



# A multi-scenario risk assessment strategy applied to mixtures of chemicals of emerging concern in the River Aconcagua basin in Central Chile

Pedro A. Inostroza<sup>a,b,\*</sup>, Sebastian Elgueta<sup>c,d</sup>, Martin Krauss<sup>e</sup>, Werner Brack<sup>e,f</sup>, Thomas Backhaus<sup>a,b</sup>

<sup>a</sup> Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

<sup>b</sup> Institute for Environmental Research, RWTH Aachen University, Aachen, Germany

<sup>c</sup> Núcleo en Ciencias Ambientales y Alimentarias (NCAA), Universidad de las Américas, Santiago, Chile

<sup>d</sup> Facultad de Medicina Veterinaria y Agronomía, Universidad de las Américas, Sede Providencia, Chile

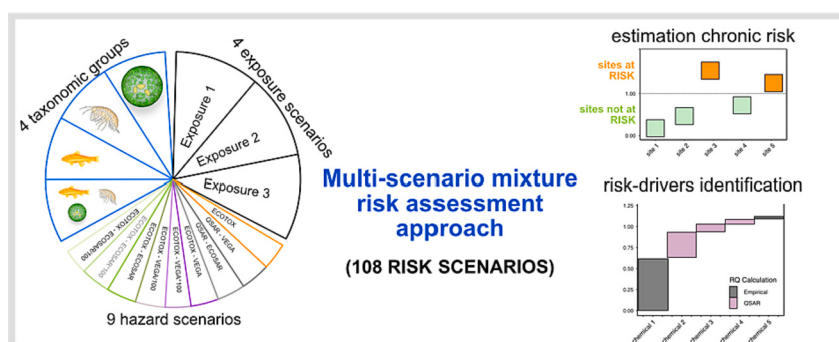
<sup>e</sup> Department of Effect-Directed Analysis, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

<sup>f</sup> Department of Evolutionary Ecology and Environmental Toxicology, Goethe University Frankfurt/Main, Frankfurt/Main, Germany

## HIGHLIGHTS

- 153 chemicals of emerging concern detected in complex multi-component mixtures.
- 108 possible mixture risk assessment scenarios were investigated.
- Non-detects, QSARs, and experimental ecotoxicological data were integrated for risk assessment.
- 8 chemicals were the main risk drivers in at least one site across the River Aconcagua basin.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Yolanda Picó

**Keywords:**  
Mixture risk assessment  
Risk drivers  
QSARs  
Micropollutants

## ABSTRACT

Environmental risk assessments strategies that account for the complexity of exposures are needed in order to evaluate the toxic pressure of emerging chemicals, which also provide suggestions for risk mitigation and management, if necessary. Currently, most studies on the co-occurrence and environmental impacts of chemicals of emerging concern (CECs) are conducted in countries of the Global North, leaving massive knowledge gaps in countries of the Global South.

In this study, we implement a multi-scenario risk assessment strategy to improve the assessment of both the exposure and hazard components in the chemical risk assessment process. Our strategy incorporates a systematic consideration and weighting of CECs that were not detected, as well as an evaluation of the uncertainties associated with Quantitative Structure-Activity Relationships (QSARs) predictions for chronic ecotoxicity. Furthermore, we present a novel approach to identifying mixture risk drivers. To expand our knowledge beyond well-studied aquatic ecosystems, we applied this multi-scenario strategy to the River Aconcagua basin of Central Chile. The analysis revealed that the concentrations of CECs exceeded acceptable risk thresholds for selected

\* Corresponding author at: Institute for Environmental Research, RWTH Aachen University, Aachen, Germany.

E-mail address: [pedro.inostroza@rwth-aachen.de](mailto:pedro.inostroza@rwth-aachen.de) (P.A. Inostroza).

organism groups and the most vulnerable taxonomic groups. Streams flowing through agricultural areas and sites near the river mouth exhibited the highest risks. Notably, the eight risk drivers among the 153 co-occurring chemicals accounted for 66–92 % of the observed risks in the river basin. Six of them are pesticides and pharmaceuticals, chemical classes known for their high biological activity in specific target organisms.

## 1. Introduction

Anthropogenic chemical pollution has profound impacts on the ecological status of surface waters at a continental scale (Malaj et al., 2014), and is therefore increasingly recognised as a driving force behind biodiversity loss (Balvanera et al., 2019; Groh et al., 2022; Sigmund et al., 2023). However, especially diffuse pollution is characterised by the presence of complex multi-component mixtures (Finckh et al., 2022; Kandie et al., 2020; Marshall and McCluney, 2021). These mixtures comprise a diverse array of organic chemicals, including pharmaceuticals and personal care products (PPCPs), pesticides, surfactants, industrial chemicals, and transformation products, collectively referred to as chemicals of emerging concern (CECs) (Ankley et al., 2008). CECs are generally acknowledged for their potential effects on aquatic life ranging from microbes (Drury et al., 2013) to higher vertebrates (Jobling et al., 1998; Schüttler et al., 2021), and exerting influences on genes and the genetic landscape of exposed organisms (Inostroza et al., 2018), even at low environmental concentrations. It's important to note that not all CECs have been identified as particularly hazardous. One of the distinguishing features of CECs is their detection in the environment, and due to data limitations, comprehensive hazard information may not always be available.

CECs enter streams and rivers through various pathways, including direct discharges from wastewater treatment plants (WWTPs) (Hug et al., 2014), emissions from industrial facilities (Kaewlaoyong et al., 2018), unintentional runoff from agricultural areas, and accidental spills (Reiber et al., 2021). Despite the degradability of many CECs, their continuous release into the aquatic environment results in a phenomenon known as “pseudo-persistence” (Boxall et al., 2004; Kolpin et al., 2002), and ultimately in chronic exposures and ecological effects. Conventional WWTPs exhibit limited effectiveness in removing some CECs (Eggen et al., 2014), since they are not designed to remove CECs (Rizzo et al., 2019), and even advanced technologies, such as phosphorus elimination, nitrification, and denitrification, are ineffective in CEC removal (Neale et al., 2017). Despite numerous studies focusing on CEC concentrations in surface waters, particularly regarding pharmaceuticals (Wilkinson et al., 2022) and pesticides (Chow et al., 2020), significant knowledge gaps persist regarding the co-occurrence and environmental risks associated with CECs, especially in developing countries. These countries often experience the highest CEC concentrations due to inadequate WWTP technologies (Wilkinson et al., 2022) and/or outdated environmental protection frameworks.

Chemicals that are included in the monitoring suite, but that are not detected occur at a concentration somewhere between zero and the chemical-analytical limit of detection. The incorporation of non-detects is often considered into risk assessment and several studies have examined their potential contribution to the mixture risk (Gustavsson et al., 2017a, 2017b; Han and Price, 2011; Price et al., 2012; Rodríguez-Gil et al., 2018). Even if all chemicals are present at concentrations below their individual “safe” levels, there may still be an unacceptable risk posed by the mixture (Rudén et al., 2019). The environmental risk associated with these mixtures can be modelled using the concentration addition model (CA), which is widely recommended as an initial precautionary approach for any mixture assessment (Backhaus and Faust, 2012; Kortenkamp et al., 2009; Rudén et al., 2019). It assumes that the combined effect of multiple substances with similar modes of action can be predicted by summing their individual effects based on their concentrations (Kortenkamp et al., 2009). CA can be applied to chemical monitoring data and allows exploring different exposure and/or hazard

scenarios, in which the reliability and validity of the risk estimates can be systematically explored, for instance, the role of non-detects and the use of Quantitative Structure-Activity Relationships (QSARs) to estimate hazard concentrations. This also permits the identification of the risk-driving chemicals from the considerable number of chemicals that are often found to co-occur.

The CA model requires the ecotoxicological characterisation of every mixture component. However, such data are often lacking for CECs, including pharmaceuticals (Spilsbury et al., 2023) and pesticides (Li et al., 2023). Those gaps are therefore often bridged by *in silico* methods such as QSARs. However, the resulting uncertainties are often not comprehensively evaluated and integrated into the risk assessment. It is important to note that QSARs for mixtures of chemicals with distinct modes of action are inherently less robust compared to predictions for a single mechanism of action (Escher and Hermens, 2002). Therefore, describing these uncertainties will identify important data gaps and their impact on the final risk estimate.

The monitoring of CECs and the assessment of their associated risks have been increasingly conducted in developed countries within Europe and North America. Nevertheless, there is a substantial lack of data regarding South America. In recent decades, Chile witnessed a notable increase in agricultural production, resulting in a corresponding increase in pesticide usage (Coria and Elgueta, 2022). The Central Valley, where the River Aconcagua basin is located, is characterised by agricultural activities, and several studies have investigated the presence of pesticides and their transformation products in surface waters within this region (Climent et al., 2019; Giordano et al., 2011; Inostroza et al., 2023a; Montory et al., 2017). An additional source of organic micro-pollutants are WWTPs (Neale et al., 2017). Although wastewater treatment facilities are widely distributed throughout the country, with a high coverage rate of 96.6 % among the urban population (OECD/ECLAC, 2016), it is noteworthy that only two-thirds of urban households are connected to advanced wastewater treatment plants (secondary or tertiary treatment). Additionally, wastewater treatment coverage remains limited in rural areas (OECD/ECLAC, 2016).

Monitoring studies that focus on assessing the environmental risks of organic chemicals in Chile's aquatic environment, particularly in streams and rivers, are scarce. In comparison, coastal areas have received slightly more attention, with some studies quantifying the presence of antibiotics (Buschmann et al., 2012), endocrine-disrupting chemicals (Bertin et al., 2011), and industrial chemicals (Salamanca et al., 2019). This may reflect the broader situation in countries of the Global South, including Chile, where outdated monitoring programs and inadequate water management frameworks persist (OECD/ECLAC, 2016).

This study, therefore, implements a multi-scenario mixture risk assessment for the River Aconcagua basin, located in Central Chile. It incorporates various exposure scenarios to account for non-detects and CECs with missing empirical ecotoxicological data. We propose a novel strategy for identifying and prioritising mixture risk drivers within complex environmental mixtures.

## 2. Material and methods

### 2.1. Case study area - River Aconcagua Basin

The River Aconcagua (143 km long) is located in Central Chile and its basin drains an area of 7338 km<sup>2</sup>. The basin is characterised by a Mediterranean climate with warm, dry summers (October to March) and

wet, cool winters (May to August) marked by intense and irregular rain (Amigo and Ramírez, 1998). There are half a million residents in this river basin, and it supports 12 % of Chile's national agriculture and 4 % of its copper production, respectively (COCHILCO, 2020). Roughly 7.6 % of the total river basin is devoted to agriculture, with >90 % of the cropland (avocado and grapes) concentrated in the Lower and Putaendo sub-basins (Webb et al., 2021). Moreover, ten middle-sized WWTPs, featuring aeration ponds and activated sludge technologies, are located across the basin, serving about 405,000 residents. However, only five of them discharge directly into the main course of the River Aconcagua and the others discharge into its tributaries (Inostroza et al., 2023a).

Surface water samples were collected once from nine sampling sites in October 2018 during the dry season. Sampling sites were selected based on land use types (e.g., streams and/or rivers running through natural parks, agricultural areas, urban, and mixed land uses). Reference sites (RS1, RS2, and RS3) were in the upper section of the river basin except for RS3 (Fig. 1). Tributary sites (T1, T2, and T3) were spread in the central part of the river basin and finally we selected three sites (R1, R2, and R3) in the main course of the River Aconcagua. In total, 153 CECs, including pesticides, pharmaceuticals and personal care products (PPCPs), surfactants, and industrial chemicals were analysed. Detailed analytical methodologies, including sample collection, storage, extraction methods, and LC- and GC-HRMS instrumentation along with the environmental measured concentrations of CECs in the River Aconcagua Basin are accessible in Inostroza et al. (2023a) and through the open-access zenodo repository (Inostroza et al., 2023b). Sample locations and sampling sites information are reported in Fig. 1 and Table S1.

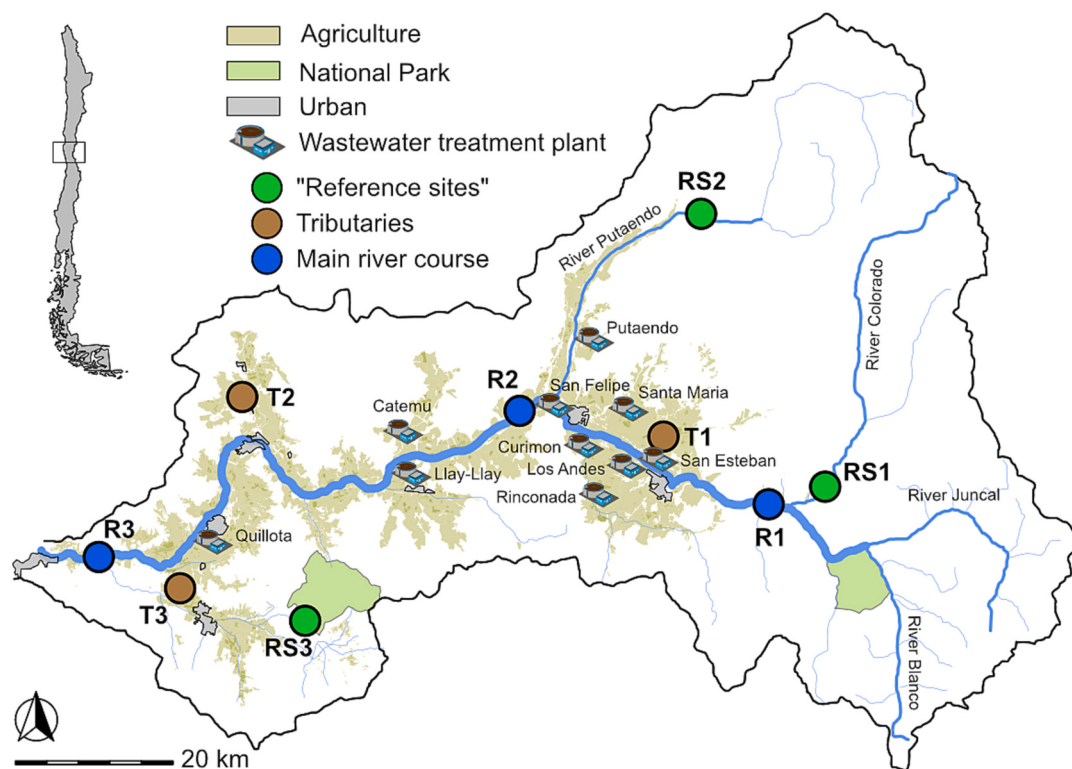
## 2.2. Retrieval and curation of empirical ecotoxicological data

Chronic experimental data were obtained from the US EPA ECOTOX Knowledgebase (ECOTOX) version "ecotox\_ascii\_09\_15\_2022" (Olker et al., 2022) for all the targeted chemicals.

Only data for freshwater organisms and only for chronic exposures were retained. The data were curated by excluding records that lacked values for exposure durations, measurement endpoints, appropriate units, or a reference for the data source, as well as limit values. To ensure uniformity, effect concentrations were normalised to  $\mu\text{mol/L}$ . Chronic effect data were identified in accordance with Australian and New Zealand guidelines (Warne et al., 2018), with exposure durations of at least 1, 14, and 21 days for algae, macroinvertebrates, and fish, respectively and endpoint ecologically relevant, including lethality, immobilisation, growth, development, population growth, and reproduction. All remaining data were recalculated to chronic EC10-equivalents, using the extrapolation factors from (Warne et al., 2018). Lethal Concentration (LC), Inhibitory Concentration (IC), Effect Concentration causing 50 % effect (EC50) values were divided by 5; Lowest Observed Effect Concentration (LOECs) were divided by 2.5; and Maximum Acceptable Toxicant Concentration (MATC) values were divided by 2. An in-house dataset was used to assign a taxonomic group (i.e., algae, macroinvertebrates, and fish) to each species group based on the reported phyla in ECOTOX and all other data were discarded. For each taxonomic group, the geometric mean was calculated for each chemical. The geometric mean was chosen over the arithmetic mean as it is considered more resistant to the impact of outliers and more suitable for skewed datasets (Leith et al., 2010).

## 2.3. Quantitative Structure Activity Relationships (QSARs)

Limiting the assessment to those chemicals for which chronic experimental data are available results in an underestimation of the mixture risk. Various academic researchers as well as regulatory authorities such as the European Chemicals Agency (ECHA) and the US EPA encourage the use of quantitative structure-activity relationships (QSARs) in order to estimate *in silico* ecotoxicological properties. QSARs were employed to predict the chronic toxicity for algae,



**Fig. 1.** Location of sampling sites through the River Aconcagua Basin. Sites featuring "low" urban/agriculture pressures ("Reference sites") in green, sites running through agricultural areas in brown, and sites from the main course of the river in light blue. Selected land use (agriculture, urban areas, and national parks) and location of wastewater treatment plants are showed in the figure.

macroinvertebrates and fish, for all chemicals detected at least once. Two QSAR platforms, the VEGA HUB (version 1.1.5 48, (Benfenati et al., 2013)) and the Ecological Structure Activity Relationships (ECOSAR) Class Program (version 2.2) were utilised for this purpose. QSAR predictions were retrieved from an online QSAR dataset and quality assurance/quality control (QA/QC) and filtering criteria are described in Svedberg et al. (2023). Chemicals were identified via their CAS numbers, and the corresponding SMILES (Simplified molecular-input line-entry system) were retrieved from PubChem and/or Webchem and then used as a chemical identifier for the QSAR calculations. If multiple predictions were provided by the software, its geometric mean was used for the mixture risk assessment. The predicted toxicities were transformed to  $\mu\text{mol/L}$  and recalculated to chronic EC10-equivalents, using the extrapolation factors from (Warne et al., 2018).

#### 2.4. Mixture risk assessment

A mixture risk assessment can be either performed separately for each of the three taxonomic groups (algae, macroinvertebrates, fish) or by accounting for the most sensitive taxonomic group (MST) for each chemical. The MST approach is conceptually similar to first calculating a Predicted No Effect Concentration (PNEC, European Chemicals Agency, 2016) or Environmental Quality Standard ( $QS_{fw,eco}$  European Commission, 2018) without applying any assessment factor and then applying Concentration Addition to these values (Gustavsson et al., 2017a).

The environmental mixture assessment was conducted using CA, for details see (Gustavsson et al., 2017a; Rudén et al., 2019; Spilsbury et al., 2020). The mixture risk for a particular taxonomic group (algae, macroinvertebrates, fish), expressed as its risk quotient ( $RQ_{STU}$ ), is defined as follows:

$$RQ_{STU} = \sum_{i=1}^n \frac{MEC_{mixture}}{EC10_{mixture}} = \sum_{i=1}^n RQ_i = \sum_{i=1}^n \frac{MEC_i}{EC10_i} \quad (1)$$

where  $MEC_i$  is the measured environmental concentration of chemical  $i$  and  $EC10_i$  denotes the corresponding geometric mean of chronic effect concentrations (EC10-equivalent) of chemical  $i$  for a particular taxonomic group (algae, macroinvertebrates or fish). The ratio  $MEC_i/EC10_i$  provides a dimensionless measure of the toxicity contribution of chemical  $i$ . This approach estimates the mixture risk quotient separately for each taxonomic group.

The ecological risk posed by the mixture of CECs was evaluated on a more integrating ecological level through the application of the concept of the most sensitive taxonomic group (MST) (Backhaus and Faust, 2012; Gustavsson et al., 2017a), in which the mixture risk quotient is defined as:

$$RQ_{MST} = \sum_{i=1}^n \frac{MEC_i}{\min(EC10_{Algae}, EC10_{Macroinvertebrates}, EC10_{Fish})} \quad (2)$$

This method corresponds to the summation of fractions of Predicted No Effect Concentrations (PNECs) without using any assessment factors (Backhaus and Faust, 2012; Gustavsson et al., 2017a). Overall, there is no procedural agreement on how to integrate uncertainties in the risk assessment for mixtures without the use of an assessment factor. Thus, the exclusion of an assessment factor in this step provides non-overestimated risk values. In line with the strategy outlined for the environmental risk assessment of industrial chemicals under REACH, we applied the MST approach by combining the data from three taxonomic groups (European Commission, 2011).

For the assessment of risks for each individual taxonomic group as well as the MST, we employed a final assessment factor of 10, again in line with the European guidelines for industrial chemicals, in order to account for the extrapolation from the laboratory to the field situation and to account for the lack of biodiversity considerations in the assessment (European Commission, 2011).

For all these calculations, ecotoxicity data for all detected chemicals

are required. Empirical data gaps were bridged by QSARs. However, as QSAR-estimates had a comparatively low accuracy, we included specific scenarios in which we assumed that the QSAR estimates were off by two orders of magnitude (Table 1, see results for a justification on why two orders of magnitude were used as the likely margin of error). All in all, nine different hazard scenarios were included in the assessment (Table 1).

Three exposure scenarios were defined, depending on how non-detects were accounted for:

- Exposure-Scenario 1: non-detects were set to zero, representing the scenario with the lowest risk that is still compatible with the analytical data.
- Exposure-Scenario 2: non-detects were set to their method detection limits (MDLs), representing the scenario with the highest risk that is still compatible with the analytical data.
- Exposure-Scenario 3: missing concentration values were estimated using Kaplan-Meier modelling (Gustavsson et al., 2017a; Helsel, 2010), providing the most accurate basis for the risk assessment but not allowing to identify individual risk drivers since single chemical risk contributions are averaged, so no individual ranking of chemicals is possible.

Summary of all possible mixture risk scenarios are presented in Fig. 2.

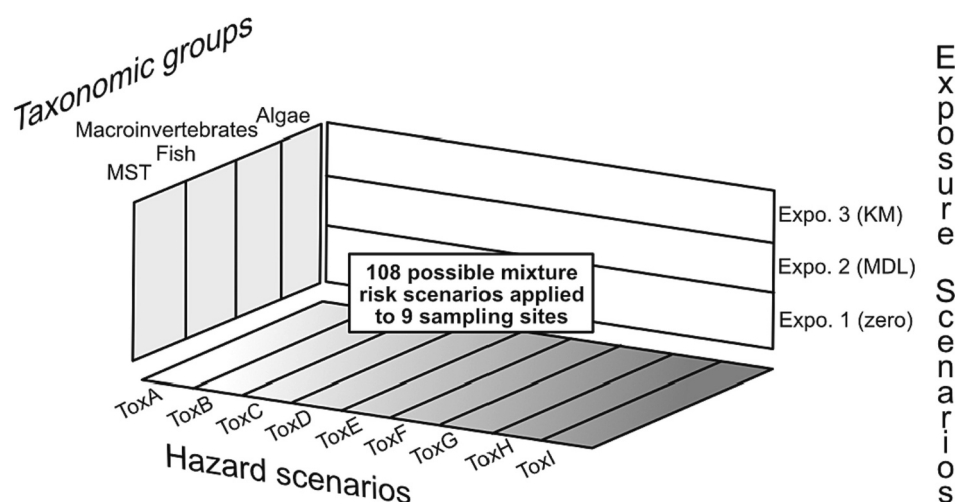
We identified two categories of mixture risk drivers: absolute and relative risk drivers. An absolute risk driver is defined as a compound that contributes to the mixture risk with an RQ of at least 0.02 (i.e., 20 % of the acceptable mixture RQ of 0.1 following the Water Framework Directive Guidance No 27 (European Commission, 2011)), at least at one site. A relative risk driver is a compound which contributes 20 % or more of the final RQ-sum, at least at one site at risk (Table 2). Given the considerable uncertainty introduced by bridging data gaps with QSAR estimates, we termed compounds that are not identified as risk drivers but could become one if the QSAR value underestimates the compound's toxicity by at least 2 orders of magnitude as *potential* mixture risk drivers (Table 2). The 2 orders of magnitude criterion for defining *potential* risk drivers is based on our correlations between experimental and QSAR-based chronic effect data. Actual risk drivers are compounds that should be prioritised for risk mitigation, while *potential* risk drivers are compounds flagged for ecotoxicological testing.

#### 2.5. Data analysis

The statistical analyses and data visualisation were performed using R version 4.2.2 (R Core Team, 2021). To assess the normality assumption, the Shapiro-Wilk Normality Test was utilised, and if the data deviated from a normal distribution, non-parametric testing was employed. The comparison of quantified environmental concentrations across chemical classes and sampling sites was conducted using the Kruskal-Wallis test (KW) and Dunn's test, which was implemented in the R-package {dunn.test} (Dinno, 2017), respectively. The Kaplan-Meier adjustment was incorporated in the analysis using the R-package

**Table 1**  
Hazard scenarios used for environmental mixture assessment.

| Scenarios | Definition                                                          |
|-----------|---------------------------------------------------------------------|
| ToxA      | Only experimental chronic toxicity data from US EPA ECOTOX Database |
| ToxB      | Only QSAR VEGA HUB chronic predictions                              |
| ToxC      | Only QSAR ECOSAR chronic predictions                                |
| ToxD      | Experimental toxicity data amended with QSAR VEGA                   |
| ToxE      | Experimental toxicity data amended with QSAR VEGA *100              |
| ToxF      | Experimental toxicity data amended with QSAR VEGA /100              |
| ToxG      | Experimental toxicity data amended with QSAR ECOSAR                 |
| ToxH      | Experimental toxicity data amended with QSAR ECOSAR *100            |
| ToxI      | Experimental toxicity data amended with QSAR ECOSAR /100            |



**Fig. 2.** Summary of all mixture risk scenarios applied to the data of each sampling site. Each assessment was performed for each taxonomic group (algae, macroinvertebrates, fish), and the most sensitive taxonomic group (MST). Exposure scenarios are defined based on how non-detects were handled (Expo1 = non-detects set to zero, Expo2 = non-detects set to the method detection limit (MDL), Expo3 = Kaplan-Meier-adjustment), for details see text. The nine hazard scenarios are based on ecotoxicological data used for calculating the risk quotients (Table 1). A total of  $4 \times 3 \times 9 = 108$  assessments was calculated for each site.

**Table 2**

Mixture risk driver definitions. It is important to highlight that it is sufficient to fulfil each criterion for at least one site.

|                                | Definition used in this study                                                                                                           |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Absolute risk driver           | A compound with an RQ of at least 0.02.                                                                                                 |
| Relative risk driver           | A compound that contributes at least 20 % to RQ-sum.                                                                                    |
| Potential absolute risk driver | A compound that is not an absolute risk driver but becomes one if their QSAR-estimated RQ-contribution is increased by a factor of 100. |
| Potential relative risk driver | A compound that is not a relative risk driver but becomes one if their QSAR-estimated RQ-prediction is increased by a factor of 100.    |

{NADA} (Helsel, 2005). The performance of the QSAR predictions was conducted through weighting Spearman's correlation coefficients between empirical observations and QSAR values from ECOSAR and VEGA. All data curation and data analysis scripts are openly available on Github ([https://github.com/ThomasBackhausLab/Mixture\\_assessment\\_analysis](https://github.com/ThomasBackhausLab/Mixture_assessment_analysis)) for further utilisation.

### 3. Results

#### 3.1. Occurrence of CEC mixtures in the River Aconcagua Basin

Detailed tables with detected and quantified CECs, concentrations, and their respective chemical identifiers are published in a separate data paper (Inostroza et al., 2023a) and are available via the open-access repository zenodo (Inostroza et al., 2023b). The data reveal the widespread occurrence of CECs in the surface waters of the River Aconcagua basin. From the 861 organic chemicals included in the analysis, 153 chemicals, including PPCPs, pesticides, and industrial chemicals were detected and quantified at least at one site. The industrial chemicals triacetaminine (intermediate and potential degradation product of plastic additives (UV stabilizers)) and benzyl dimethyl ketal (UV photosensitizer) as well as the disinfectant didecyltrimethylammonium (DDA) were detected at all sites (Fig. S1). The number of detected and quantified chemicals varied across sampling sites. We detected and quantified between 46 and 80 chemicals in tributary streams, between 39 and 71 in the main river course, and only between 18 and 28 at the reference sampling sites. The high number of CECs in tributary streams is likely due to intensive agriculture and the influence of WWTP discharges near the sampling sites. The low number of CECs found at RS1,

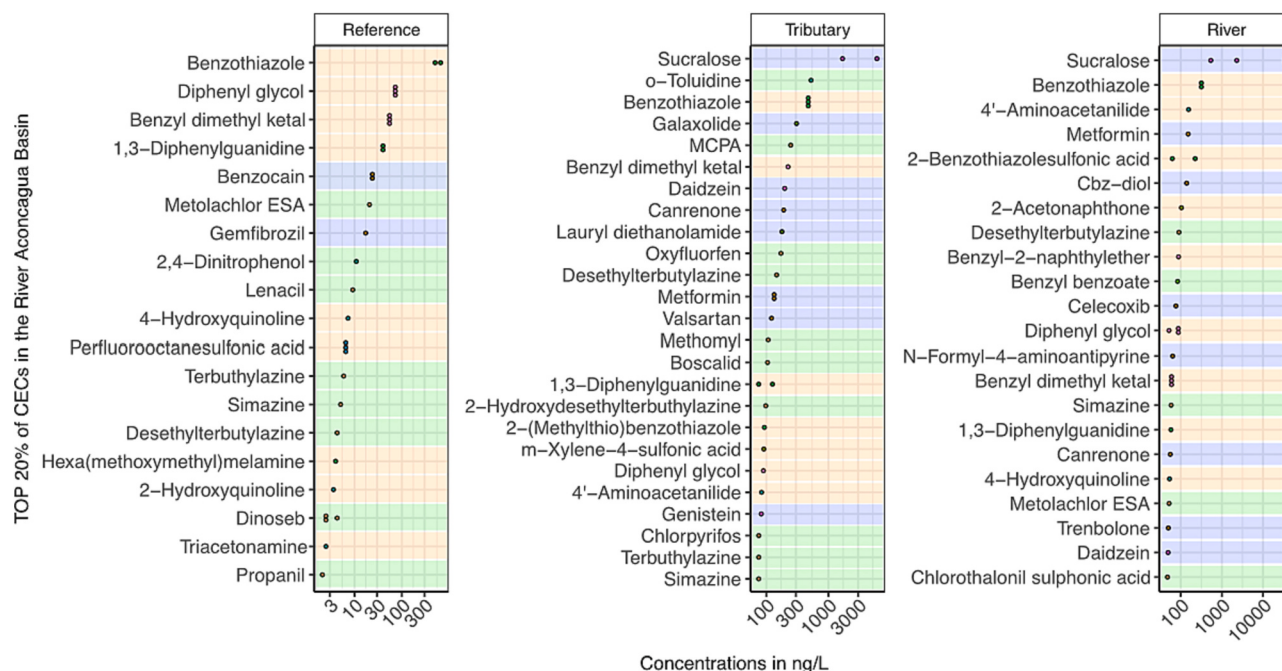
RS2, and RS3 sites is a result of the lower urbanisation in the region and supports the use of these sites as “reference sites”.

The highest single measured environmental concentrations were recorded in the main river course (35,625 ng/L) followed by tributaries (6038 ng/L), and reference sites (655 ng/L). The highest CEC concentrations (top 20 %) are plotted in Fig. 3. The top concentrations corresponded to sucralose and benzotriazole in the River Aconcagua basin. The artificial sweetener sucralose reached the highest concentrations in the main river course (538–35,625 ng/L) and tributary streams (1688–6038 ng/L), most likely as a consequence of its discharge from the WWTPs spread along the river basin. Benzotriazole, a vulcanisation accelerator but also used as a UV stabiliser and pesticide, was found in almost similar concentrations in the reference sites (501–655 ng/L) and tributaries (452–496 ng/L), while the main river course was slightly less exposed (37–345 ng/L).

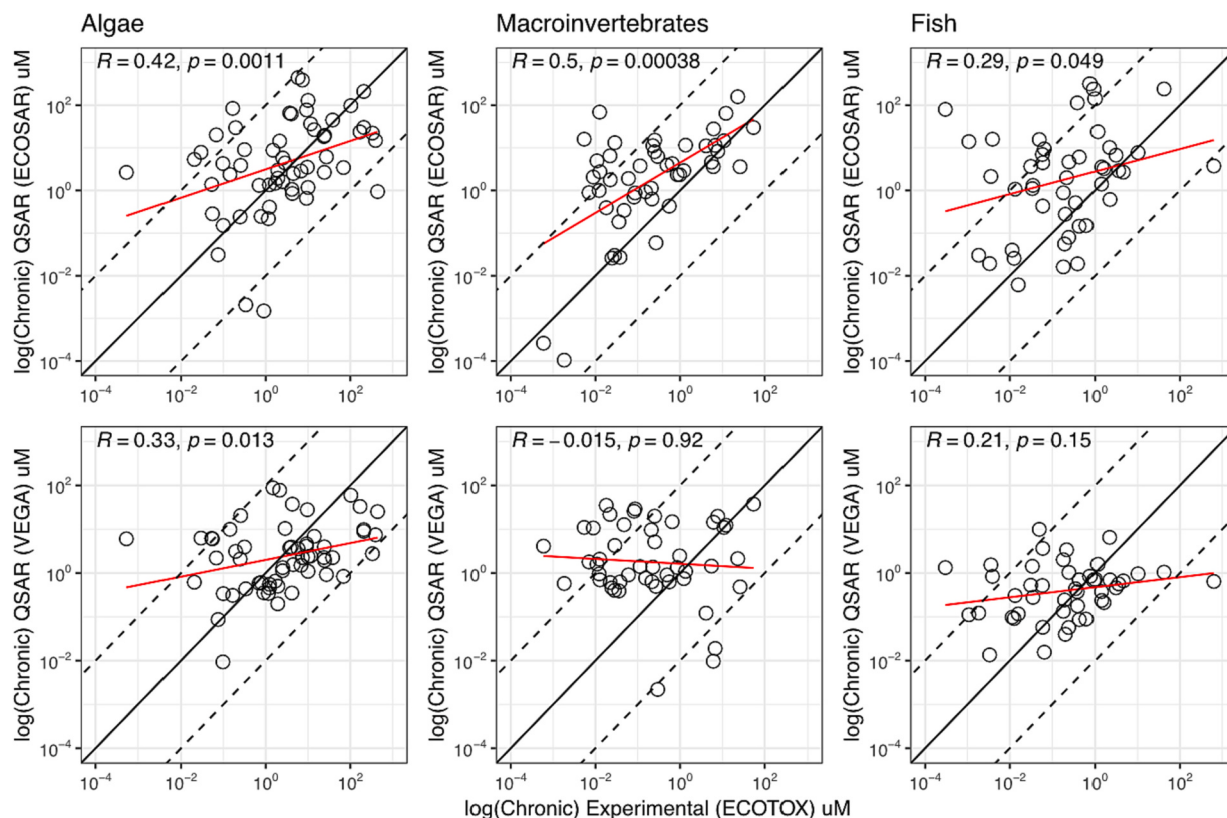
#### 3.2. Ecotoxicological assessment

Chronic data for all three main taxonomic groups (algae, macroinvertebrates, and fish) were retrieved for only 34 chemicals (22 % of the quantified chemicals) and only for those chemicals the most sensitive taxonomic group can be identified. For a few additional chemicals, we could retrieve partial datasets from the ECOTOX database (Olker et al., 2022), with either data only for algae and macroinvertebrates (7 chemicals), algae and fish (2 chemicals), or macroinvertebrates and fish (2 chemicals). In the end, we are facing the dilemma that chemical-analytical sensitivity and capacity allow screening for hundreds of chemicals of which only a small fraction can be assessed for their risks due to a lack of ecotoxicological data.

In order to evaluate the performance of the QSAR models, we compared the QSAR-estimates to the available experimental chronic data (Fig. 4). Unfortunately, all QSAR models show a relatively poor performance (Spearman's  $\rho \leq 0.5$ ) and only ECOSAR predictions are significantly correlated with the experimental data ( $p$ -value  $< 0.05$ ) (Fig. 4). ECOSAR outperforms VEGA for all three taxonomic groups, showing consistently higher Spearman's correlation coefficients. Overall, 81 % and 85 % of the ECOSAR and VEGA predictions, respectively, deviate less than two orders of magnitude from the experimental data. On this basis, we defined nine hazard scenarios for the mixture risk assessment, each with a different strategy to bridge the gaps in the empirical data (Table 1).



**Fig. 3.** Selected highest concentrations (top 20 %) of CECs quantified in at least one sampling site in the River Aconcagua Basin. Main CECs classes are coloured, green represents pesticides, blue pharmaceuticals and personal care products, and orange industrial chemicals. MCPA = 2-methyl-4-chlorophenoxyacetic acid and Cbz-diol = 10,11-Dihydroxycarbamazepine.



**Fig. 4.** Correlations between experimental and QSAR-based chronic effect data. Upper row shows the results from QSARs estimated using ECOSAR, lower row shows the result from VEGA HUB. Solid lines represent perfect agreement between the experimental and in silico predictions and dashed lines denote  $\pm$  two orders of magnitude deviation. Red lines represent the linear regression model. The non-parametric Spearman's rank correlation ( $R$ ) was calculated for significance testing, with the resulting  $p$ -value provided in each figure.

3.3. Mixture risk assessment

In total, we calculated 108 mixture risk scenarios (3 exposure scenarios  $\times$  9 hazard scenarios  $\times$  4 mixture evaluations (one for each of the three taxonomic groups plus the MST evaluation)), which were applied to each of the 9 sampling sites included in this study (Fig. 2). The resulting 972 mixture evaluations are presented in the supporting information (Table S2). RQs are calculated without an assessment factor and we used a value of 0.1 for the  $RQ_{STU}$  and  $RQ_{MST}$  sum as the acceptability chronic criterion, which corresponds to applying an assessment factor of 0.1 as limit, in line with the European Chemical Agency (2016) and the European Commission (2018).

Our findings indicate that the sensitivity of the chemical-analytical methods was high enough to characterise the chemicals in the samples, as reflected in the marginal differences observed in risk values generated by Exposure-Scenario 3 (Kaplan-Meier) compared to Exposure-Scenarios 1 (MECs < MDL equal zero) and 2 (MECs < MDL, equal MDL) (with a ratio of risk estimates ranging between 1.03 and 1.06, Table 3). Consequently, non-detects had only a negligible impact on the mixture risk assessment. The Kaplan-Meier scenario was therefore used for the overall mixture risk assessment, while Exposure-Scenario 1 was used for the identification of mixture risk drivers (which cannot be done using Kaplan-Meier estimates). Not surprisingly, risk estimates based on the ToxA scenario (which includes only chemicals with empirical ecotoxicological data) resulted in the lowest risk estimates (Table S2), simply because only a small fraction of the detected chemicals were included. The ToxB and ToxC scenarios (i.e., the mixture assessment based entirely on QSAR-estimates) systematically under-predict mixture risks, in comparison to the corresponding scenarios in which empirical data were preferred (ToxD and ToxG).

Because ECOSAR slightly outperformed VEGA for the CECs included in this study (Fig. 4), we base the actual mixture risk evaluation on the ToxG scenario (empirical data gaps filled by ECOSAR estimates), Table 3. The lowest risk was estimated at the reference sites with  $RQ_{Expo3-ToxG-MST}$  values ranging between 0.0012 and 0.0056. Reference sites were located in the upper section of the River Aconcagua (RS1 and RS2) and in one national park (RS3), all sites with lower anthropogenic pressure compared to the rest of the selected sites. Tributary streams, mainly running through agricultural and small urban areas, had higher  $RQ_{Expo3-ToxG-MST}$  values, with a site ranking of  $T1 > T3 > T2$  with  $RQ_{Expo3-ToxG-MST}$  values of 0.54, 0.18, and 0.067, respectively. Similarly, the main river course had high  $RQ_{Expo3-ToxG-MST}$  values, where sites ranked from  $R3 > R1 > R2$  with  $RQ_{Expo3-ToxG-MST}$  values of 0.69, 0.087, and 0.036, respectively (Table 3). Interesting R1 and R2 had similar risk values, even though R2 is located downstream of WWTPs but there were none upstream of R1. Site R3 (located in the main river course close to the river mouth), site T1 (located in an agricultural area) and site T3 (located in an area with mixed land use) are considered to be at risk

( $RQ_{Expo3-ToxG-MST} \geq 0.1$ ).

As expected,  $RQ_{Expo3-ToxG-MST}$  values always exceed the corresponding value of the individual taxonomic groups (Table 3). In all three sites where  $RQ_{Expo3-ToxG-MST}$  exceeded 0.1 at least one taxonomic group also exceeded a risk quotient of 0.1. That is, the regulatory conclusions from the assessments are identical, independent of whether the mixture risk assessment was performed for each taxonomic group or directly with a view on the whole ecosystem. Values for  $RQ_{Exp3-ToxG-Algae}$  were always below the risk threshold of 0.1. We, therefore, consider photo-synthetic organisms are not exposed to high risk from exposure to CEC mixtures in this study.  $RQ_{Exp3-ToxG-Macro}$  and  $RQ_{Exp3-ToxG-Fish}$  were consistently higher (Table 3).

3.4. Mixture risk drivers

We identified eight absolute risk drivers (Table 4). With two exceptions (galaxolide and daidzein), they all belong to groups of substances (pesticides and pharmaceuticals) that are used because of their high biological activity in certain target organisms. The top 3 comprise trenbolone, daidzein, and chlorpyrifos with maximum RQ values of 0.62, 0.20, and 0.12, respectively. That is, all three compounds occurred at concentrations that exceed the maximum acceptable level, even if only the exposure to the individual chemical is taken into account (assuming the application of an assessment factor of at least 10).

We also determined 19 substances as *potential* absolute risk drivers (Table S3). Those are substances without a full set of empirical ecotoxicity data, but which could potentially be risk drivers, under the worst-case assumption that their QSAR-based hazard estimate underestimates their actual toxicity by a factor of 100 (Fig. 4). Twelve substances did not have any empirical data on chronic toxicity, five chemicals had chronic information for one taxonomic group, and only two chemicals (i.e., fungicides boscalid and myclobutanil) have chronic information for two taxonomic groups. Especially relevant are pesticides, biocides and pharmaceuticals with a maximum  $RQ_{Expo1-ToxI-MST} \geq 0.1$  for which either the experimental data on the likely target organism (= the most sensitive organism group) are missing in case of pesticides, such as chlorfenapyr (an insecticide) and allethrin (an insecticide); or chemicals for which we don't know their target organisms, such as telmisartan (a pharmaceutical), octocrylene (a personal care product), benzyl-2-naphthyl ether (an industrial chemical), or *N,N*-dimethyltetradecylamine *N*-oxide (TDAO, a surfactant). Empirical ecotoxicological data are urgently needed for these substances.

Eight relative risk drivers were determined across the nine sites (Table S4). All of them, with two exceptions, were also categorised as actual absolute risk drivers. The two exceptions (1,3-diphenylguanidine and chlorfenapyr) were also identified as *potential* absolute risk drivers. We also identified six chemicals as *potential* relative risk drivers (Table S5) of which four were also categorised as *potential* absolute risk

**Table 3**  
 $RQ_{STU}$  estimates for the different exposure scenarios based on the ToxG scenario (empirical chronic data amended with ECOSAR QSAR values). In Expo 1: MECs < MDL were set to 0 (most conservative scenario), Expo 2: MECs < MDL were set to the MDL (worst case scenario), Expo 3: Kaplan-Meier estimation of mixture risk (most realistic scenario). Sites at risk were defined as  $RQ_{STU} \geq 0.1$ . RQ values exceeding 0.1 are boldfaced. Reference, tributary, and main river course sites were abbreviated as RS, T, and R, respectively.

| ToxG (empirical data amended with QSAR-data (ECOSAR)) |                    |        |                    |                    |              |              |                    |              |                    |              |              |              |
|-------------------------------------------------------|--------------------|--------|--------------------|--------------------|--------------|--------------|--------------------|--------------|--------------------|--------------|--------------|--------------|
| Sites                                                 | Algae              |        |                    | Macroinvertebrates |              |              | Fish               |              |                    | MST          |              |              |
|                                                       | Expo-1             | Expo-2 | Expo-3             | Expo-1             | Expo-2       | Expo-3       | Expo-1             | Expo-2       | Expo-3             | Expo-1       | Expo-2       | Expo-3       |
| RS1                                                   | $1 \times 10^{-3}$ | 0.005  | 0.001              | 0.003              | 0.015        | 0.004        | 0.001              | 0.024        | 0.001              | 0.005        | 0.040        | 0.005        |
| RS2                                                   | $3 \times 10^{-4}$ | 0.004  | $3 \times 10^{-4}$ | 0.001              | 0.013        | 0.001        | $5 \times 10^{-4}$ | 0.024        | $4 \times 10^{-4}$ | 0.001        | 0.037        | 0.001        |
| RS3                                                   | $2 \times 10^{-4}$ | 0.004  | $3 \times 10^{-4}$ | $8 \times 10^{-4}$ | 0.012        | 0.001        | $6 \times 10^{-4}$ | 0.024        | $7 \times 10^{-4}$ | 0.001        | 0.036        | 0.001        |
| T1                                                    | 0.011              | 0.014  | 0.011              | <b>0.293</b>       | <b>0.297</b> | <b>0.293</b> | <b>0.271</b>       | <b>0.285</b> | <b>0.271</b>       | <b>0.534</b> | <b>0.554</b> | <b>0.535</b> |
| T2                                                    | 0.001              | 0.005  | 0.001              | 0.012              | 0.021        | 0.012        | 0.062              | 0.072        | 0.062              | 0.067        | 0.087        | 0.067        |
| T3                                                    | 0.010              | 0.014  | 0.011              | <b>0.148</b>       | <b>0.151</b> | <b>0.148</b> | 0.041              | 0.063        | 0.042              | <b>0.179</b> | <b>0.204</b> | <b>0.180</b> |
| R1                                                    | 0.028              | 0.029  | 0.028              | 0.005              | 0.016        | 0.005        | 0.057              | 0.079        | 0.058              | 0.086        | <b>0.116</b> | 0.086        |
| R2                                                    | $5 \times 10^{-4}$ | 0.004  | $5 \times 10^{-4}$ | 0.003              | 0.014        | 0.003        | 0.034              | 0.056        | 0.034              | 0.036        | 0.070        | 0.036        |
| R3                                                    | 0.006              | 0.010  | 0.007              | 0.062              | 0.070        | 0.062        | <b>0.629</b>       | <b>0.640</b> | <b>0.630</b>       | <b>0.686</b> | <b>0.705</b> | <b>0.686</b> |

**Table 4**  
Absolute mixture risk drivers, i.e., chemicals with an individual  $RQ_{MST} \geq 0.02$  in at least one site. Chemicals are listed in descending order of their  $RQ_{Expo1-ToxG-MST}$  (mean across all sites). ECOTOX and ECOSAR columns describe the experimental and predictive EC10-equivalent concentrations available for calculating RQs for algae, crustaceans, and fish. Experimental (ECOTOX) and QSAR ECOSAR concentrations in  $\mu M$ .  $minRQ =$  minimum risk quotient,  $meanRQ =$  mean risk quotient,  $maxRQ =$  maximum risk quotient,  $N =$  number of sites where the respective chemical was an absolute risk driver.  $RQ_{MST-ToxA} =$  risk values considering only experimental data (ToxA),  $RQ_{MST-ToxC} =$  risk values considering only QSAR ECOSAR (ToxC), and  $RQ_{MST-ToxG} =$  risk values considering experimental data amended with QSAR ECOSAR (ToxG).

| Name           | CAS Number  | Class            | minRQ | meanRQ | maxRQ | N | $RQ_{MST-ToxA}$ | $RQ_{MST-ToxC}$ | $RQ_{MST-ToxG}$ | Algae<br>ECOTOX | Macroinv.<br>ECOTOX | Fish<br>ECOTOX | Algae<br>ECOSAR | Macroinv.<br>ECOSAR | Fish<br>ECOSAR |
|----------------|-------------|------------------|-------|--------|-------|---|-----------------|-----------------|-----------------|-----------------|---------------------|----------------|-----------------|---------------------|----------------|
| Trenbolone     | 10,161-33-8 | Anabolic steroid | 0.045 | 0.330  | 0.615 | 2 | 0.615           | 0.000           | 0.615           | 0.248           | 0.001               | 0.000          | 16.696          | 5.777               | 79.675         |
| Chlorpyrifos   | 2921-88-2   | Insecticide      | 0.118 | 0.118  | 0.118 | 1 | 0.118           | 2.057           | 0.118           | 0.248           | 0.001               | 0.012          | 0.240           | 0.000               | 0.025          |
| Daidzein       | 486-66-8    | Phytoestrogen    | 0.032 | 0.094  | 0.199 | 3 | 0.199           | 0.000           | 0.199           | 0.777           | 0.028               | 0.003          | 37.523          | 17.450              | 15.898         |
| Galaxolide     | 1222-05-5   | Musk fragrance   | 0.041 | 0.041  | 0.041 | 1 | 0.041           | 0.062           | 0.041           | 0.777           | 0.028               | 0.398          | 0.245           | 0.030               | 0.019          |
| Diazinon       | 333-41-5    | Insecticide      | 0.022 | 0.041  | 0.061 | 2 | 0.061           | 0.139           | 0.061           | 9.189           | 0.000               | 0.433          | 0.653           | 0.000               | 0.144          |
| Methomyl       | 16,752-77-5 | Insecticide      | 0.036 | 0.036  | 0.036 | 1 | 0.036           | 0.001           | 0.036           | 440.749         | 0.018               | 0.183          | 0.940           | 0.393               | 0.882          |
| Terbutylazine  | 5915-41-3   | Herbicide        | 0.021 | 0.029  | 0.037 | 2 | 0.021           | 0.000           | 0.021           | 0.052           | 0.008               | 3.569          | 1.384           | 2.062               | 2.909          |
| Clarithromycin | 81,103-11-9 | Antibiotic       | 0.026 | 0.026  | 0.026 | 1 | 0.026           | 0.000           | 0.026           | 0.000           | 0.000               | 0.000          | 2.650           | 1.453               | 1.266          |

drivers.

The mixture risk drivers at those sites where risk cannot be excluded ( $RQ_{Expo1-ToxI-MST} \geq 0.1$ ) are shown in Fig. 5 and in those with  $RQ_{Expo1-ToxI-MST} < 0.1$  (no risk) are presented in Fig. S2. All three sites at risk (i.e., T1, T3, and R3) show distinct patterns and possess different risk drivers (Fig. 5). The number of absolute as well as relative risk drivers never exceeded 4. This is a typical pattern in environmentally realistic mixtures, which is sometimes called the Pareto-principle of mixture toxicity, relating to the power-law probability distribution named after the Italian engineer Vilfredo Pareto (Rudén et al., 2019 and references therein). However, the chemicals actually identified as risk drivers varied across sites, in dependence on land-use patterns and land-use intensity.

4. Discussion

4.1. Exposure and hazard assessment

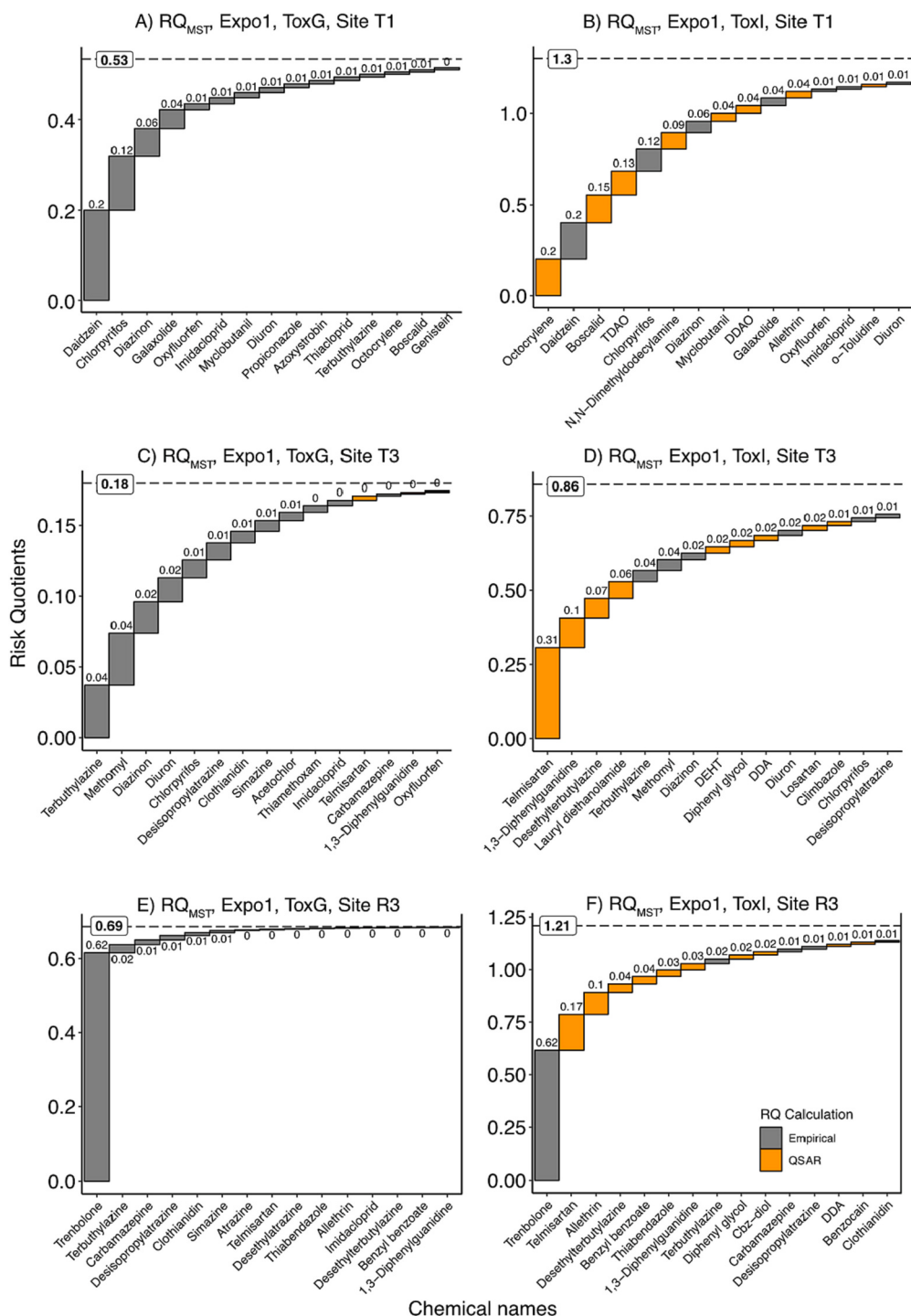
The nature of the chemicals found and the fact that we observed a clear pollution gradient from the “reference sites” to the main river and the tributaries shows the impact of human activities on the chemical status of the River Aconcagua basin. The overall CEC fingerprints did not substantially differ from those previously determined in Europe, North America, and some African countries (Carpenter and Helbling, 2018; Finckh et al., 2022; Kandie et al., 2020; Loos et al., 2013). This similarity can be attributed to the widespread and global use of these chemicals in daily life, industry, and agriculture, as well as the use of a target list based on commonly measured CECs in European aquatic environments. The CECs that we detected at the highest concentrations, sucralose and benzothiazole, are ubiquitous in surface waters around the globe, in similar concentration ranges (Finckh et al., 2022; Loos et al., 2013; Yu et al., 2023).

Although we have the analytical sensitivity for screening hundreds of CECs in the aquatic environment, the Achilles' heel is the lack of ecotoxicological data for assessing CEC hazards. A similar situation has been described in previous publications, including studies that assessed WWTP effluents (Finckh et al., 2022), agricultural streams after rain events (Neale et al., 2020), and emission-based mixture risk assessments (Gustavsson et al., 2023). The use of chronic effect estimates derived from QSARs bridges the gap in chronic effect data, which enabled us to conduct the mixture assessment separately for each of the three taxonomic groups (algae, macroinvertebrates, fish) and MST for all the CECs included in our study. Nevertheless, our results show that the accuracy of the chronic QSAR estimates needs improvement, findings that are in agreement with previously published studies, with some exceptions in the field of endocrine disruption (Cronin, 2017). QSAR models that estimate acute ecotoxicity perform better (Melnikov et al., 2016; Zhou et al., 2021).

4.2. Mixture risk assessment and risk drivers

Our study presents a systematic and comprehensive strategy for the environmental risk assessment of chemical mixtures. This strategy encompasses 108 distinct mixture risk scenarios, taking into account different possibilities on how to account for the potential contribution of CECs that were not detected, different strategies to account for data gaps and different ecotoxicological perspectives (focus on individual taxonomic groups and MST). Conversely, the incorporation of QSAR predictions into the mixture assessment framework is generally applied (Finckh et al., 2022; Reiber et al., 2021; von der Ohe et al., 2011), as recommended by the European Chemical Agency (European Chemicals Agency, 2021). In addition to using QSARs for filling data gaps, we introduce a novel application of QSARs to predict potential mixture risk drivers. This approach identifies chemicals that may have adverse effects at the concentrations at which they are detected and for which therefore empirical data are urgently needed.

Three of the nine sites can be considered at risk (Sum  $RQ \geq 0.1$  in



**Fig. 5.** Mixture risk assessment estimates for the sites at risk ( $RQ_{MST} \geq 0.1$ ) in the River Aconcagua basin. (A)(C)(E):  $RQ_{MST}$  predictions based on non-detects set to zero (Expo1) and experimental chronic data amended with ECOSAR values (ToxG). (B)(D)(F):  $RQ_{MST}$  predictions based on non-detects set to zero (Expo1) and experimental chronic data amended with ECOSAR values (ToxI) that were multiplied by a factor of 100 (i.e., QSAR ECx estimates were divided by a factor of 100) (ToxI). Site T represents tributaries and Site R the main river course. Colours represent the source of the toxicity data. Grey bars: empirical data, orange data: QSAR estimates.

scenario ToxG, using KM-based exposure estimates) in the River Aconcagua basin. Overall, limited differences in the potential risks of the chemical mixtures were observed when non-detects were included (scenario 1) or excluded (scenario 3) in the analysis. The small number of risk drivers found at each of those sites raises the question of whether the risk is indeed an issue that is driven by mixtures, or whether this is a single-substance problem, and the analytical performance was simply good enough to detect a myriad of chemicals that happen to co-occur but

that are irrelevant from a risk perspective. If risk mitigation measures would focus exclusively on those compounds that are present at unacceptable concentrations (individual  $RQ > 0.1$ ) in order to reduce their individual  $RQ$  values to a maximum of 0.1, the remaining  $RQ$  sums would be 0.23 (site T1), 0.18 (site T3) and 0.17 (site R3). That is, even if single-substance-oriented risk mitigation measures would be consistently implemented so that all chemicals are present at individually “safe” concentrations, all three sites would still be at risk. This leads to

the conclusion that the risks encountered at the sites are a combination of a single substance problem (unacceptably high concentrations of a few individual substances), and a mixture problem (unacceptably high sum RQ values even after successful single substance risk mitigation).

Component-based mixture risk assessments, such as the one implemented in this study, inherently underestimate the actual site-specific risks, given that most likely not all relevant chemicals are included in the analytical profile. For instance, the present study is based on a selection of target compounds that was developed largely from a European perspective (Beckers et al., 2018; Krauss et al., 2019), and therefore does not include all pesticides used in Chilean agriculture. The present study also focuses exclusively on environmental pollution by synthetic organic chemicals and overlooks the role of metals as risk-contributing contaminants. At the same time, the assumption of a concentration-additive behaviour of the mixture might lead to a risk overestimation, although this might be comparatively small (see discussion in Backhaus and Faust, 2012; Rudén et al., 2019).

The top absolute risk drivers ( $RQ_{MST} \geq 0.02$ , Table 4) are CECs that are known to cause harmful effects on aquatic life. Trenbolone stands out as a veterinary drug that enters river basins through livestock farming, which has been recognised as an endocrine disruptor, capable of altering hormone and steroid synthesis in fish (Ankley et al., 2008; Overturf et al., 2015). Chlorpyrifos is a chlorinated organophosphate insecticide that is well-known for its neurotoxicity to invertebrates and fish (Echeverri-Jaramillo et al., 2020; Scott and Sloman, 2004). Daidzein is a natural phytoestrogen primarily found in the *Fabaceae* family, including soybeans, peas, and red clover. Chlorpyrifos and daidzein have been previously identified as risk drivers in the aquatic environment (Caracciolo et al., 2023; König et al., 2017). In addition, the absolute risk drivers diazinon, terbuthylazine, and clarithromycin have been also identified as major risk drivers in WWTP's effluents (Beckers et al., 2018; Finckh et al., 2022). Chlorpyrifos is a priority substance of the EU Water Framework Directive and the sunscreen octocrylene, identified as a *potential* absolute risk driver, is listed on the 3rd watch list under the WFD (European Commission, 2008, 2022). Regarding Chilean water quality and environmental protection goals, none of the identified risk drivers are currently defined as priority substances or under evaluation at national or river-basin level.

## 5. Conclusions

Our study advances our understanding of environmental risks caused by the co-occurrence of CECs in freshwater systems in South America. In the River Aconcagua basin, we detected a total of 153 CECs from various chemical classes, including pesticides, pharmaceuticals, personal care products, and chemicals used in industrial processes. The overall pattern of CEC occurrence did not differ significantly from other small streams and rivers worldwide. However, we observed clear site-specific differences in concentrations and mixture composition.

To comprehensively evaluate the risk associated with CEC mixtures, we introduced an integrative strategy for mixture risk assessment. This approach systematically assesses both the exposure and hazard components of the risk assessment process. Due to the lack of experimental ecotoxicological data, we utilised QSAR modelling, as recommended by various environmental agencies, to fill data gaps. The QSAR models lacked accuracy across different taxonomic groups, and we incorporated those uncertainties into the mixture risk assessment, by defining various hazard scenarios.

Based on our analysis, we identified three sites at risk in the River Aconcagua basin. These conclusions are supported by the different risk scenarios and their interlinkage. Furthermore, our findings endorse the use of the most sensitive taxonomic group ( $RQ_{MST}$ ) as a comprehensive ecological risk metric for predicting the risks posed by complex environmental mixtures. This metric successfully captured the taxonomic groups that were most vulnerable to the determined exposures.

Risk scenarios based solely on QSAR ecotoxicological data

consistently underestimated the actual risk. Therefore, we propose the use of QSAR predictions amended with experimental ecotoxicological data as a worst-case scenario for risk estimation. QSAR models proved to be valuable for identifying chemicals that potentially contribute to the predicted risk (*potential* risk drivers). Additionally, we recommend evaluating the performance of available QSAR platforms, especially those offering chronic models, before integrating their predictions into the risk assessment process.

We found that only a few chemicals were responsible for driving the mixture risk. However, the results show that mitigation measures focused solely on single chemicals are insufficient if water bodies are impacted by complex mixtures of chemicals. It is crucial to acknowledge that chemical pollution risks are a combination of (1) the problem of unacceptably high concentrations of comparatively few individual substances and (2) the problem caused by a complex melange of chemicals, co-occurring at seemingly low concentrations.

## CRedit authorship contribution statement

**Pedro A. Inostroza:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sebastian Elgueta:** Methodology, Writing – review & editing. **Martin Krauss:** Data curation, Methodology, Writing – review & editing. **Werner Brack:** Resources, Writing – review & editing. **Thomas Backhaus:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

All research data is available in open repositories (see Material and methods section)

## Acknowledgments

We thank Alba Lopez Mangas and Monica del Aguila for fieldwork support, Margit Petre and Jörg Ahleim (UFZ) for LVSPE training as well as cartridge extractions. This work was supported by the FRAM Centre for Future Chemical Risk Assessment and Management at the University of Gothenburg and ANID Project FOVI 230197. The QExactive Plus LC-HRMS used at UFZ is part of the major infrastructure initiative CITEPro (Chemicals in the Terrestrial Environment Profiler) funded by the Helmholtz Association.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.171054>.

## References

- Amigo, J., Ramírez, C., 1998. A bioclimatic classification of Chile: woodland communities in the temperate zone. *Plant Ecol.* 136, 9–26. <https://doi.org/10.1023/A:1009714201917>.
- Ankley, G.T., Erickson, R.J., Hoff, D.J., Mount, D.R., Lazorchak, J.M., Beaman, J., Linton, T.K., 2008. Draft White Paper: Aquatic Life Criteria for Contaminants of Emerging Concern, Part I, General Challenges and Recommendations. Prepared by the Office of Water and Office of Research and Development Emerging Contaminants Workgroup. US Environmental Protection Agency, Washington, DC.

- Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. *Environ. Sci. Technol.* 46, 2564–2573. <https://doi.org/10.1021/es2034125>.
- Balvanera, P., Pfaff, A., Viña, A., García Frapolli, E., Hussain, S.A., Merino, L., Minang, P. A., Nagabhatla, N., Sidorovich, A., 2019. Chapter 2.1 Status and Trends –Drivers of Change. Zenodo. <https://doi.org/10.5281/zenodo.5517423>.
- Beckers, L.-M., Busch, W., Krauss, M., Schulze, T., Brack, W., 2018. Characterization and risk assessment of seasonal and weather dynamics in organic pollutant mixtures from discharge of a separate sewer system. *Water Res.* 135, 122–133. <https://doi.org/10.1016/j.watres.2018.02.002>.
- Benfenati, E., Manganaro, A., Gini, G., 2013. VEGA-QSAR: AI inside a platform for predictive toxicology, in: *Proceedings of the Workshop Popularize Artificial Intelligence Co-Located with the 13th Conference of the Italian Association for Artificial Intelligence*. Turin, pp. 21–28.
- Bertin, A., Inostroza, P.A., Quiñones, R.A., 2011. Estrogen pollution in a highly productive ecosystem off central-south Chile. *Mar. Pollut. Bull.* 62, 1530–1537. <https://doi.org/10.1016/j.marpollbul.2011.04.002>.
- Boxall, A., Sinclair, C.J., Fenner, K., Kolpin, D., Maund, S.J., 2004. When synthetic chemicals degrade in the environment. *Environ. Sci. Technol.* 38, 368A–375A. <https://doi.org/10.1021/es040624v>.
- Buschmann, A.H., Tomova, A., López, A., Maldonado, M.A., Henríquez, L.A., Ivanova, L., Moy, F., Godfrey, H.P., Cabello, F.C., 2012. Salmon aquaculture and antimicrobial resistance in the marine environment. *PLoS One* 7, e42724. <https://doi.org/10.1371/journal.pone.0042724>.
- Caracciolo, R., Escher, B.I., Lai, F.Y., Nguyen, T.A., Le, T.M.T., Schlichting, R., Tröger, R., Némery, J., Wiberg, K., Nguyen, P.D., Baduel, C., 2023. Impact of a megacity on the water quality of a tropical estuary assessed by a combination of chemical analysis and in-vitro bioassays. *Sci. Total Environ.* 877, 162525 <https://doi.org/10.1016/j.scitotenv.2023.162525>.
- Carpenter, C.M.G., Helbling, D.E., 2018. Widespread micropollutant monitoring in the Hudson River estuary reveals spatiotemporal micropollutant clusters and their sources. *Environ. Sci. Technol.* 52, 6187–6196. <https://doi.org/10.1021/acs.est.8b00945>.
- Chow, R., Scheidegger, R., Doppler, T., Dietzel, A., Fenicia, F., Stamm, C., 2020. A review of long-term pesticide monitoring studies to assess surface water quality trends. *Water Res.* X 9, 100064. <https://doi.org/10.1016/j.wroa.2020.100064>.
- Climet, M.J., Herrero-Hernández, E., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., Pedreros, P., Urrutia, R., 2019. Residues of pesticides and some metabolites in dissolved and particulate phase in surface stream water of Cachapoal River basin, Central Chile. *Environ. Pollut.* 251, 90–101. <https://doi.org/10.1016/j.envpol.2019.04.117>.
- COCHILCO, 2020. *Yearbook: Copper and Other Mineral Statistics*. The Chilean Copper Commission, Santiago.
- Coria, J., Elgueta, S., 2022. Towards safer use of pesticides in Chile. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-022-18843-6>.
- Cronin, M.T.D., 2017. (Q)SARs to predict environmental toxicities: current status and future needs. *Environ. Sci.: Processes Impacts* 19, 213–220. <https://doi.org/10.1039/C6EM00687F>.
- Dinno, A., 2017. *dunntest* (dunn.test for R). Package to perform Dunn's nonparametric pairwise multiple comparisons.
- Drury, B., Rosi-Marshall, E., Kelly, J.J., 2013. Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers. *Appl. Environ. Microbiol.* 79, 1897–1905. <https://doi.org/10.1128/AEM.03527-12>.
- Echeverri-Jaramillo, G., Jaramillo-Colorado, B., Sabater-Marco, C., Castillo-López, M.Á., 2020. Acute toxicity of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol alone and in combination using a battery of bioassays. *Environ. Sci. Pollut. Res.* 27, 32770–32778. <https://doi.org/10.1007/s11356-020-09392-x>.
- Eggen, R.L.L., Hollender, J., Joss, A., Schärer, M., Stamm, C., 2014. Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. *Environ. Sci. Technol.* 48, 7683–7689. <https://doi.org/10.1021/es500907n>.
- Escher, B.I., Hermens, J.L.M., 2002. Modes of action in ecotoxicology: their role in body burdens, species sensitivity, QSARs, and mixture effects. *Environ. Sci. Technol.* 36, 4201–4217. <https://doi.org/10.1021/es015848h>.
- European Chemicals Agency, 2016. *Guidance on Information Requirements and Chemical Safety Assessment. Part E: Risk Characterisation*. European Chemical Agency, Helsinki doi:ECHA-2016-G-04-EN.
- European Chemicals Agency, 2021. The use of alternatives to testing on animals for the REACH regulation. European Chemicals Agency, Helsinki. <https://doi.org/10.2823/092305>.
- European Commission, 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on Environmental Quality Standards in the Field of Water Policy.
- European Commission, 2011. *Technical Guidance for Deriving Environmental Quality Standards*, Guidance Document no: 27 under the Common Implementation Strategy for the Water Framework Directive (2000/60/EC).
- European Commission, 2018. *Technical guidance for deriving environmental quality standards*. European Commission. <https://doi.org/10.2875/018826>.
- European Commission, 2022. Commission Implementing Decision (EU) 2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C(2022) 5098). *Official Journal of European Union L197* (65), 117–120.
- Finckh, S., Beckers, L.-M., Busch, W., Carmona, E., Dulio, V., Kramer, L., Krauss, M., Posthuma, L., Schulze, T., Slootweg, J., Von der Ohe, P.C., Brack, W., 2022. A risk based assessment approach for chemical mixtures from wastewater treatment plant effluents. *Environ. Int.* 164, 107234 <https://doi.org/10.1016/j.envint.2022.107234>.
- Giordano, A., Richter, P., Ahumada, I., 2011. Determination of pesticides in river water using rotating disk sorptive extraction and gas chromatography-mass spectrometry. *Talanta* 85, 2425–2429. <https://doi.org/10.1016/j.talanta.2011.07.087>.
- Groh, K., vom Berg, C., Schirmer, K., Thili, A., 2022. Anthropogenic chemicals as underestimated drivers of biodiversity loss: scientific and societal implications. *Environ. Sci. Technol.* 56, 707–710. <https://doi.org/10.1021/acs.est.1c08399>.
- Gustavsson, M., Kreuger, J., Bundschuh, M., Backhaus, T., 2017a. Pesticide mixtures in the Swedish streams: environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation. *Sci. Total Environ.* 598, 973–983. <https://doi.org/10.1016/j.scitotenv.2017.04.122>.
- Gustavsson, M., Magnér, J., Carney Almqvist, B., Eriksson, M.K., Sturve, J., Backhaus, T., 2017b. Chemical monitoring of Swedish coastal waters indicates common exceedances of environmental thresholds, both for individual substances as well as their mixtures. *Mar. Pollut. Bull.* 122, 409–419. <https://doi.org/10.1016/j.marpollbul.2017.06.082>.
- Gustavsson, M., Molander, S., Backhaus, T., Kristiansson, E., 2023. Risk assessment of chemicals and their mixtures are hindered by scarcity and inconsistencies between different environmental exposure limits. *Environ. Res.* 225, 115372 <https://doi.org/10.1016/j.envres.2023.115372>.
- Han, X., Price, P.S., 2011. Determining the maximum cumulative ratios for mixtures observed in ground water wells used as drinking water supplies in the United States. *Int. J. Environ. Res. Public Health* 8, 4729–4745. <https://doi.org/10.3390/ijerph8124729>.
- Helsel, D.R., 2005. *Nondetects and Data Analysis: Statistics for Censored Environmental Data*. John Wiley & Sons.
- Helsel, D.R., 2010. Summing nondetects: incorporating low-level contaminants in risk assessment. *Integr. Environ. Assess. Manag.* 6, 361–366. <https://doi.org/10.1002/ieam.31>.
- Hug, C., Ulrich, N., Schulze, T., Brack, W., Krauss, M., 2014. Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening. *Environ. Pollut.* 184, 25–32. <https://doi.org/10.1016/j.envpol.2013.07.048>.
- Inostroza, P.A., Vera-Escalona, I., Wild, R., Norf, H., Brauns, M., 2018. Tandem action of natural and chemical stressors in stream ecosystems: insights from a population genetic perspective. *Environ. Sci. Technol.* 54, 7962–7971. <https://doi.org/10.1021/acs.est.8b01259>.
- Inostroza, P.A., Elgueta, S., Muz, M., Krauss, M., Brack, W., Backhaus, T., 2023a. Dataset comprising pesticides, pharmaceuticals and personal care products, and industrial chemicals detected in streams and rivers of Central Chile. *Data Brief* 50, 109600. <https://doi.org/10.1016/j.dib.2023.109600>.
- Inostroza, P.A., Elgueta, S., Muz, M., Krauss, M., Brack, W., Backhaus, T., 2023b. Dataset of chemicals of emerging concern detected in streams and rivers of Central Chile. <https://doi.org/10.5281/zenodo.8088841>.
- Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* 32, 2498–2506. <https://doi.org/10.1021/es9710870>.
- Kaewlaoyong, A., Vu, C.T., Lin, C., Liao, C.S., Chen, J.-R., 2018. Occurrence of phthalate esters around the major plastic industrial area in southern Taiwan. *Environ. Earth Sci.* 77, 457. <https://doi.org/10.1007/s12665-018-7655-4>.
- Kandie, F.J., Krauss, M., Massei, R., Ganatra, A., Fillinger, U., Becker, J., Liess, M., Torto, B., Brack, W., 2020. Multi-compartment chemical characterization and risk assessment of chemicals of emerging concern in freshwater systems of western Kenya. *Environ. Sci. Eur.* 32, 115. <https://doi.org/10.1186/s12302-020-00392-9>.
- Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environ. Sci. Technol.* 36, 1202–1211. <https://doi.org/10.1021/es011055j>.
- König, M., Escher, B.I., Neale, P.A., Krauss, M., Hilscherová, K., Novák, J., Teodorović, I., Schulze, T., Seidensticker, S., Kamal Hashmi, M.A., Ahlheim, J., Brack, W., 2017. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. *Environ. Pollut.* 220, 1220–1230. <https://doi.org/10.1016/j.envpol.2016.11.011>.
- Kortenkamp, A., Backhaus, T., Faust, M., 2009. *State of the Art Report on Mixture Toxicity*. Report to the EU Commission, Directorate General of the Environment.
- Krauss, M., Hug, C., Bloch, R., Schulze, T., Brack, W., 2019. Prioritising site-specific micropollutants in surface water from LC-HRMS non-target screening data using a rarity score. *Environ. Sci. Eur.* 31, 45. <https://doi.org/10.1186/s12302-019-0231-z>.
- Leith, K.F., Bowerman, W.W., Wierda, M.R., Best, D.A., Grubb, T.G., Sikarske, J.G., 2010. A comparison of techniques for assessing central tendency in left-censored data using PCB and p,p'DDE contaminant concentrations from Michigan's Bald Eagle Biosentinel Program. *Chemosphere* 80, 7–12. <https://doi.org/10.1016/j.chemosphere.2010.03.056>.
- Li, Y., Fan, T., Ren, T., Zhang, N., Zhao, L., Zhong, R., Sun, G., 2023. Ecotoxicological risk assessment of pesticides against different aquatic and terrestrial species: using mechanistic QSTR and iQSTTR modelling approaches to fill the toxicity data gap. *Green Chem.* <https://doi.org/10.1039/D3GC03109H>.
- Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* 47, 6475–6487. <https://doi.org/10.1016/j.watres.2013.08.024>.
- Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of

- freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U. S. A.* 111, 9549–9554. <https://doi.org/10.1073/pnas.1321082111>.
- Marshall, M.M., McCluney, K.E., 2021. Mixtures of co-occurring chemicals in freshwater systems across the continental US. *Environ. Pollut.* 268, 115793. <https://doi.org/10.1016/j.envpol.2020.115793>.
- Melnikov, F., Kostal, J., Voutchkova-Kostal, A., Zimmerman, J.B., Anastas, P.T., 2016. Assessment of predictive models for estimating the acute aquatic toxicity of organic chemicals. *Green Chem.* 18, 4432–4445. <https://doi.org/10.1039/C6GC00720A>.
- Montory, M., Ferrer, J., Rivera, D., Villouta, M.V., Grimalt, J.O., 2017. First report on organochlorine pesticides in water in a highly productive agro-industrial basin of the Central Valley, Chile. *Chemosphere* 174, 148–156. <https://doi.org/10.1016/j.chemosphere.2016.12.125>.
- Neale, P.A., Munz, N.A., Ait-Aissa, S., Altenburger, R., Brion, F., Busch, W., Escher, B.I., Hilscherová, K., Kienle, C., Novák, J., Seiler, T.-B., Shao, Y., Stamm, C., Hollender, J., 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. *Sci. Total Environ.* 576, 785–795. <https://doi.org/10.1016/j.scitotenv.2016.10.141>.
- Neale, P.A., Braun, G., Brack, W., Carmona, E., Gunold, R., König, M., Krauss, M., Liebmann, L., Liess, M., Link, M., Schäfer, R.B., Schlichting, R., Schreiner, V.C., Schulze, T., Vormeier, P., Weisner, O., Escher, B.I., 2020. Assessing the mixture effects in vitro bioassays of chemicals occurring in small agricultural streams during rain events. *Environ. Sci. Technol.* 54, 8280–8290. <https://doi.org/10.1021/acs.est.0c02235>.
- OECD/ECLAC, 2016. OECD Environmental Performance Reviews: Chile 2016. <https://doi.org/10.1787/9789264252615-en>.
- von der Ohe, P.C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R.-U., Ginebreda, A., De Cooman, W., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. *Sci. Total Environ.* 409, 2064–2077. <https://doi.org/10.1016/j.scitotenv.2011.01.054>.
- Olker, J.H., Elonen, C.M., Pilli, A., Anderson, A., Kinziger, B., Erickson, S., Skopinski, M., Pomplun, A., LaLone, C.A., Russom, C.L., Hoff, D., 2022. The ECOTOXicology knowledgebase: a curated database of ecologically relevant toxicity tests to support environmental research and risk assessment. *Environ. Toxicol. Chem.* 41, 1520–1539. <https://doi.org/10.1002/etc.5324>.
- Overturf, M.D., Anderson, J.C., Pandelides, Z., Beyger, L., Holdway, D.A., 2015. Pharmaceuticals and personal care products: a critical review of the impacts on fish reproduction. *Crit. Rev. Toxicol.* 45, 469–491. <https://doi.org/10.3109/10408444.2015.1038499>.
- Price, P., Han, X., Junghans, M., Kunz, P., Watts, C., Leverett, D., 2012. 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. *Environ. Sci. Eur.* 24, 34. <https://doi.org/10.1186/2190-4715-24-34>.
- R Core Team, 2021. R: A Language and Environment for Statistical Computing.
- Reiber, L., Knillmann, S., Kaske, O., Atencio, L.C., Bittner, L., Albrecht, J.E., Götz, A., Fahl, A.-K., Beckers, L.-M., Krauss, M., Henkelmann, B., Schramm, K.-W., Inostroza, P.A., Schinkel, L., Brauns, M., Weitere, M., Brack, W., Liess, M., 2021. Long-term effects of a catastrophic insecticide spill on stream invertebrates. *Sci. Total Environ.* 768, 144456. <https://doi.org/10.1016/j.scitotenv.2020.144456>.
- Rizzo, L., Malato, S., Antakyali, D., Beretsou, V.G., Dolić, M.B., Gernjak, W., Heath, E., Ivancev-Tumbas, I., Karaolia, P., Lado Ribeiro, A.R., Mascolo, G., McArdell, C.S., Schaar, H., Silva, A.M.T., Fatta-Kassinos, D., 2019. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. *Sci. Total Environ.* 655, 986–1008. <https://doi.org/10.1016/j.scitotenv.2018.11.265>.
- Rodríguez-Gil, J.L., Cáceres, N., Dafouz, R., Valcárcel, Y., 2018. Caffeine and paraxanthine in aquatic systems: global exposure distributions and probabilistic risk assessment. *Sci. Total Environ.* 612, 1058–1071. <https://doi.org/10.1016/j.scitotenv.2017.08.066>.
- Rudén, C., Backhaus, T., Bergman, P., Faust, M., Molander, L., Slunge, D., 2019. Future chemical risk management - Accounting for combination effects and assessing chemicals in groups (No. SOU 2019:45). Stockholm, Swedish Government Official Reports.
- Salamanca, M., Chandía, C., Hernández, R.A., Vargas, F., 2019. First long-term record of halogenated organic compounds (AOX, EOX, and PCDD/F) and trace elements (Cd, Cr, Cu, Fe, Hg, Pb, Ni, and Zn) in marine biota of the coastal zone of southcentral Chile. *Mar. Pollut. Bull.* 146, 442–453. <https://doi.org/10.1016/j.marpolbul.2019.06.074>.
- Schüttler, A., Jakobs, G., Fix, J.M., Krauss, M., Krüger, J., Leuthold, D., Altenburger, R., Busch, W., 2021. Transcriptome-wide prediction and measurement of combined effects induced by chemical mixture exposure in zebrafish embryos. *Environ. Health Perspect.* 129, 47006. <https://doi.org/10.1289/EHP7773>.
- Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquat. Toxicol.* 68, 369–392. <https://doi.org/10.1016/j.aquatox.2004.03.016>.
- Sigmund, G., Ågerstrand, M., Antonelli, A., Backhaus, T., Brodin, T., Diamond, M.L., Erdelen, W.R., Evers, D.C., Hofmann, T., Hueffer, T., Lai, A., Torres, J.P.M., Mueller, L., Perrigo, A.L., Rillig, M.C., Schaeffer, A., Scheringer, M., Schirmer, K., Tlili, A., Soehl, A., Triebkorn, R., Vlahos, P., vom Berg, C., Wang, Z., Groh, K.J., 2023. Addressing chemical pollution in biodiversity research. *Glob. Chang. Biol.* 29, 3240–3255. <https://doi.org/10.1111/gcb.16689>.
- Spilsbury, F.D., Warne, M.St.J., Backhaus, T., 2020. Risk assessment of pesticide mixtures in Australian rivers discharging to the Great Barrier Reef. *Environ. Sci. Technol.* 54, 14361–14371. <https://doi.org/10.1021/acs.est.0c04066>.
- Spilsbury, F.D., Inostroza, P.A., Svedberg, P., Cannata, C., Rogas, A.M.J., Backhaus, T., 2023. Defining the data gap: what do we know about environmental exposure, hazards and risks of pharmaceuticals in the European aquatic environment? *Water Res.* 121002. <https://doi.org/10.1016/j.watres.2023.121002>.
- Svedberg, P., Inostroza, P.A., Gustavsson, M., Kristiansson, E., Spilsbury, F., Backhaus, T., 2023. Dataset on aquatic ecotoxicity predictions of 2697 chemicals, using three quantitative structure-activity relationship platforms. *Data Brief* 51, 109719. <https://doi.org/10.1016/j.dib.2023.109719>.
- Warne, M.St., Batley, G.E., van Dam, R.A., Chapman, J.C., Fox, D.R., Hickey, C.W., Stauber, J.L., 2018. Revised method for deriving Australian and New Zealand water quality guideline values for toxicants – Update of 2015 version. In: Prepared for the Revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian State and Territory Governments, Canberra.
- Webb, M.J., Winter, J.M., Spera, S.A., Chipman, J.W., Osterberg, E.C., 2021. Water, agriculture, and climate dynamics in Central Chile's Aconcagua River Basin. *Phys. Geogr.* 42, 395–415. <https://doi.org/10.1080/02723646.2020.1790719>.
- Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M., Luarte, T., Petrosyan, V., Perikhanian, Y., Mahon, C.S., McGurk, C.J., Hofmann, T., Kormoker, T., Iniguez, V., Guzman-Otazo, J., Tavares, J. L., De Figueiredo, F.G., Razzolini, T.P., Maria, Dougnon, V., Gbaguidi, G., Traoré, O., Blais, J.M., Kimpe, L.E., Wong, M., Wong, D., Ntchantcho, R., Pizarro, J., Ying, G.-G., Chen, C.-E., Pérez, M., Martínez-Lara, J., Otamanga, J.-P., Poté, J., Ifo, S.A., Wilson, P., Echeverría-Sáenz, S., Udikovic-Kolic, N., Milakovic, M., Fatta-Kassinos, D., Ioannou-Tfoa, L., Belušová, V., Vymazal, J., Cárdenas-Bustamante, M., Kassa, B.A., Garric, J., Chaumot, A., Gibba, P., Kunchulia, I., Seidensticker, S., Lyberatos, G., Halldórsson, H.P., Melling, M., Shashidhar, T., Lamba, M., Nastiti, A., Supriatin, A., Pourang, N., Abedini, A., Abdullah, O., Gharbia, S.S., Pilla, F., Chefetz, B., Topaz, T., Yao, K.M., Aubakirova, B., Beisenova, R., Olaka, L., Mulu, J. K., Chatanga, P., Ntuli, V., Blama, N.T., Sherif, S., Aris, A.Z., Looi, L.J., Niang, M., Traore, S.T., Oldenkamp, R., Ogunbanwo, O., Ashfaq, M., Iqbal, M., Abdeen, Z., O'Dea, A., Morales-Saldaña, J.M., Custodio, M., De la Cruz, H., Navarrete, I., Carvalho, F., Gogra, A.B., Koroma, B.M., Cerkvenik-Flajs, V., Gombac, M., Thwala, M., Choi, K., Kang, H., Celestino Ladu, J.L., Rico, A., Amerasinghe, P., Sobek, A., Horlitz, G., Zenker, A.K., King, A.C., Jiang, J.-J., Kariuki, R., Tumbo, M., Tezel, U., Onay, T.T., Lejjou, J.B., Vystavna, Y., Vergeles, Y., Heinzen, H., Pérez-Parada, A., Sims, D.B., Figy, M., Good, D., Teta, C., 2022. Pharmaceutical pollution of the world's rivers. *Proc. Natl. Acad. Sci.* 119, e2113947119. <https://doi.org/10.1073/pnas.2113947119>.
- Yu, X., Yu, F., Li, Z., Shi, T., Xia, Z., Li, G., 2023. Occurrence, distribution, and ecological risk assessment of artificial sweeteners in surface and ground waters of the middle and lower reaches of the Yellow River (Henan section, China). *Environ. Sci. Pollut. Res.* 30, 52609–52623. <https://doi.org/10.1007/s11356-023-26073-7>.
- Zhou, L., Fan, D., Yin, W., Gu, W., Wang, Z., Liu, J., Xu, Y., Shi, L., Liu, M., Ji, G., 2021. Comparison of seven in silico tools for evaluating of daphnia and fish acute toxicity: case study on Chinese Priority Controlled Chemicals and new chemicals. *BMC Bioinformatics* 22, 151. <https://doi.org/10.1186/s12859-020-03903-w>.