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An application of a decision tree for assessing effects from exposures to multiple substances to the assessment of human and ecological effects from combined exposures to chemicals observed in surface waters and waste water effluents

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Abstract

Background: A decision tree has been developed for evaluating risks posed by combined exposures to multiple chemicals. The decision tree divides combined exposures of humans and ecological receptors into groups where one or more components are a concern by themselves, where risks from the combined exposures are of low concern, and where there is a concern for the effects from the combined exposures but not from individual chemicals. This paper applies the decision tree to real-world examples of exposures to multiple chemicals, evaluates the usefulness of the approach, and identifies issues arising from the application.

Results: The decision tree was used to evaluate human health and ecological effects from the combined exposure to 559 mixtures of substances measured in surface waters and effluents. The samples contained detectable levels of 2 to 49 substances. The key findings were, 1) the need for assessments of the combined exposures varied for ecological and human health effects and with the source of the monitoring data, 2) the majority of the toxicity came from one chemical in 44% of the exposures (human health) and 60% of exposures (ecological effects), 3) most cases, where risk from combined exposures was a concern, would have been identified using chemical-by-chemical assessments. Finally, the tree identified chemicals where data on the mode of action would be most useful in refining an assessment.

Conclusions: The decision tree provided useful information on the need for combined risk assessments and guidance on the questions that should be addressed in future research.

Keywords: Mixtures, Maximum cumulative ratio, Surface waters, Human health effects, Ecotoxicological effects

Background

Humans and ecological receptors are continuously exposed to multiple chemicals; however, regulatory programmes have traditionally focused on regulating chemicals on a chemical-by-chemical basis. As a result, there is a concern that instances may occur where individual chemicals do not cause adverse effects but the combined effects of the exposures could pose a risk to human health and the environment. Such risks would not be detected in a chemical-by-chemical approach. In response to this concern, a number of organizations have investigated the issue of combined exposures to multiple chemicals [1-5].

The Mixtures Industry Ad-hoc Team (MIAT) was created by the European Chemical Industry Council (*Conseil Européen des Fédérations de l'Industrie Chimique*, CEFIC) to address the issues associated with combined exposures to multiple chemicals. In 2010 the MIAT

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began the development of a decision tree for combined exposures to chemicals (hereafter referred to as the "decision tree"). The decision tree was based on concepts taken from a number of published approaches including those developed by a joint group of three Scientific Committees to the European Commission (SCs) [6], the World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) [1,2], and recent publications on new quantitative tools (the Maximal Cumulative Ratio (MCR) and use of the Threshold of Toxicological Concern (TTC) in the assessment of risks from combined exposure) [7-9]. A detailed description of the decision tree is given in a companion paper to this publication [10].

This paper discusses the application of the decision tree to "real world" examples of combined exposures, the specific findings that were obtained from its application, the value of these findings, and issues that arose during the exercise. The application addresses a common problem encountered in combined risk assessment, exposure to a mixture of chemicals that co-occur in an environmental medium. The assessment addresses risks to both humans and ecological receptors exposed, or potentially exposed, to mixtures of substances reported from water monitoring programmes in Europe. These monitoring programmes measured the concentrations of multiple substances in samples of surface waters and effluents from wastewater treatment plants (WwTPs) that are discharged to surface waters following treatment.

No one application can illustrate all of the steps of the decision tree. In this application, the decision tree explores a large portion (but not all) of the tree. Specifically, it addresses how the toxicity and ecotoxicity of combined exposures can be evaluated when there is limited data on mode of action (MoA) and exposure. The steps of the decision tree addressed in this assessment are given in red in Figure 1.

Terminology

The combined effects of exposures to multiple chemicals have been the subject of discussion, research, and regulation by a number of organizations for more than 50 years. One unfortunate result of this history is the proliferation of confusing and sometimes conflicting terminology [1,2]. In this paper the term 'combined exposures' is defined as a person's or another organism's exposures to multiple chemicals that are received from either a single source or multiple sources. When doses of multiple chemicals are received from one source (multiple chemicals co-occurring in a medium or a commercial product) they can be referred to as a mixture exposure. Thus mixture exposures are a subset of combined exposures.

Results

As discussed in the methods section, the data on the mixture exposures are composed of seven data sets (CH 1–1, CH 1–2, CH 2–1, CH 2–2, JRC, UKCIP, and EA). The data sets differ in the number of compounds analyzed in each sample and the waters surveyed. A summary of the information on the compositions of the mixtures and the availability of toxicity information on the mixture components is provided in Table 1. A list of the specific reference values (RVs) used for the human and ecological assessments including the specific values and the source of the values is given in Additional file 1: Appendix A.

The seven datasets developed for this project focused on mixtures that have large numbers of detected substances. The datasets include 559 samples, 362 samples of surface waters and 197 samples of WwTP effluents. The number of analytes ranged from 21 to 123, the number of detected substances in samples ranged from 2 to 69, with an average (median) of 20.4 (16). The number and composition of analytes in each survey of analytes varied across surveys and a total of 222 substances were analyzed for in one or more surveys and 163 were detected in one or more samples. The substances include a wide range of inorganics, and polar and nonpolar organic chemicals. RVs for human health were identified for 100 of the 222 analytes. The RVs of 110 additional substances were conservatively estimated using the Cramer classes of the substances [8]. Five polycyclic aromatic hydrocarbons did not have non-cancer human health values. These substances are not representative of the data used in setting the values for the Cramer classes [11,12]. As a result, the Cramer class approach could not be used for these substances. The remaining seven substances are common cations and anions (sodium, magnesium, chloride, orthophosphate, sulphate, potassium, and calcium). These substances have low toxicity and are not expected to pose a risk to humans or ecological receptors at the concentrations reported (0.08 to 500 ppb).

Ecotoxicity criteria were identified for 143 of the 222 substances. Excluding the seven substances identified above, there were 72 substances without criteria. The majority of the substances with missing criteria were measured in two datasets: CH 1-2 and CH 2-1. CH 1-2 and CH2-1 also had the the most compounds that were measured but were not detected.

The reason that larger numbers of substances were not detected in any sample is that these surveys also included substances that had not been frequently measured in the past but they 1) were known to be released in household wastewaters and are likely to be introduced to surface waters via WwTPs, 2) had been measured infrequently in surface waters but occurred at high

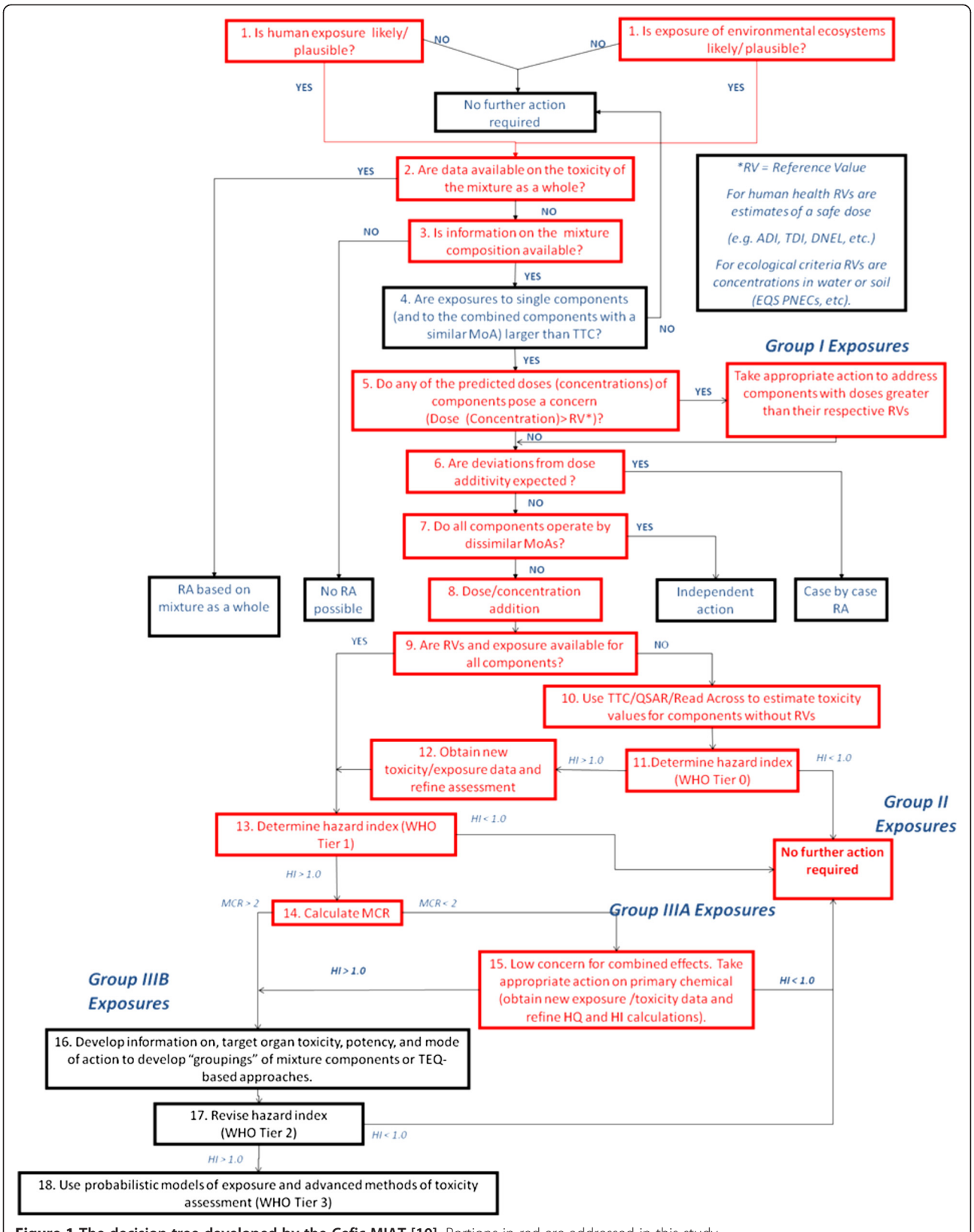

Figure 1 The decision tree developed by the Cefic MIAT [10]. Portions in red are addressed in this study.

Table 1 Summary of the characteristics of the 559 mixtures included in the seven datasets

	Datasets						
Dataset name	**CH 1-1**	**CH 1-2**	**CH 2-1**	**CH 2-2**	**JRC**	**UK CIP**	**EA river**
Number of samples	74	10	24	9	122	120	200
Number of analytes	21	123	63	24	35	47	40
Number of analytes detected in more than one sample	21	69	45	21	35	46	40
With human toxicity data	21	69	45	21	35	34*	36
With ecotoxicity data	16	39	37	20	35	39*	40
Analytes never detected	0	54	18	3	0	1	0
With human toxicity data	-	54	18	3	-	1	-
With ecotoxicity data	-	37	8	1	-	1	-
Number of substances detected in samples							
Lowest number of substances detected in a sample	2	55	6	4	4	16	12
Highest number of substances detected in a sample	20	69	32	19	35	42	30
Average number of substance detected in a sample	15.3	62.6	24.1	12.6	22	27.5	20.4

* Seven of the missing standards are for common ions (sodium, magnesium, chloride, orthophosphate, sulphate, potassium, and calcium) that are of low concern for human health and ecological effects.

concentrations, or 3) have specific toxicological concerns (e.g. mutagenicity, carcinogenicity, hormonal activity, or immunotoxicity). All of the other surveys used to produce the datasets focused on substances known to occur in the sampled waters and effluents. As a result, the majority of the analytes were detected in at least one sample for the five datasets.

Human health effects

The 559 mixtures were evaluated using the decision tree. The initial finding was that 2% (nine mixtures) were of concern and had a single substance that was a concern under the exposure assessment assumptions (Group I). Three substances, chromium, estrone and ethinylestradiol, exceeded the human health criterion in one or more of the nine mixtures and all nine came from surface water samples.

The remaining 98% had a predicted Hazard Index (HI) of less than 1, indicating low concern for the mixture as well as the individual chemicals, and thus fell into Group II. None of the mixtures fell into Groups IIIA or IIIB (concern for the mixture, low concern for individual chemicals, with/without dominating chemical). The above findings are based on the assumption that the concentrations of substances that were analysed for, but not detected (NDs) were zero. When the concentrations of NDs were set at the limit of detection (LOD) divided by $2^{0.5}$ there was little or no change in the fractions of mixtures that fell into the four groups (Table 2).

Plotting the MCR values against the HI for combined exposures provides a graphical description of the relationships between the four Groups [10]. Figure 2 presents the MCR-HI plot for the mixtures. In these plots HI is plotted on a log scale and MCR is plotted on a linear scale with a minimum value of 1. The four groups fall into separate regions bounded by the functions,

Table 2 Percent of the exposures to mixtures that fall into Group I (concern for individual chemicals), Group II (low concern for individual chemicals and combined exposures), and Group III (concern for combined exposures but not for exposures to individual chemicals) for human health effects, under different assumptions for addressing chemicals that may be present at levels below their detection limits (nondetects)

	Percent of mixtures		
	All samples	**Surface water samples**	**WWTP effluent samples**
In Group I			
Nondetects = 0	2%	2%	0
Nondetects = Detection limit/$2^{0.5}$	3%	3%	0
In Group II			
Nondetects = 0	98%	98%	100%
Nondetects = Detection limit/$2^{0.5}$	97%	97%	100%
In Group IIIA			
Nondetects = 0	0	0	0
Nondetects = Detection limit/$2^{0.5}$	0	0	0
In Group IIIB			
Nondetects = 0	0	0	0
Nondetects = Detection limit/$2^{0.5}$	0	0	0

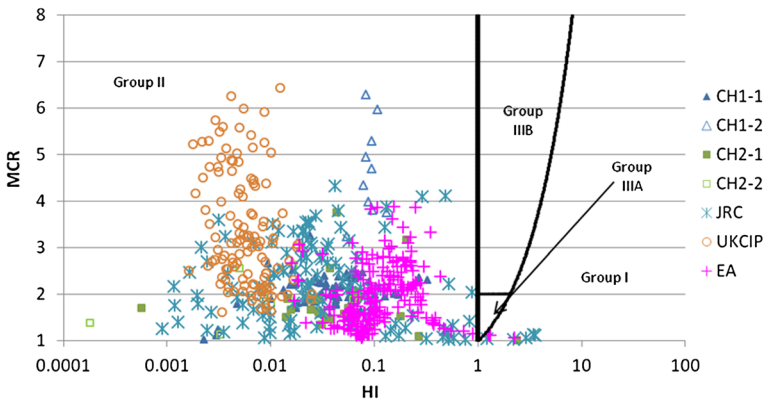

Figure 2 Human health effects: Log linear plot of MCR versus HI for the combined exposures to mixtures in the seven datasets (559 mixtures) with the delineation of the regions where mixture exposures fall into Groups I, II, IIA, and IIB.

MCR = HI, HI = 1, and MCR = 2 (for values of HI between 1 and 2).

As Figure 2 indicates, the results from the exposures to the mixtures in the seven different datasets tend to fall into distinct groups reflecting the differences in the number and nature of the analytes investigated in each survey and the levels of contaminants in the waters being surveyed. The average number of substances detected in the 559 samples was 20. As discussed in Price and Han [8], the theoretical upper bound of MCR for an individual exposure is equal to the number of chemicals that reach a receptor. In this assessment MCR, therefore, values of 20 or higher were possible. The average value of MCR was only 2.4. In addition, 44% of the MCR values were less than two. These values indicate that only a few compounds made significant contributions to the HI values for individuals exposed to the mixtures.

Ecological effects

The results for the assessment of ecological effects are quite different from the human health effects (Table 3). The majority (68%) of the mixture exposures contained one or more substances that were of concern in the Tier 1 ecological assessment (Group I). The percentages falling into Group I were larger for WwTP effluents (78%) than for surface water samples (63%). The substances that exceed their criteria include a range of metals, pharmaceuticals, organic chemicals, herbicides, dietary components, and human hormones. Only 19% of the exposures to the mixtures had an HI that was less than one (Group II). Practically all of the samples in Group II came from surface waters. The percentage of mixtures falling into Group III, was 12%; however, the percentage was much larger for effluents (20%) than for surface waters (8%). The 12 % of mixture exposures in Group III were evenly divided between Groups IIIA (one dominating chemical) and IIIB (no chemical dominates).

The above findings are based on the assumption that the concentrations of NDs were zero. When the concentrations of NDs were set at $LOD/2^{0.5}$, there was a reduction in the number of mixture exposures in Group IIIA and an increase in the number in Group 1 (Table 3), indicating that the contributions from NDs had an impact on some but not all of the findings.

Figure 2 presents the MCR - HI plot for the ecological effects of the mixtures. The different datasets fell into separate clusters reflecting differences in the number and nature of the analytes investigated in each survey as well as differences in the waters surveyed. As the figure indicates, the majority of the samples in Group I come from the EA and the four Swiss datasets, while Group II is dominated by samples from the JRC dataset, and the majority of Group III comes from the UKCIP and JRC datasets. The average number of substances detected in the 559 samples was 20; however, the average value of MCR was 1.8 for both surface water and effluent samples. Almost three quarters of the samples (72%) had MCR values that were less than two. The values of MCR for the ecological effects are smaller than the MCR values for human health (see above). This indicates that while the ecological endpoints for the mixture exposures to the ecological receptors had much higher HI values than human exposures, the HI values were more often dominated by the HQ values from a single compound.

The HI values vary greatly across the four groups. As expected, the HI values are quite large in Group I (mean of 15) and small in Group II (mean 0.4). The HI values in Groups IIIA and IIIB were greater than Group II but much smaller than Group I (means of 1.3 and 1.8 respectively). The largest HI value for either IIIA or IIIB is 3.4. This indicates that while toxicity is a concern for many mixtures in this study, the mixtures with the

Table 3 Percent of the exposures to mixtures that fall into Group I (concern for individual chemicals), Group II (low concern for individual chemicals and effects), and Group III (concern for combined exposures but not for exposures to individual chemicals) for ecological effects, under different assumptions for addressing chemicals that may be present at levels below their detection limits (nondetects)

	Percent of mixtures		
	All samples	Surface water samples	WWTP effluent samples
In Group I			
Nondetects = 0	68%	63%	78%
Nondetects = Detection limit/$2^{0.5}$	73%	65%	86%
In Group II			
Nondetects = 0	19%	29%	3%
Nondetects = Detection limit/$2^{0.5}$	18%	27%	1%
In Group IIIA			
Nondetects = 0	6%	4%	10%
Nondetects = Detection limit/$2^{0.5}$	3%	2%	4%
In Group IIIB			
Nondetects = 0	6%	4%	10%
Nondetects = Detection limit/$2^{0.5}$	7%	5%	9%

greatest concerns had at least one individual chemical with HQ values above 1.0.

Approximately 12% of the mixture exposures were predicted to have toxicities of concern (HI > 1) that would not have been identified unless a combined assessment had been performed (Group III). For the mixture exposures that fall into Group IIIA, the primary drivers were six substances: Diclofenac, Clarithromycin, Tramadol, terbuthylazine desethyl, Terbuthylazine, and PBDEs. This suggests that for these mixtures, risk assessors should focus on refining the exposure and toxicity estimates for these compounds.

The majority of the mixtures that fall into Group IIIB occurred in the UKCIP and JRC datasets. According to the decision tree, Group IIIB mixture exposures should be passed on to a WHO Tier 3 assessment. In these assessments, the chemicals are grouped into categories with MoAs. However, such analyses were beyond the scope of this assessment. The mean MCR values for the IIIB mixtures in the two datasets were 3.0 for UKCIP and 3.3 for JRC. This suggests that the HI values for the IIIB mixtures, i.e. their predicted toxicity, are largely driven by the HQ values, i.e. the single substance toxicity, from the top four substances.

Table 4 presents the substances that had the four highest HQ values for the 12 and 10 Group IIIB mixtures in the UKCIP and JRC datasets respectively. As the table indicates, the chemicals that appear in the top four HQ values are a small fraction of the total number of analytes. Approximately six substances in each of the two surveys make up more than 90% of the substances with the top four HQ values. The critical decisions on groupings should focus on these substances and it will be most important to develop or identify the MoAs for these compounds. The remaining analytes in the mixtures contributed very little to the predicted toxicity of the mixture exposures and they can be assumed to be additive without significantly affecting the estimates of toxicity.

Discussion

As discussed in the companion paper [10], the issue of assessing the risks from combined exposures to chemicals is complex and the decision tree must address a number of issues such as determining the potential for co-exposures to chemicals, making the best use of whole mixture toxicity data, developing screens for very low level exposures, and addressing information on MoA and chemical interaction. It is difficult to explore all of these issues in a single example. In this exercise, we have investigated the value of the decision tree for addressing instances where relatively little data are available on the combined exposures and on the MoA of the individual chemicals and no data were available on the toxicity of the mixtures as a whole. As a result, the key steps in the tree are Step 5 (identifying Group 1 exposures) and Steps 8–16 (identification of mixture exposures that fall into Groups II, IIIA, and IIIB).

The results of this application provide a number of findings on the risks to humans and ecological receptors from the reported mixtures. One of the key findings in the paper is that assessments of human and ecological effects assessments can result in different decisions at various steps in the tree for the same mixture. This occurs because of differences between the availability of RVs, different exposure pathways, and the availability of methodologies that support Tier 0 assessments. In this analysis, the ability to perform a Tier 0 assessment using the TTC for human health assessments allowed the use of this option for the evaluation of substances without human health RV values. In contrast, for the ecological endpoint we had to perform a Tier 1 assessment for the chemical with RVs but could not consider the impact of the 72 remaining chemicals. The data in the assessment of human health suggest that the risks from combined exposures to the measured substances in the samples were low. The values of HI were less than one for 98% of the mixture exposures even when conservative Tier 1

Table 4 Substances that have one of the four top Hazard Quotients in one or more of the Group IIIB mixture exposures in the UKCIP and JRC datasets and the number of mixtures where they are in the top four Hazard Quotients

UKCIP Dataset (WWTP effluents)		JRC Dataset (Surface waters)	
Substance[1]	Number mixtures in Group IIIB (out of 12)	Substance[1]	Number mixtures in Group IIIB (out of 10)
Copper	12	Diclofenac	9
Total PDBEs	12	Terbuthylazine	9
Zinc	11	Terbuthylazine desethyl	9
Propanolol	5	Nonylphenol	6
Iron	3	Bisphenol A	4
Nickel	3	Estrone	3
Fluoxetine	2	Isoproturon	3
		tert-Octylphenol	3

[1]All substances are present at levels below their RV values (HQ less than 1) but make significant contributions to mixtures with HI values greater than 1.

exposure assumptions were made and when an assumption of additivity was applied to all of the components. This finding was not changed when the possible contributions of NDs were considered. The analysis also indicated that the historical chemical-by-chemical approach would have identified every mixture exposure that has an HI greater than one (no mixture exposures fell into Group III). Thus the human health effects of the combined measured substances would have been sufficiently addressed by chemical-by-chemical approaches and had little need for a separate assessment of the combined exposures.

The data on the ecological effects of the mixtures in the samples were quite different from those for human health. The majority of the mixtures, 82%, were shown to have HI values greater than one in a Tier 0 combined assessment. Three quarters of the mixtures with HI values greater than one (or 68% of all mixtures) had individual substances with exposure levels exceeding their RVs (Group I). This indicates that a chemical-by-chemical assessment would have identified most, but not all, mixtures with HI values greater than one. The HI values of these predicted exposures in Group I ranged from 1 to greater than 1000 and had an average value of 15. In contrast the ranges and averages for Groups IIIA and IIIB were much smaller. For Group IIIA the range was 1–1.8 with an average value of 1.3. For Group IIIB the range of values is 1–3.4 with a mean of 1.7. This suggests that a chemical-by-chemical approach would "catch" the mixture exposures with the largest HI values.

In contrast, the mixtures that would be missed in a chemical-by-chemical approach (Groups IIIA and IIIB) had considerably lower HI values (average of 1.7 and range of 1–4).

As indicated in Figure 2, the results were quite different for the different surveys. For example the JRC survey reported a large range of mixture exposures which result in some mixture exposures falling in each of the four groups. In contrast, the EA dataset has almost all of its mixture exposures falling into Group I and the UKCIP dataset largely fell into Group IIIB and Group I. The decision tree also demonstrates that the mixtures in Group IIIB are dominated by a small number of substances (three to four chemicals). These substances should be the focus of efforts to group chemicals based on MoA [1,2]. These findings can give direction to risk assessors on where to focus efforts in risk management.

The application of the tree to ecological effects made the assumption that the effluents were discharged into a body of water that resulted in a 10-fold dilution of the effluent. This assumption is used as a default value in many assessments [13]. However, for some smaller rivers under low-flow conditions the degree of dilution could be smaller than 10. In addition, for rivers receiving multiple discharges the receiving water may already contain one or more of the compounds from discharges that occurred upstream [14]. The impact of this reduced capacity to dilute substances will result in higher values of HQ and HI for ecological receptors immediately below the point of discharge. This will, in turn, reduce the number of mixture exposures that fall into Group II. The affected exposures will move into Group I, IIIA, or IIIB. In addition, some mixture exposures in Groups IIIA and IIIB will move into Group I. In this analysis reducing the assumption of dilution from 10 to 2 caused the percentage of effluents in Group II to drop from 3% to 1%, Group IIIA to drop from 10% to 0% and the percent of effluents in Group IIIB to drop from 10% to 1%. The percentage for effluents in Group I increased from 78% to 98%. This suggests that under such conditions the effluent mixture exposures would almost always have at least one substance that would fail a chemical-by-chemical assessment.

Under the decision tree, the findings of HI and HQ values greater than one in Steps 5 and 11 calls for refining the exposure information used in the assessment. The refinements could include:

- Determine the actual degree of dilution that occurs for an effluent;
- Take additional samples to determine the long-term average concentration in a surface body of water;
- Determine if other mechanisms of removal are occurring (e.g. volatilization, degradation); and
- Determine if the RVs for the chemicals in a mixture apply to a common ecological receptor.

These steps are not possible in this illustration since we did not have the site-specific information or the ability to collect additional data. As a result, the illustration has focused on organizing of the exposures into the four Groups and identifying which chemicals are the drivers of mixture toxicity for Group IIIB.

The data used in this exercise are mixtures of chemicals measured in a range of surface waters and WwTP effluents in a number of European countries. The mixtures are composed of a large number of diverse substances that include certain traditional water contaminants (metals) and micropollutants such as pharmaceuticals, herbicides, and substances in the human diet (caffeine and Sucralose). As discussed in the methods section below, the samples from six of the seven datasets (excluding JRC) were selected because they had large numbers of detected substances. Within the surveys, mixtures with larger numbers of detected substances were favoured since they provide a greater challenge for the decision tree. As discussed below, the goal of having samples with largest possible number of detected substances is likely to have biased the data towards samples that represent more stressed waters. As a result, the results reported here are not necessarily representative of the range of mixtures in that occur in the Swiss and UK surface waters or WwTP effluents. In addition, the findings in this paper are based, necessarily, on the chemicals that were measured in the five surveys. None of the surveys attempted to exhaustively identify all naturally occurring or synthetic chemicals present in each of the samples. In an actual assessment of combined exposures, efforts need to be made to determine that all of the relevant chemical exposures are included.

The application of the tree was performed using existing data. In certain cases these data were sufficient to allow the application of the tree. Human health RVs were identified or conservatively estimated for all substances except seven substances of little concern and five polycyclic aromatic hydrocarbons. In contrast, ecological risk assessment does not have a widely accepted equivalent of the TTC and for two datasets, CH 1–2 and CH2-1, RVs were missing for 30% and 40% the detected substances. Because there was no method for evaluating these substances, they were not included in the analysis. The absence of the values, therefore, will result in underestimates of the measure of total toxicity (HI) and the fraction of mixture exposures with MHQ values greater than 1, and could affect the value of MCR. As a result the findings for these datasets should be viewed with less confidence than the findings for other data sets. However, the percentage of exposures that fall in Group 1 will not be underestimated. In cases where many RVs are missing, using whole mixture toxicity tests might be a reasonable approach to reduce uncertainty [10].

In this exercise, the decision tree developed by the MIAT was shown to offer a useful way of taking available information on mixtures and applying it in a systematic way to the assessment of combined exposures to chemicals. The tree uses a tiered approach that seeks to screen out combined exposures of low concern and exposures known to be a concern based on the effects of individual chemicals. This allows risk managers to focus on mixtures where assessments of combined exposures are most necessary. The approach also directs future efforts to refine the assessments by identifying which chemicals make significant contributions to combined exposures of a receptor.

All combined exposures should be evaluated for a decision in risk assessment. In practice, however, there is a need for prioritization and screening tools. The analyses in this paper demonstrate that the need for assessments of combined exposures differs for the human health and ecological receptors and differs by survey. The next steps will be to use the findings from this application and other applications of the decision tree to develop predictions of when and where assessments of risks from combined exposures are most needed. Specifically, where are Group IIIB mixture exposures most likely to occur and what are the chemicals that drive such exposures?

Conclusions

The decision tree is shown to be a useful tool to evaluate combined exposures to chemicals and their impacts on human health and the environment. In this illustration, the tree was able to demonstrate that the value in performing assessment of the combined exposures varies by endpoints (human health and ecological effects) and varies from survey to survey. The tree has the ability to identify which endpoints and which sources have combined exposures that pose concerns that would be missed by a chemical-by-chemical approach. In addition, the tree can identify specific substances where MoA data would be most useful in refining the assessment. The analysis also supports the earlier findings that only a few substances contribute to the toxicity of mixtures in surface and groundwater and in many instances a single substance is responsible for the majority of the toxicity of a mixture [7,8].

Methods

In this project, the decision tree as provided by the Cefic MIAT was applied to ecological receptor and human exposures to mixtures of chemicals observed in either surface waters or effluents discharged by wastewater treatment facilities in Europe. This section presents how each step of the decision tree was performed in the

application. Because the example reported here is an assessment of exposures to discrete mixtures, the exposures by definition co-occur to the same individual humans and ecological receptors (Step 1). To the best of our knowledge, the mixtures investigated in this case study have not been tested for either human health effects or ecological effects or such tests have not been made publically available. It is not possible, therefore, to use the whole-mixture option (Step 2). The compositions of the mixtures, however, are known allowing the use of mixture toxicity models to estimate toxicities (Step 3). In this example, we have assumed that exposures to the mixtures do not qualify for an exclusion based on the TTC (Step 4). Therefore, all of the estimates of mixture exposures reach Step 5.

In Step 5, chemicals with exposure levels exceeding their RVs are identified using the exposure assumptions described below. In an actual risk assessment, these estimates of exposure and the RVs would be subject to refined chemical-specific risk assessments. However, such efforts require detailed site-specific information on where the samples were collected (e.g., the degree of dilution, environmental fate, the presences or absence of environmental receptors, and detailed toxicity data). Such information was not available for the monitoring data and performing such assessments is beyond the scope of this paper. Therefore, in this paper the exposures to the mixtures that do not pass Step 5 are not analyzed further.

In this assessment, we have assumed that none of the pairs of components of the mixtures have non-additive interactions (Step 6). In addition, we have not researched the MoAs on all of the chemicals and in many instances the MoA for human and ecological effects are not well defined. As a result we cannot conclude that all chemicals will follow an independence model (Step 7). Therefore an additive model was used as the default assumption to assess human health and ecological effects (Step 8).

In Step 9 a determination is made on whether RVs are available for each chemical in each mixture. In the case of human health assessments, Cramer classes provide an alternative source of conservative estimates of oral toxicity [2,9]. Thus, the assessment of mixture exposures to humans is an example of a Tier 0 assessment (Steps 10 and 11) and the assessment of ecological effects is a Tier 1 assessment (Step 13). Mixture exposures that are found not to pose a concern are assigned to Group II.

In Step 14, the MCR value is determined for the remaining mixture exposures. The primary component (the chemical that makes the largest contribution to the total risk of the individual) is determined for mixture exposures that result in MCR values of less than two (Group IIIA). The estimate of the toxicity and exposure for this chemical can be refined as additional data allow. In this study, we do identify the substances that were primary drivers for one or more mixture exposures.

Mixture exposures with MCR values greater than two (Group IIIB) should be grouped based on MoA data. In this assessment, we have not attempted to identify data on the MoA of the various substances; however, the substances that are the top contributors to HI values for these mixture exposures are identified. These are the substances where MoA data would be most valuable for defining chemical groupings [1,5].

Finally, while the decision tree only calls for the calculation of the MCR on the exposures to mixtures that fall into Group III, in this manuscript we have calculated the values for all of the mixture exposures. The determination of MCR values for all of the mixture exposures allows all of the mixtures to be plotted using a HI-MCR plot (Figure 3). This plot provides a

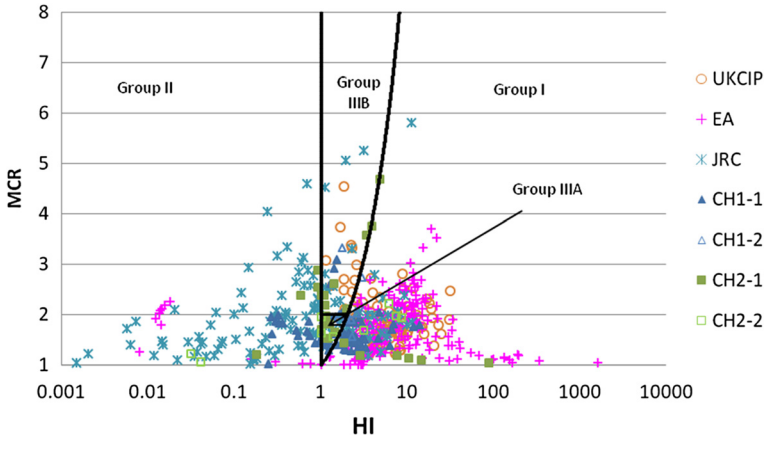

Figure 3 Ecotoxicological endpoints: Log linear plot of MCR versus HI for the combined exposures to mixtures in the seven datasets (559 mixtures) with the delineation of the regions where mixture exposures fall into Groups I, II, IIA, and IIB.

useful graphical description of how MCR values vary across the four groups and across the results for the different studies.

Risk characterisation

The potential for the occurrence of adverse effects in humans are evaluated using the Hazard Index/Hazard Quotient (HI/HQ) approach. The HQ for human health effects is defined as an individual's dose of a chemical divided by the RV.

$$HQ = \frac{Dose}{RV} \quad (1)$$

The potential for exposure to humans used in this assessment is based on the WHO guidance for Tier 1 assessments of mixtures [1,2]. This guidance calls for the use of conservative screening assumptions. Therefore, we have made the conservative assumption that the sampled surface waters would be used directly as a water supply and that individuals would be exposed to the chemicals in mixtures from the consumption of drinking water. Human exposures to the mixtures reported to occur in the effluents was based on the assumption that a 10-fold dilution of the effluent would occur in the surface water receiving the effluent before the receiving water would be used as a drinking water supply [13]. No allowance was made for losses in the receiving waters due to biodegradation or other mechanisms or removal during the treatment of surface waters in drinking water treatment plants. The concentration of the chemicals in the surface water before receiving the effluent was assumed to be zero. Oral doses to humans were determined based on the assumption that an adult with a body weight of 60 kg would consume two litres of water a day.

HQs of the components are summed to provide a measure of combined exposure, the HI.

$$HI = \Sigma HQ \quad (2)$$

The values of HI and HQ are used to determine the MCR value for the exposures to the mixture using the following equation.

$$MCR = \frac{HI}{MHQ} \quad (3)$$

where MHQ is the maximum of the HQ values calculated for an individual's exposures to multiple chemicals.

A similar approach was used for ecological effects. HQ is defined as the ratio of the concentration of a chemical in a surface water sample to the chemical's RV. In the case of effluents, the receptor is assumed to be present in surface water where the effluent concentrations of chemicals are diluted by a factor of 10 [13].

$$HQ = \frac{Concentration}{RV} \quad (4)$$

Non-detects (NDs)

In the assessment of mixtures, a common issue is how to address the impact of substances which may be present at levels below the LOD of the analytical techniques used in the survey (non-detects or NDs). Risk assessors should not assume an absence of the chemicals in the samples for NDs [15]. However, assuming the presence of every substance that is not detected could result in overestimating the toxicity of a mixture. This issue is especially important when there are a large number of NDs that could drive the estimates of the toxicity of the mixture and the MCR values. In order to investigate the impact of NDs on HI and MCR values, the data were analyzed using two assumptions: Case 1 where concentrations of NDs were set as 0 and Case 2 where the concentrations are assumed to be equal to the LOD divided by $2^{0.5}$ [15].

Materials

Monitoring data on environmental mixtures

In this paper, data were collected on mixtures of substances measured in samples of surface water and WwTP effluents. The goal of this effort was to obtain a range of "real world" mixtures that would provide a robust application of the value of the decision tree. Specifically this project sought out samples where large numbers of substances had been detected. Access to the raw data was obtained from a JRC publication [16] and regulatory agencies and water companies in Switzerland and the United Kingdom.

Data from two surveys, CH 1 and CH 2, were obtained from the Environmental Chemistry Department of Eawag, Dübendorf, Switzerland.[a] The samples were taken from either rivers or WwTP effluents. Both surveys were designed to follow the fate and transport of micropollutants in urban sewage through treatment in WwTPs and resulting levels in rivers receiving the effluents [14]. Results from each of the two surveys were apportioned into four datasets (CH 1–1, CH 1–2, CH 2–1, and CH 2–2) based on differences in the number of substances analysed in a sample. For these data quantification limits (LOQ) were reported, which are 3.3 times the detection limit. These values were used instead of LODs in the study of the investigation of the impacts of NDs.

The third survey was performed by United Kingdom Water Industry Research (UKWIR) in collaboration with the Environment Agency of England and Wales as part of the UK water industry's Chemical Investigations

Programme (CIP). The objective of this programme is to improve the management and control of concentrations of Water Framework Directive (WFD) Priority Substances and other substances of potential concern that may be entering the environment via WwTP effluents. The dataset used in this project (UKCIP) is a small subset of the effluent monitoring data collected as part of the screening of effluents. The screening data was collected from approximately 170 WwTPs in England and Wales to determine the concentrations of existing and proposed Water Framework Directive priority substances, UK specific pollutants, and a small number of 'emerging' substances (e.g. pharmaceuticals). The dataset consists of the concentrations of substances measured in 120 discrete WwTP samples collected and analysed in 2010 and 2011.

The fourth survey was performed by the General Quality Assessment monitoring scheme of U.K. Environment Agency of England and Wales. The program began in 1988 to monitor the water quality of rivers and canals throughout England and Wales and includes more than 7,000 sampling sites. The dataset (EA) used in this project is derived from data collected between 2006 and 2010. These data, which included more than 300,000 samples, were processed to identify samples with the highest number of individual substances measured. The final dataset consists of the top 200 samples in terms of numbers of measured substances. Many of these samples were taken from locations immediately downstream from WwTP effluent discharges or in the vicinity of known sources of water pollution.

The fifth survey was an EU-wide study of the occurrence of polar organic persistent pollutants in European river waters [16]. Samples were taken from over 100 European rivers, streams or similar water bodies from 27 European Countries and were analysed for 35 polar organics. Samples were taken from both pristine waters and waters affect by WwTP discharges [16].

In total, seven datasets based on these five surveys were analyzed in this work. These data consist of samples of either surface water or effluents containing detectable levels of large numbers of diverse substances.

Implications of the seven datasets

The mixtures in this study were selected only for the purpose of demonstrating the application of the decision tree to diverse datasets containing mixtures of substances. It is important to emphasise that none of the seven datasets are representative of effluents or surface waters of the EU or individual countries. The JRC dataset was intended to represent a range of water types but was not the result of a statistically representative sampling plan [16]. The samples in the remaining six data sets were selected with the goal of maximizing the number of detected substances. All of the data came from surveys that focused on specific micropollutants and the analyses varied both by substance type (polar vs. non-polar, organic versus inorganic) and the number of analytes. As a result, the mixtures reported do not reflect all of the natural or anthropogenic substances present in the sampled bodies of water or effluents. Finally, the datasets from the first four surveys (CH-1, CH-2, UKCIP and EA) are the property of the identified organizations. Individuals wishing further information on the data should contact the organizations identified above.

Human health and ecotoxicity RVs

The application of the decision tree requires data on the RVs. A literature and internet based search was performed to identify current standards, criteria, or guidance values that could serve as the basis for the RVs. The mixtures in this project came from analyses of either grab samples or in a few instances flow proportional 24-hour composite samples; therefore, the most appropriate standards that could be used are acute toxicity standards. However, acute oral values for humans were not available for most of the substances. Therefore, chronic human health values were used in this assessment as conservative estimates of acute toxicity RVs.

When human health criteria were not available, the Cramer class portion of the TTC was used to conservatively estimate the RVs of the compounds. This was done by assigning each compound to one of the three Cramer classes based on the compound's structures, assuming an uncertainty factor of 100, and using the point of departures established for each of the three Cramer classes [9].

The ecotoxicity criteria were based on standards or criteria for intermittent exposures (MAC-EQS or PNEC$_{intermittent}$ - based on acute ecotoxicity data) when they were available; otherwise, criteria for chronic exposure (AA-EQS, PNEC – based on chronic ecotoxicity data) are used. All ecotoxicity RVs reflect the lowest reported effect (either acute or chronic) for any of the tested receptors (e.g. fish, daphnids, and algae) available in various substances. As a result, the critical receptor on which an RV is set varies from substance to substance.

List of definitions

Additive models: Chemicals have the same effect on the target organism and differ only in potency; hence the combined effect of two agents can be estimated from the sum of potency weighted doses of both agents.

Analyte: A chemicals measured in a sample.

Combined exposures: The measurement of the doses that reach an individual or an ecological receptor at the

same time or over a period of time sufficiently short that the effects of one chemical add to the effect of another.

Effluents: Discharges to surface water from WWtP following treatment.

Independence joint action: If two or more substances elicit the same endpoint via different modes of action the combined effect can be estimated by the Bliss independence model [17].

Intermittent exposure: An exposure is intermittent in cases where a chemical is released to the environment in pulses separated by periods of little or no exposure.

Micropollutants: Anthropogenic chemicals found at low levels (generally ppb or less) in waste water effluents or surface waters.

Mixture: A combination of chemicals present in an object (e.g., a consumer product) or an environmental medium.

Mixture exposure: The combination of doses received by a receptor from exposure to a mixture.

Primary chemical: The chemical with the largest HQ value for an individual.

Receptor: For human health – a population of individuals receiving the combined exposure. For ecological assessments - an organism or taxonomic group.

Reference Value: In human health risk assessment, reference values are doses that are not anticipated to cause adverse effects in humans and are developed based on a point of departure divided by uncertainty" or "adjustment" factors. Examples of these values are Allowable Daily Intakes, Tolerable Daily Intakes, or Derived No Adverse Effect Levels. In ecological risk assessment, reference values are concentrations in environmental compartments (soil, sediments, or water) that are not anticipated to cause adverse effects for receptors in the environmental compartments. Examples of RVs are Environmental Quality Standards or Predicted No Effect Concentrations.

Tier 0 Assessment: Defined in WHO approach to mixtures as an initial approach to assessing toxicity (or exposure) where data gaps are filled using conservative assumptions [2].

Tier 1 Assessment: Defined in WHO approach to mixtures as an initial approach to assessing toxicity (or exposure) where data on toxicity and exposure data are available for each component of a mixture [2].

Tier 2 Assessment: Defined in WHO approach to mixtures as a refined assessment that only assumes additivity with in groups of chemicals where a common mode of action occurs. In the case of ecological risk assessment this would also include grouping the effects of the chemicals based on specific receptors [2].

Tier 3 Assessment: Defined in WHO approach to mixtures as an advanced assessment of combined risks that uses techniques such as biologically based dose response and probabilistic models of variation in dose and susceptibility [2].

Endnotes

[a]UCHEM: Unpublished monitoring data. 2010. Please contact Juliane.hollender@eawag.ch for further information on these data.

Additional file

Additional file 1: Appendix A. Human Health and Ecological Reference Values Used in This Study.

Abbreviations

AA-EQS: Annual Average – Environmental Quality Standards; Cefic: Conseil Européen des Fédérations de l'Industrie Chimique (European Chemical Industry Council); JRC: Joint Research Centre; HI: Hazard Index; HQ: Hazard Quotient; LOD: Level of Detection; MAC-EQS: Maximum Allowable Concentrations – Environmental Quality Standards; MCR: Maximum Cumulative Ratio; MHQ: Maximum Hazard Quotient; MIAT: Mixtures Industry Ad Hoc (Team); MoA: Mode of Action; ND: Non-detect; $PNEC_{intermittent}$: Predicted No Adverse Effect Level; RV: Reference Value; SCs: Scientific Committee on Consumer Safety, Scientific Committee on Health and Environmental Risks, and Scientific Committee on Emerging and Newly Identified Health Risks; TTC: Threshold of Toxicological Concern; UKCIP: United Kingdom Water Industry Research Chemical Investigations Programme; WHO: World Health Organization; WwTP: Waste Water Treatment Plants.

Competing interests

Paul Price and Xianglu Han are employees of The Dow Chemical Company. The Dow Chemical Company produces many chemicals including some of the compounds included in this study. However, since this study is not about any specific chemical the authors declare that they have no competing interests. The remaining authors declare that they have no competing interests.

Authors' contributions

The authors of this paper were members of a team that performed the project. CW and DL identified and obtained access to the data on effluents and surface waters in the UK and identified and organized the data on RV values for human health and ecological effects for metals. MJ and PK identified and gained access to the CH data and identified and organized the RV values on ecological effects for non-metals. XH performed the majority of the data analyses. PP was the project director, performed data analysis, and was the primary writer for the manuscript. All of the authors made significant contributions to the drafting and editing of the manuscript. All authors read and approved the final manuscript.

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