; Heys et al., 2016; Kienzler et al., 2016). Approaches for considering mixtures in risk assessment could range from full component based analysis and modelling, to the inclusions of an additional assessment factor placed on a single substance hazard value or environmental quality standard (a so called "Mixture Assessment Factor") to account for co-exposure effects

(Hassold et al., 2021; Van Gestel et al., 2010).

Within component based mixture ecotoxicology, the current dominants paradigm for predicting joint effects is based on two different concepts of additivity (Van Gestel et al., 2010): Concentration Addition for chemicals that act on the same biological target (similar mode of action), and Independent Action for chemicals that act sepraately on the same endpoint (dissimilar mode of action). These two models have been assessed for their capacity to predict the effects of carefully selected mixtures of chemicals with similar (Altenburger et al., 2000) or dissimilar (Faust et al., 2003) modes of action. Such studies have verified the predictive capability of these two models within their strict mechanistic contexts, although their application to environmental mixtures can be challenging because toxic mechanisms and their relationship to species sensitivity are often poorly known (Spurgeon et al., 2020).

Despite their limitations, concentration addition and independent action have been routinely used to predict mixture risks in freshwaters (Baas et al., 2016; Finizio et al., 2022; Gustavsson and Engwall, 2006;

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Silva and Cerejeira, 2015; Vallotton and Price, 2016) and these predictions compared to ecological effects (De Zwart et al., 2008; DeZwart et al., 2006; Kortenkamp et al., 2019). These assessments have found that observed effects are often consistent with mixture predictions, although in some cases models underestimate effects. Such conservatism may be due to factors such as chemical-chemical interactions leading to synergism; the time scale of the exposures; or combined stressor effects (Schipper et al., 2014; Stockdale et al., 2010). Overall, however, these verification studies indicate that mixture analysis can support a more realistic risk assessment than a traditional chemical by chemical approach.

To add to this body of knowledge, we here conduct an analysis to assess the proportional contribution of organic chemicals measured in groundwater and surface water collected in England to predicted overall mixture hazard. Our study was not intended to conduct a definitive site specific risk assessment. Any such analysis would require an extended approach that includes the use of fully quantitative measurements and of regulatory relevant hazard values, such as the substance specific HC5 or an approach that predicts the number of species that may be affected by an exposure, such as the multi-substance potentially affected fraction (msPAF). The assessment was based on a comparison of semi-quantitative gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) measured chemical concentrations in samples to the ecological hazard value (chronic SSD HC50) available for that chemical as published by Posthuma et al. (2019), as the most robust hazard value to use for such an assessment. The hazard quotients (HQs) calculated were then used in combination with an additive model to predict the mixture effect. From this analysis, some key trends relating to the presence of multiple chemicals in waters were calculated and visualised. These included: determining how mixtures increased the frequency of identified hazard compared to a single chemical assessment; assessing the relationship between mixture complexity and overall mixture hazard and assessing the degree to which mixture hazard exceeds that for the most important single chemical. The implications of these results for risk assessment are outlined, including how they may inform on the magnitude of any future Mixture Assessment Factor.

2. Materials and methods

2.1. Environment agency groundwater and surface water monitoring data-set

The Environment Agency Groundwater Quality Monitoring Network in England comprises ~2000 borehole, well or spring locations. These sites have been selected to be representative of the groundwater quality in that locality, with obvious point sources of pollution excluded from the program. Approximately 10,800 samples were taken for GC-MS analysis between 2009 and October 2019 and ~800 samples for LC-MS analysis between 2014 and October 2019. The samples for the surface water monitoring program have been collected from rivers, lakes and ponds across England. Samples have been collected for a wide range of reasons including site assessment, compliance monitoring, operational monitoring and water resource management. Visited sites have been sampled at a range of frequencies including regular, periodic and event driven sampling for specific requirements not design within the current project (which merely aimed to incorporate all available data). Within the surface water program, approximately ~23,000 samples have been collected and analysed by GC-MS and ~ 2800 by LC-MS.

All chemical analysis was conducted by the Environment Agency's accredited laboratory in Star Cross, UK, using broad target based semi-quantitative screening methods that are described in detail in Lapworth et al. (2018), Moreau et al. (2019) and White et al. (2019). A full list of analytes measured by the two methods across the full data-set are provided in the Supplementary information of Spurgeon et al. (2022). For brevity a summary of the full method used for the analysis is provided below.

The GC-MS method used for all measurements uses a liquid-liquid extraction method. An internal standard (D_{10}-phenanthrene) is added to each sample which is then extracted using dichloromethane (50 mL). The solvent is removed, and the aqueous layer acidified (pH ~ 1–2). Extraction is then repeated on the acidified sample. The extracts are combined and then evaporated slowly to avoid volatile compound loss under a nitrogen stream. The extract is dried using anhydrous sodium sulphate prior to analysis. The GC-MS target based (multi-residue) method, measures almost all GC-amenable pesticides and hundreds of other organic contaminants to detection limits as low as 0.01 μg/L. Deconvolution reporting software is used for the mass spectral analysis. Over time the method has added further substances to the analytical suite and also lowered many detection limits. Within the full data-set, 491 analytes were found in one or more samples in groundwater and 515 in surface water.

The LC-MS (Q-TOF) method measures almost all LC amenable pesticides, as well as many pharmaceutical and perfluorinated chemicals. For sample analysis, Waters Oasis HLB SPE cartridges are conditioned with methanol and then Ultra High Purity (UHP) water. The water sample is then loaded onto the cartridge, which is then washed with UHP water and dried under nitrogen. The column is eluted twice, firstly with 0.1% formic acid in a 1:1 mix of methanol: acetonitrile followed by dichloromethane. The dichloromethane and methanol: acetonitrile extracts are then successively evaporated under nitrogen and UHP water added for the analysis. Target compound identification is made by retention time, accurate mass and isotope distributions. Estimates of concentration is based on quantitative ion response and the measurement of the internal standard. Quantification limits are compound specific and are typically between 0.001–0.1 μg/L. Changes over time have added substances to the suite and lowered detection limits. Overall measurements have quantified 290 and 315 chemicals above the limit of detection in groundwaters and surface waters respectively.

2.2. CAS number assignment and excluded substances

CAS numbers were assigned to each measured substance. Any measured values for substances lacking a CAS number, or for which units were not provided, were excluded from the analysis. Such values always comprised <5% of the total number of measured values. Two sulphur compounds, S8 (CAS number 10,544–50–0; cyclooctasulphur) and S6 (CAS number 13,798–23–7; hexathiane) that were detected by GC-MS were excluded from the assessment as neither is an organic chemical. The steroids cholesterol and squalene were also excluded due to their potentially ubiquitous natural endogenous origin. The range of substances included for measurement was 1144 from the combined number of GC-MS and LC-MS detected analytes. This number excludes duplicate substances detected by both methods. Of this total number of determinands, 684 and 769 were detected above the limit of detection in the groundwater or surface water samples by one or both methods.

2.3. Collection of hazard values

Hazard values relating to surface water ecological receptors were collected for all substances included in the two analytical suites. The hazard values were used for both the surface water and groundwater data-sets. Use for groundwater was considered relevant because: (i) specific values were not available for the sub-surface environment; and, (ii) groundwater resources will contribute to surface water flows, hence, values for surface water receptors are relevant. The hazard values were taken from the larger set of HC$_{50}$ values published by Posthuma et al. (2019). These authors derived these values from a set of species sensitivity distributions (SSD) generated from the available aquatic ecotoxicological data in a well-structured and highly valuable assessment. The hazardous concentration for 50% of species (HC50) values from these SSDs was selected above lower effect thresholds, such as the HC5,

because the HC50 is placed in the middle of the distribution and so is more robust than values sitting more within the distribution tail. For site specific risk assessments, the use of values with a higher protection goal, such as the HC5, would provide a more precautionary approach.

2.4. Calculation of mixture risk

The overall approach to mixture risk calculation is shown in Fig. 1. Briefly, concentrations of all of the substances present above the limit of detection in ground and surface water by GC-MS and LC-MS were compared to the chronic SSD HC50 for that substance (if available) to generate a set of hazard quotients (measured concentration in groundwater or surface water / chronic SSD HC50 value) for all of the chemicals in every analysed sample. To calculate the mixture risk, two models were considered: concentration addition and independent action. Concentration addition was selected rather than independent action for two reasons. First, of the two models, concentration addition generally predicts slightly greater effects for a given mixture than independent action, except when response curves are shallow. Hence the use of concentration addition is precautionary. Secondly, concentration addition only requires a single hazard metric (i.e. a chronic SSD HC50) to calculate contributions to the mixture effect, rather than a full response relationship as is needed for independent action, but which was not readily available for all substances considered here (Van Gestel et al., 2010). Based on a concentration addition approach, all detected chemicals with a chronic SSD HC50 could be included in the mixture analysis. This equated to 725 of the 1144 analysed chemicals (n.b. a full list of chemicals and extracted HC50 values can be found in the Supplementary information of Spurgeon et al. (2022)). Concentration addition was implemented following the basic equation of Altenburger et al. (2000). A limitation of the use of concentration addition (also applicable to independent action), is that if there is no hazard value available for a chemical, then that substance simply cannot be included within the mixture calculation. This means mixture assessment for a given sample may frequently underestimate risk due to the presence of chemicals lacking a hazard value. Similarly, chemicals that are not detected by the GC-MS and LC-MS scans (e.g. trace metals) are also not included. Hence while informative, our overall approach cannot be taken as an exhaustive assessment of risk for any sample or site.
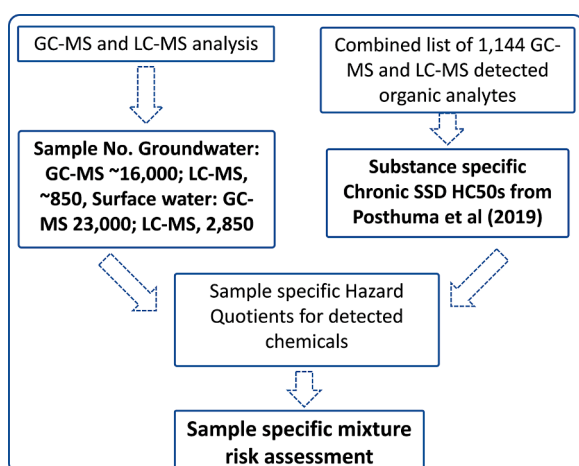

Fig. 1. Schematic of the approach used for worst-case risk ranking. The exposure term for hazard quotient calculation (left branch of the diagram) is identified as the highest measured concentration and detection frequency from the measured GC-MS and LC-MS data. The hazard value (right branch of the diagram) is collected for multiple metrics from easily available toxicological and ecotoxicological resources. Calculated HQ are used for mixture assessment using an established and widely used additive mixture toxicity modelling approach.

3. Results

3.1. Calculation of mixture effects

Substance HQs were calculated for all chemicals present above the LOD in a sample by comparing the measured concentration for that substance to its reported chronic SSD HC50. This analysis was conducted separately for all GC-MS and LC-MS analysed groundwater and surface water samples. Individual substance HQs were then summed using concentration addition to derive the mixture hazard quotient (hereafter HQmix). The distribution of HQmix values for all measured samples was then plotted from lowest to highest, to allow the distribution of mixture risk to be visualised (Fig. 2).

In the groundwater GC-MS data-set, individual substance HQs and calculated HQmix values indicate that, as expected, a greater number of sites have an HQmix value $\geq$ 1 than is the case for any single chemical. The GC-MS HQmix was $\geq$ 1 in 64 (0.63%) of the analysed groundwater samples (Fig. 2a), indicating that there were multiple samples in which GC-MS measured chemicals would be predicted to impact (in some way) 50% of the species present (based on the mixture exposure exceeding the calculated hazard value 50% of species). For LC-MS substances, no site has an HQmix $\geq$ 1. However, an HQmix of $\geq$ 0.1 was found for 89 of 878 sites (10.1%)(Fig 2c). This is greater than the number of sites showing this degree of mixture hazard for any single LC-MS measured chemical. The greater number of sites with HQmix values $\geq$ 1 or $\geq$0.1 in both the GC-MS and LC-MS measured chemicals in groundwater, indicates additional cumulative hazard from mixtures above that for any single chemical.

In surface water, there was also a greater number of sites with a chronic SSD HC50 HQmix $\geq$ 1 than sites with an HQ $\geq$ 1 for any single chemical. For the GC-MS data, a HQmix $\geq$ 1 is found in 876 samples (4.3%) (Fig. 2b), although of these, 572 samples were characterised by the presence of rotenone at piscicidal levels. This high predicted risk for this chemical is based on an HC50 value that comes from a high quality SSD derived from measured data (Posthuma et al., 2019). The presence of high rotenone levels is associated with samples taken for analysis to confirm concentrations used for invasive fish species control. The detection of high hazard at these rotenone treated sites was useful, as it confirm method capacity to identify sites with high chemical associated risk, although such sites cannot be considered representative of wider surface water chemical status. The remaining 304 samples (1.4%) that had an HQmix > 1, all contained multiple GC-MS detected chemicals (but not rotenone) that contributed to the overall mixture effect. In the surface water LC-MS measurement data-set, a value of HQmix $\geq$ 1 is found in a single sample (0.03%)(Fig. 2d), whereas no single chemical has a chronic SSD HC50 HQ $\geq$ 1. A greater number of sites with an HQmix $\geq$ 0.1 are also found compared to the values of HQ $\geq$ 1 for any single substance. Calculation of HQmix for both measurement methods and sample types indicates that mixture assessment identifies a greater scale of potential impacts due to the presence of multiple chemicals than for any single chemical.

The identification of a greater number of sites with HQmix $\geq$ 1 by GC-MS compared to LC-MS may simply be the product of the greater number of samples analysed by GC-MS (~16,000 for groundwater, ~22,000 for surface water) compared to LC-MS (~800 for groundwater, ~2200 for surface water). This greater number gives an order of magnitude or more potential to find locally high concentrations of one or more chemicals giving an HQmix > 1. Alternatively, substances detected by GC-MS may be more likely to exceed hazard thresholds because of their specific physicochemical characteristics. Further work would be needed to tease apart the relevance of both of these factors and their role in determining mixture risk.
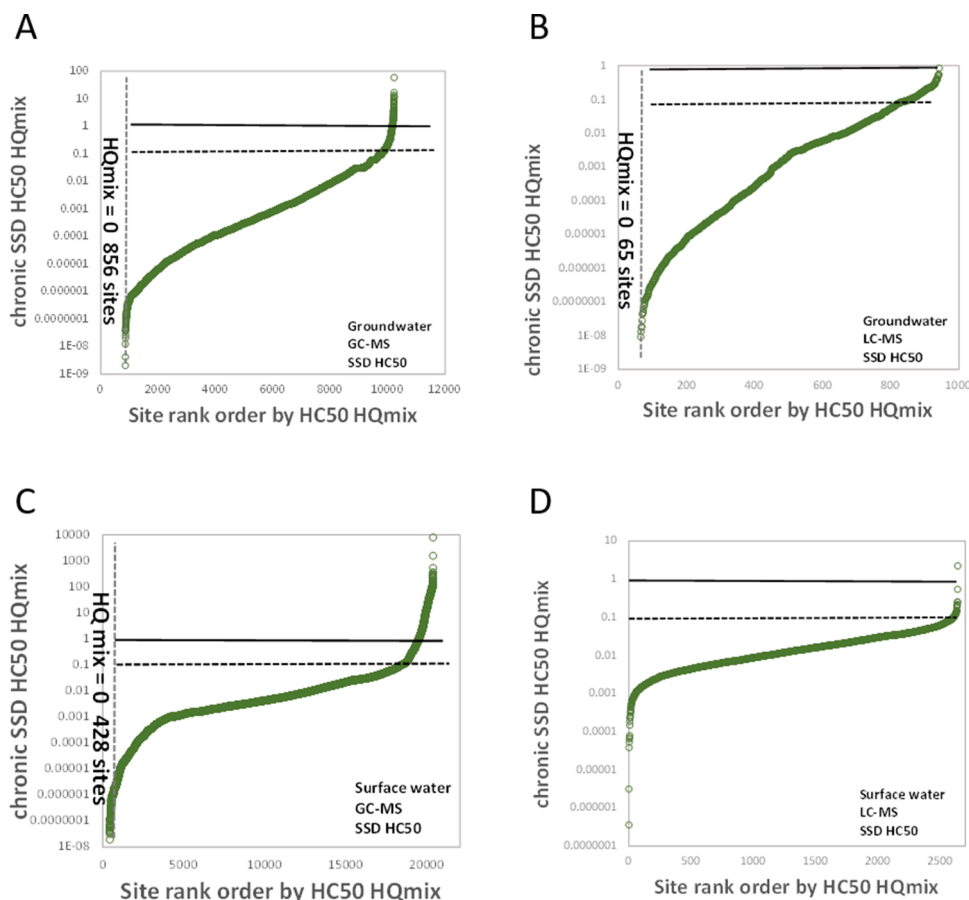
Fig. 2. Cumulative distributions of HQmix for the GC-MS (left) and LC-MS (right) analysed samples for the groundwater (top row) and surface water (bottom row) data-sets; horizontal solid lines indicate HQmix = 1, horizontal dashed lines indicates HQmix = 0.1, vertical dash lines indicates the number of sites where HQmix = 0 with the text providing the number of such sites.

3.2. Contribution of the most important single chemical to the mixture effect

To assess the importance of the most toxic chemical to mixture risk, the cumulative distribution of the proportional contribution of the most important individual substance HQ to HQmix was calculated for all groundwater and surface water GC-MS and LC-MS sample measurements (Fig. 3). If HQmix is equal to the HQ of the most important (toxic) chemical (hereafter HQmax), then that chemical contributes all of the mixture effect. In such cases, the evaluation effectively is a single substance assessment and no longer needs to consider mixtures. In those cases where HQmax = 0.5, then the most toxic substance contributes half of the mixture effect and where HQmax = 0.2 then one fifth of the total mixture effect is due to the most important chemical.

For GC-MS chemicals in groundwater, the most important chemical (HQmax) contributes to ≤ 20% of HQmix in 2 of 9365 (0.02%) samples and ≤ 50% of HQmix in 516 samples (5.5%)(Fig. 3a). This compares to 6013 (62%) of samples where HQmax is between 50% and 100% of HQmix, indicating the most "risky" substance contributes ≥ half of the mixture effect and 3034 samples (32.4%) where HQmax = 1, indicating that only a single substance contributes. For GC-MS detected chemicals in surface water, there are 9 of 19,911 (0.05%) of samples where HQmax contributes ≤ 20% of HQmix and 1984 (10%) of samples where HQmax is ≤ 50% of HQmix (Fig. 3c). This compares to 15,241 sample (76.5%) where HQmax is ≥50%, but less 100% of HQmix and 2677 (13.4%) sites where HQmax = 1 indicating only a single chemical contribution.

For LC-MS detected chemicals in groundwater, HQmax always contributes ≥ 20% to HQmix. There are 55 of 818 sites (7.2%) when HQmax ≤ 0.5 indicating that the most toxic chemical contributes less

than 50% to the mixture effect (Fig. 3b). This compares to 645 sites (78.9%) where HQmax is ≥ 50% of HQmix, and only a single sample where HQmax = 1 indicating the contribution of a single substance (although 433 (53.2% of all samples) have an HQmax ≥ 90% of HQmix, indicating that one chemical contributes a large proportion of the mixture effect). For the LC-MS analytes in surface water, 32 samples (1.2%) have an HQmax that contributes ≤ 20% to HQmix and there are 1010 samples (38.2%) where the most "toxic" chemical contributes <50% of HQmix (Fig 3d). This compares to 1602 (60.6%) where HQmax is ≥ 50%, but less <100% of HQmix. There is also a single sample where HQmax = 1 indicating the presence of a single substance, although 159 (6%) have an HQmax >90& of HQmix indicating the dominance of a single chemical.
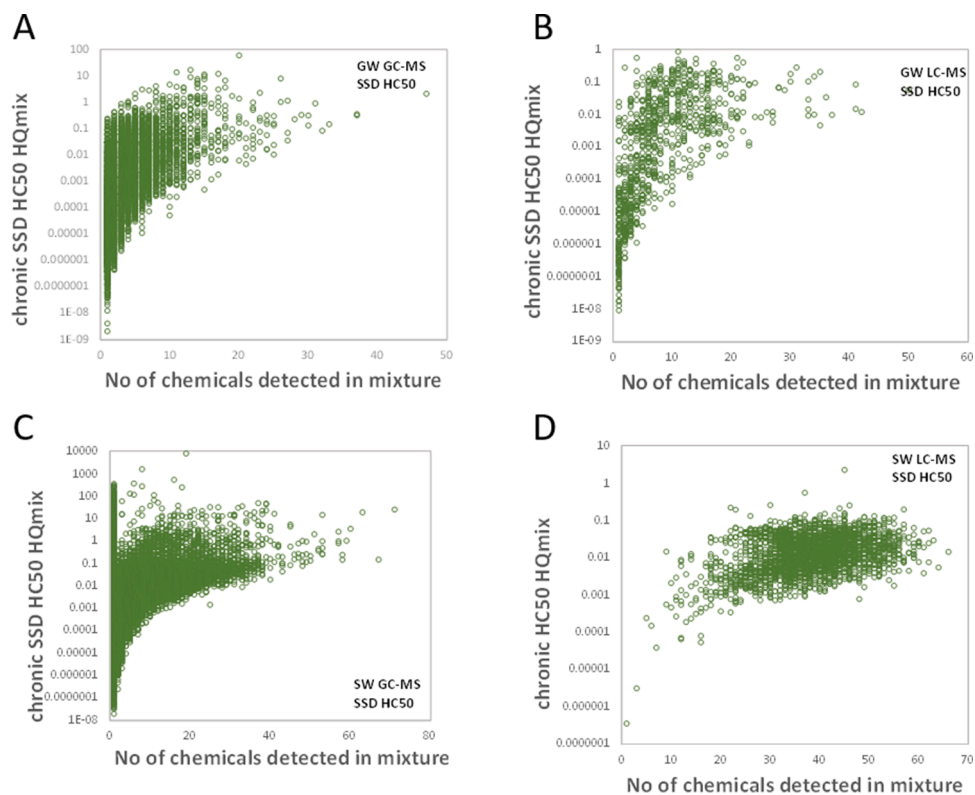
3.3. Relationship of HQmix with the number of chemicals detected in the sample

Mixture HQmix may be expected to increase as mixture complexity increases given that more chemicals are present at detectable concentrations to contribute to the overall effect. To test this hypothesis, chronic SSD HC50 calculated values of HQmix were plotted against the number of chemicals present in the sample. An increase in the number of chemicals detected was weakly associated with a higher value of HQmix (Fig. 4a–d). There are, however, numerous samples at which high values of HQmix are found, even when the number of chemicals contributing is relatively low (<5), especially in the GC-MS data-set. As mixture complexity increases, the number of samples with low HQmix values tends to reduce, such that for mixtures of ≥10 substances, relatively low values of HQmix are rarely found (Fig. 4a–d). This pattern of a reduced
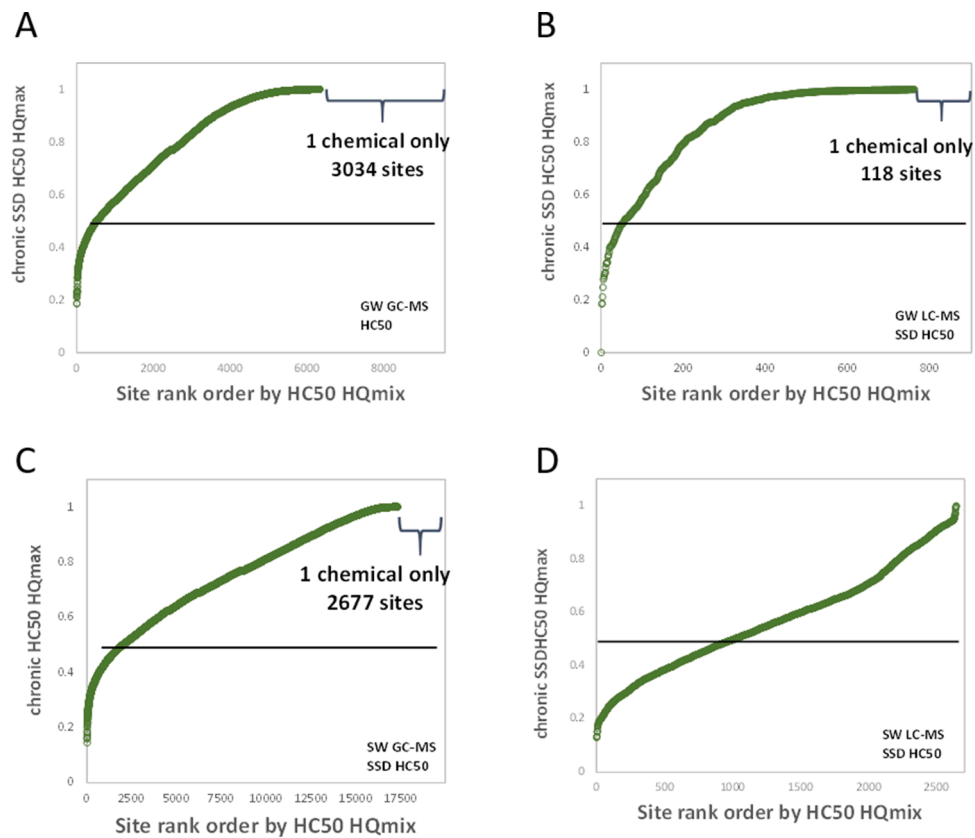
Fig. 3. Cumulative distribution of the proportional contribution of the most toxic chemical (HQmax) to the overall mixture effect (HQmax), ordered by site rank HQmax for GC-MS (left) and LC-MS (right) analysed samples for the groundwater (top) and surface water (bottom) data-sets; cases where HQmax = 1 are sites at which a single contributing chemical is present.

Fig. 4. Relationship between the number of chemicals detected above the limit of detection in any sample and the contribution of the overall mixture effect (HQmix) in that sample calculated for the GC-MS (left) and LC-MS (right) analysed samples for the groundwater (top) and surface water (bottom) data-sets.

number of low HQmix sites at high complexity is consistent across measurement methods and sampled environments. This suggest that as chemical number increases, overall mixture effect tends to increase. Although some cases do still occur where multiple low levels are present compared to respective HC50s and a small mixture effect remains.

3.4. Number of chemicals detected and the contribution of the most toxic chemical (HQmax)

To assess how the most toxic single chemical contribution changes with mixture complexity, HQmax was plotted against the number of chemicals contributing to the mixture effect (Fig. 5a–d). Even when there are relatively few chemicals present (≤ 3), there are still cases where the most important chemical contributes around 0.4 of HQmix. These samples represent cases of substantial mixture effect at low mixture complexity. As the number of substances in the mixture increases to >10, there is a trend for HQmax to decrease, although this pattern is not strong. Indeed even when the mixture becomes quite complex (≥ 15 chemicals), there still remains samples where the most important chemical still contributes $\geq 50\%$ of HQmix in all data-sets (Fig. 5a–d).

4. Discussion

Chemical mixture assessment is a complex task. Any mixture analysis, by its nature, will have specific assumptions and limitations. This is the case with the approach adopted here. The method was based on the use of concentration addition. This model is particularly well suited for mixture assessments made using single value hazard thresholds, because the mixture effects can be calculated without access to full concentration response relationship, something needed for independent action, but which is rarely available (Van Gestel et al., 2010). This means that the decision of model choice for risk assessment is primarily a pragmatic one based on data availability rather than any mechanistic considerations.

Despite the practical benefits of using an approach based on concentration addition, there are also some theoretical and practical issues with the use of this model that are relevant to the interpretation of outcomes.

The concentration addition model assumes that substances exert effects through a similar mode of action. Given the range of substances detected, a common mechanism is rarely (if ever) likely to be applicable. This means that assessment done using the concentration addition model may not accurately predict the true mixture effect. Mixture assessment using concentration addition may result in a more precautionary mixture effect prediction than use of independent action, although previous studies have suggested that the absolute magnitude of this difference is often relatively small (Backhaus, 2016; Cedergreen et al., 2008). Concentration addition is generally accepted to result in a more conservative mixture effect prediction than the independent action model and so is well suited for initial screening and risk characterisation (Van Gestel et al., 2010). The assessment conducted with concentration addition here, may mean that the magnitude of mixture effects compared to worst case single chemical may be overestimated compared to the true situation.

A further issue with a concentration addition approach is that the model assumes that all chemicals in the mixture act independently without interacting. As a consequence, synergistic or antagonistic effects are not accounted for. There is a widely accepted potential for synergism or antagonism to occur in a small, but potentially significant, number of mixture cases (Belden et al., 2007; Cedergreen, 2014; Martin et al., 2021). Studies have shown that the effects of synergistic or antagonistic interactions can be cumulative (Cedergreen et al., 2012). However, probability also means that when mixture complexity rises, the probability for counteracting synergistic or antagonistic interactions also increases. As a result, more complex mixtures may trend more to additivity than simpler mixtures in which a single synergistic or antagonistic interaction may dominate (Tian et al., 2012; Warne and Hawker, 1995).

The mixture assessment was conducted by comparing the reported concentrations of a chemical detected with the chronic SSD HC50 value
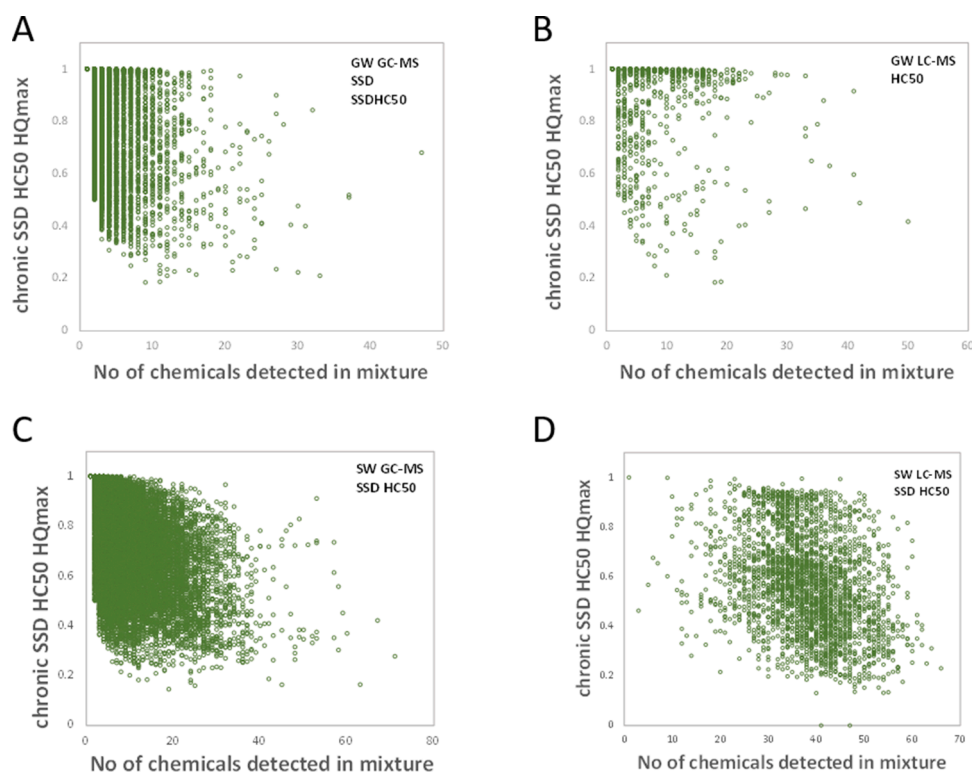

Fig. 5. Relationship between the number of chemicals detected above the limit of detection in any sample and the contribution of the most toxic chemical (HQmax) in that sample calculated for the GC-MS (left) and LC-MS (right) analysed samples for the groundwater (top) and surface water (bottom) data-sets.

for that substance. An obvious limitation of the mixture analysis is, therefore, that the assessment can only consider substances that are both detected above the limit of detection and also for which Posthuma et al. (2019) have reported a chronic SSD HC50 value (only the case for ~80% of detected chemicals). The GC-MS and LC-MS methods used provided the potential to detect a wide range of different organic chemicals. However, not all organic chemicals that are potential pollutants are included in the analysis. As a result, some substances are missing from the assessment, in addition to those present at concentrations below the analytical detection limit.

Given that different suites of substances are detected by each method, the outcome of the analysis is operationally defined by the diversity of chemicals included in the analytical suite. Hence, each method only captures a snapshot of the potential mixture risk. Within each analysis, certain classes of substances more amenable to detection by other methods are excluded. Thus, the LC-MS method measures many pesticide and pharmaceuticals, but not many classical persistent organic pollutants (PCBs, PBDEs, PAHs), while for GC-MS, the opposite is the case. A merger of HQs could potentially have been conducted for substances detected by both methods in cases where samples were taken at the same time and location. However, there are technical issues with such a merger. For example, differences in the detection limits between the analysis methods would result in the inclusion of a greater number of LC-MS than GC-MS chemicals. This difference in the number of chemicals included due to method sensitivity would be important when considering specific aspects of the mixture effect, such as how many substances contribute and any relationships between that number and the contribution of the most important single chemical. Further, the different physicochemical properties of the substance in each of these target screens could accentuate any such differences. Even if analyses outcomes were combined, this would still be an underestimation of total mixture effect, since the target screen substance selection misses not only some organic chemicals, but also the contribution of inorganic chemicals, which are frequently highlighted as the substances most likely to present relative high risk in surface waters (Donnachie et al., 2014; Johnson et al., 2017). A further limitation of the overall approach is the semi-quantitative nature of the concentrations measurements made. Further substance calibrations would be needed (and are underway) to verify measurements made for specific substance groups of high interest (e.g. PFAS compounds), to provide greater certainty on the analytical accuracy of the individual substance measurements.

Although there are challenges for mixture analyses and interpretation using concentration addition, the approach has an established history for pragmatic and conservative field based mixture assessment (DeZwart et al., 2006; Piliere et al., 2014; Schell et al., 2018; Schipper et al., 2014). Past mixture studies have used a range of metrics derived from single species studies (e.g. the LC$_{50}$ for *Daphnia* sp.) as the hazard term for mixture assessment (Schell et al., 2018; Verro et al., 2009). The approach here benefits from assessing mixture effects against a hazard value derived based on a wider set of toxicity information organised in a species sensitivity distribution, rather than on a metric derived for a single species. As such it provides a more holistic basis for assessing mixture effects at the ecosystem level.

Chemicals are generally present in the environment as mixtures. The results of the groundwater and surface water monitoring programs clearly illustrate the presence of multiple chemicals in both environments. For example, measurements by LC-MS almost always showed the presence of >5 chemicals and in some samples up to 70 chemicals were found within the analysed samples (see Fig 4). Only when the detection limits are relatively high, as in the GC-MS analysis, is the ubiquitous presence of a cocktail of chemicals not seen. However, this is almost certainly due to the inability of this method to detect chemicals at low concentrations, rather than a true absence of a complex chemical mixture at the sampled locations. Use of concentration addition to predict a combined hazard for the detected mixtures, as HQmix, indicated that, as would be expected, there was a greater hazard from

mixtures than was the case for any single substance. By comparing worst case single chemical and mixture effect predictions, it is possible to comment on the scale of this additional mixture hazard compared to the worst case single chemical case (Backhaus, 2016; Drakvik et al., 2020).

For the GC-MS and LC-MS data-sets, assessment of the contribution of the most toxic chemical (HQmax) to the mixture effect (HQmix) indicated that in < 50% of samples, the presence of further chemicals beyond the most toxic substance causes a < 2 fold increase in indicated hazard (i.e. >50% of sites have an HQmax ≤ 50% of HQmix). In only a small proportion of samples (<1.5%), does the presence of further chemicals results in a > 5 fold increase in the predicted mixture hazard compared to that for the most toxic substance (i.e. HQmax ≤ 20% of HQmix). Such information may be important if developing an assessment factor that could account for mixture effects, as it represents the ratio between the most important compound and the total mixture hazard. Within the analysis, the nature of the substances making the largest contribution to the mixture effect vary between analytical measurement methods and the sampled environments. Spurgeon et al. (2022) conducted a worse case risk ranking of substances in both groundwater and surface water using the same set environmental measurement data. This analysis indicated that a number of industrial (e.g. PAHs), plastics associated chemicals and legacy pesticides were highly ranked in groundwater; more personal care products and pharmaceuticals were highly ranked in surface waters; and perfluoroalkyl and polyfluoroalkyl and current use pesticides were highly ranked in both compartments. These classes of substance are similarly often major contributors to the mixture hazard predicted here for the groundwater and surface water samples.

The results from this analysis on the magnitude of difference between HQmax and HQmix is consistent with the conclusions of previous comparative single chemical and mixture effect studies. For example, Backhaus and Karlsson (2014) used a similar approach as used here to calculate "Mixture Assessment Factors" for pharmaceuticals. From their analysis, these authors concluded that a maximum value of 4.3 was needed to scale from worst case single chemical to the additive mixture effect. Rorije et al. (2022) also came to the conclusion that the most toxic chemical often contributed a substantial proportion of the overall mixture effect, based on an analysis conducted for European fresh water ecosystems using the NORMAN chemical monitoring database. These authors were, however, reticent to define a value of the magnitude of any assessment factor that could be placed on the most important chemical to protect against mixture effects; although further analysis was planned towards this objective. The results found in these previous studies on the nature of proportionate contribution of chemicals to mixture effects are clearly mirrored in the current work. Thus there is only ≅ 1% of samples in which the most important chemical contributed <20% to the overall mixture effects. The coalescence of the conclusions from these multiple studies suggests that a mixture assessment factor of five placed on the HQ of the most important chemical could adequately protect for possible mixture effects. As, however, the process needed to identify the most important chemical matches that used to identify the additive sum of mixture effects, the actual mixture effect can also be equally readily calculated. In monitoring studies, such a mixture assessment can provide a more robust analysis, that is neither over-protective or more rarely under-protective, than considering only the individual chemicals detected at a given site.

5. Conclusions

1 Hazard quotients were determined using chronic SSD HC50 values for >80% of the substances detected by semi-quantitative GC-MS and LC-MS analysis of groundwater and surface water samples. A concentration addition approach was used to calculate the mixture effect from the individual substance HQ values.

2 Additional hazard compared to that for any single chemical was found for the calculated mixture effect, although the magnitude of this additional risk was not always large.

3 Consistent patterns were present in all data-sets. Thus, the most toxic substance contributed >50% of the mixture effect in ~99% of cases, and greater mixture complexity was associated with increased mixture effect, although only weakly so.

4 The consistent finding that the most toxic chemical very often contributed most of the mixture effect suggests that a Mixture Assessment Factor of five placed on the HQ for the most important single substance would be sufficient to account for any mixture effects in the large majority of cases.

CRediT authorship contribution statement

David Spurgeon: Visualization, Conceptualization, Formal analysis, Writing – review & editing. **Helen Wilkinson:** Writing – review & editing. **Wayne Civil:** Data curation, Writing – review & editing. **Lorraine Hutt:** Writing – review & editing. **Elena Armenise:** Writing – review & editing. **Natalie Kieboom:** Writing – review & editing. **Kerry Sims:** Writing – review & editing. **Tim Besien:** Visualization, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors confirm that they have no competing financial or non-financial interests.

Availability of supporting data

Data-sets providing the hazard values used for the analysis are available on request from the corresponding author. The Environment Agency's GC-MS and LC-MS target screen data-sets are available for download here: https://data.gov.uk/dataset/0c63b33e-0e34-45bb-a779-16a8c3a4b3f7/water-quality-monitoring-data-gc-ms-and-lc-ms-semi-quantitative-screen.

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