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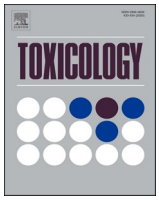

Considerations for future quantitative structure-activity relationship (QSAR) modelling for heavy metals – A case study of mercury

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ABSTRACT

With increasing annual chemical development and production, safety testing demands and requirements have also increased. In addition to traditional animal testing, quantitative structure-activity relationship (QSAR) modelling can be used to predict the biological effect of a chemical structure, based on the analysis of quantitative characteristics of structure features. Whilst suitable for e.g., pharmaceuticals, other compounds can be more challenging to model. The naturally occurring heavy metal mercury speciates in the environment, with some toxic species accumulating in aquatic organisms. Although this is well known, only little data is available from (eco)toxicological studies, none of which account for this speciation behaviour. The present work highlights the current toxicity data for mercury in aquatic animals and gaps in our understanding and data for future QSAR modelling. All publicly available ecotoxicology data was obtained from databases and literature. Only few studies could be determined that assessed mercury toxicity in aquatic species. Of these, likely speciation products were determined using PHREEQc. This highlighted that the mercury exposure species was not always the predominant species in the medium. Finally, the descriptors for the modelled species were obtained from ChemDes, highlighting the limited availability of such details. Additional testing is required, accounting for speciation and biological interactions, to successfully determine the toxicity profile of different mercury species in aquatic environments. In the present work, insufficient mercury-species specific data was obtained, to conduct QSAR modelling successfully. This highlights a significant lack of data, for a heavy metal with potentially fatal repercussions.

1. Introduction

Of the roughly 60,000 chemicals and their derived waste currently in global commerce, many can have harmful effects on environmental or organismal health (UNEP, 2019). In the European Union alone, 62% of the chemicals consumed annually were classified as hazardous to health, of which 35% were further classified as ecosystem hazards (EEA, 2017). One example of the detrimental impact that the environmental release of such a chemical can have, occurred in the 1950 s in the Minamata region, Japan. The effluent of a chemical factory contained mercury, which was transformed into methylmercury in the aquatic environment by bacterial communities (Yokoyama, 2018). Methylmercury is a known (developmental) neurotoxin which bioaccumulates in marine ecosystems, with most of the human exposure linked to dietary uptake from seafood. Methylmercury can cross from the bloodstream into the brain or placenta, leading to developmental alterations in foetuses, as well as affecting children and adults (Kitamura et al., 2020). By 2006, more than 900 people had died and 2 million people suffered from health conditions because of these release events (McCurry, 2006). To avoid such events from reoccurring, the 'Registration, Evaluation, Authorisation, and Restriction of Chemicals' (REACH; EC, 2007) regulation calls for a testing of all existing and novel chemicals. Even beyond the scope of REACH, research on the ecotoxicological impact of compounds has increased, focussing on the effect of compounds on organisms at molecular, individual, population, community, ecosystem, and biosphere levels.

Established tests assess effects of acute and chronic exposure, deriving the lethal (LC) and effect concentrations (EC), or examine bioaccumulation and bioconcentration (Van Straalen, 2003). To this end, the Organization for Economic Cooperation and Development

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(OECD) developed test guidelines (TG) for different terrestrial and aquatic model organisms (OECD, 1981). These aim to provide a better understanding of the impact that environmentally present compounds may have on individuals and populations within the ecosystems (Van Straalen, 2003). Ecotoxicological studies rely on a range of test species from three different trophic levels (de Roode et al., 2006). In aquatic tests, this includes primary producers such as algae species (e.g., *Desmodesmus subspicatus* and *Raphidocelis subscapitata*) and aquatic plants (e.g., *Lemna minor*) in the first instance. Here, growth inhibition tests such as OECD TG 201 (OECD, 2011) or OECD TG 221 (OECD, 2006) can be conducted. Further, acute (OECD TG 202; OECD, 2004) and chronic (OECD TG 211; OECD, 2012) toxicity tests for daphnids are commonly employed. Lastly, various fish species are commonly assessed in ecotoxicological studies, with the selected assay depending on the aim and environment in question. The acute toxicity of a compound over a period of 96 h post fertilisation (hpf) can be determined in the fish embryo acute toxicity (FET) test (OECD TG 236; OECD, 2013a) using developing zebrafish (*Danio rerio*) embryos. Similarly acute toxicity to juvenile fish over 96 h is tested in the fish acute toxicity (AFT) test (OECD TG 203; OECD, 2019) for different fish species (including fathead minnow *Pimephales promelas,* medaka *Oryzias latipes,* three-spined stickleback *Gasterosteus aculeatus,* bluegill *Lepomis macrichirus*, or rainbow trout *Oncprhynchus mykiss/Salmo gairdneri*). For long-term assessment, the fish early life stage (FELS) toxicity test (OECD TG 210; OECD, 2013b) assesses all sub-lethal and lethal effects in developing *D. rerio* over a period of the first 35 days post fertilisation.

Although such assays provide valuable insights into the toxicity of tested compounds, these test methods classify as animal tests (apart from the OECD TG 236; Braunbeck and Lammer, 2006), which is not in line with the current call for alternative test methods (Braunbeck, 2009). Additionally, range finding experiments are required for novel and untested compounds, which may require a high number of organisms to be sacrificed along with being costly and time consuming (de Roode et al., 2006). A method for the generation of additional (eco-)toxicological insights which has been applied increasingly to various scientific quests in recent years is quantitative structure-activity relationship (QSAR) modelling, where physical and/or chemical properties are utilised to estimate toxic effect concentrations mathematically (EFSA, 2010). This relies on existing toxicological datasets which are utilised to predict the behaviour of an untested compound based on its descriptor properties. Such descriptors include molecular weight, water solubility, the octanol-water partitioning coefficient, boiling and melting point, and vapour pressure (Piir et al., 2018), as well as topological descriptors such as e.g., the Zagreb or the Weiner index (Vračko, 2015). Classical QSAR models are based on multilinear regression (MLR), but with technological advancements and machine learning, such models have developed, now commonly based on algorithms such as k-nearest neighbours (k-NN), linear discriminant analysis (LNA), decision trees (DT), or random forests (RF) (Piir et al., 2018). Ecotoxicology QSARs may, for example, inform scientific data gaps and pollution prevention measures, reduce the reliance of laboratory studies and the cost, time, and labour required in compound development (Cronin et al., 2003).

With the plethora of studies addressing e.g., the risks of acute and chronic exposure to heavy metals such as mercury, the lack of QSAR modelling approaches may be linked to other parameters. Mercury is a naturally occurring heavy metal also found in hydrocarbon reservoirs and can be present in different forms (i.e., species) depending on environmental parameters (Benoit et al., 1999; EA, 2019; Fitzgerald et al., 2007; Hammerschmidt and Fitzgerald, 2004; Zhao et al., 2017). According to the Global Mercury Assessment of 2018, anthropogenic emissions have increased the global atmospheric mercury by 450 %, leading to increased terrestrial and oceanic deposition of 310 % (UNEP, 2018). This is further impacted by the speciation behaviour of mercury under different conditions, leading to the formation of species that are more, or less, bioavailable than others. In the marine aquatic environment common mercury species include insoluble forms (such as cinnabar (HgS), calomel (Hg_2Cl_2), mercuric oxide (HgO), and elemental mercury (Hg^0)), as well as soluble mercury-chloride complexes ($HgCl_2$, $HgCl_3^-$, $HgCl_4^{2+}$), and dissolved or particulate organic matter complexes (DOM-Hg and POM-Hg, respectively) (Gworek et al., 2016). More bioavailable mercury species can also be absorbed and methylated by bacteria into the neurotoxin methylmercury (Bełdowska and Falkowska, 2016; Benoit et al., 1999). The speciation behaviour highlights a potential challenge with applying QSAR modelling to compounds such as mercury. However, only one publication could be determined predicting mercury toxicity to nematodes through QSAR modelling (Tatara et al., 1997). They successfully predicted toxicities based on the log k(OH) however, further indicated that speciation affects the accuracy of such approaches. Publications assessing mercury toxicity will frequently provide information regarding the initial mercury compounds used for the exposure, but not the species formed in the medium through e.g., photodegradation, metabolism, or chemical speciation.

1.1. Aims and objectives

This work aims to highlight the issues with considering mercury as a single metal species in QSAR modelling, rather than assessing the speciation in environmental conditions. To this end, publicly available ecotoxicological data for mercury was obtained and chemical speciation modelling conducted, to determine all mercury species potentially present in the media used in the respective studies. Then, descriptors were determined for these species, aiming to conduct QSAR on the obtained data.

2. Materials and methods

A literature search for published data on ecotoxicological studies with mercury was conducted. The data gathered was then used to model the possible chemical speciation of mercury in the exposure medium using PHREEQc (Parkhurst and Appelo, 2013). The modelled species were then researched, and descriptors obtained using the ChemDes platform (Dong et al., 2015). For data analysis and visualisation, the packages 'tidyverse' (Wickham et al., 2019), and 'ggpubr' (Kassambara, 2023) were used in R-Studio version 4.2.1 (R Core Team, 2019).

2.1. Ecotoxicology data search

Published literature and databases were searched, to obtain the 50 % effect and lethal concentrations (EC_{50} and LC_{50}, respectively) for acute toxicity of mercury. Only databases that were publicly accessible and free of charge were used: The CompTox Chemistry Dashboard (Williams et al., 2017), PubChem (Kim et al., 2023), and eTox (Cases et al., 2014). References were checked to avoid the duplication of datasets. Databases were searched for data linked to any mercury compound and the data downloaded unaltered. References were reviewed and the data updated where necessary. The literature search was conducted on Web of Science, PubMed, and Scopus. Search terms were: 'Mercury' AND 'Acute toxicity' AND 'Plankton' OR 'Algae' OR 'Daphnia' OR 'Artemia' OR 'Crustacean' OR 'Zebrafish' OR 'Fish'. Here all publications available in German or English were included. In addition to directly determined publications, cross-references made to publications were also reviewed. All publications that could be accessed are included in A.1.

2.2. Speciation modelling

Mercury speciation was modelled using PHREEQC version 3.6.2 (Parkhurst and Appelo, 2013), with the minteq.v4 (Felmy et al., 1984) and Thermodem V1.10 (Blanc, 2017) databases. PHREEQC is a hydro-geochemical modelling software, where specific reaction environments are constructed and considered reactants defined. The thermodynamic log K reaction constant is then used to simulate both static and dynamic chemical reactions. Compound speciation in the aquatic

environment can be simulated in PHREEQc using an ion-association aqueous model. The minteq.v4 database was used for speciation analyses of inorganic mercury compounds, while the Thermodem V1.10 database was used for speciation analyses of organic mercury species. Sufficient information about the exposure medium was only provided for MBL and Steinberg medium (Rodrigues et al., 2013), artificial water (Hassan et al., 2012; Kalčíková et al., 2012; Simão et al., 2021; Törökné, 2004; von Hellfeld et al., 2020; Xiong et al., 2022), and Elendt M7 medium (Elendt, 1990). To model the speciation behaviour, 1 mMol of either inorganic Hg(II) or organic CH_3Hg^+ was added to 1 litre of each medium, to make the output comparable between the different experimental set-ups. Output species were selected in PHREEQc, based on chemical feasibility, removing all non-existing species. The output was given in mMol and transformed into percentage fractions of total mercury. Here, a full dissociation of added Hg compounds at static temperature, volume, and pH was assumed. The modelled solutions do not consider the possible interaction of solutes and exposure vessels as well as potential changes elicited by biotic activity (e.g., oxygen depletion, metabolism) or abiotic influences (e.g., photodegradation).

2.3. Descriptor determination

For each mercury species modelled in the PHREEQc speciation approach outlined above, a structure data file ('.sdf' format) of the species' 2D structure was obtained from PubChem. From the 28 species modelled as present in the different media, shape files for 16 could be obtained, the remaining 12 species were not listed on PubChem. This data gap could be addressed with structure drawing programmes. However, in the present study the lack of toxicity data for all speciation compounds limited the suitability of such methods. Thus, the 16 obtained shape files were entered into ChemDes, to generate constitution, topology, connectivity, kappa, and molecular indices descriptors. Geometrical descriptors from the 3D coordinates of atoms in the given molecule were not considered, as they require e.g., geometric optimisation (Danishuddin, 2016). All obtained results were retained, although identical read-outs for many descriptors were obtained for all species.

28 species of mercury were modelled to be formed within the exposure media used in the cited studies. Toxicity data for 4 species were determined in the literature search, but for only 2 of these ($HgCl_2$ and CH_3HgCl) enough information was published, to be suitable for QSAR modelling, in accordance with published best practice (Piir et al., 2018). However, none of the determined toxicity data could be used for QSAR modelling in this case, as either only one mercury species was tested in each test organism species, or only effect or lethal concentrations, but not both, were published. Due to a lack of sufficient descriptor data, no QSAR modelling could be conducted as part of this study, highlighting significant data and knowledge gaps.

3. Results

3.1. Ecotoxicological data

31 publications describing mercury toxicity in different test organisms were determined, published between 1974 and 2022, in which 44 tests were run, assessing different endpoints (Fig. 1A). Of all listed studies, only 20 followed an OECD TG, with TG 202 being most frequently employed (7), followed by 201, 212, and 236 (3), then 203, 210, 221, and 235 (1) (Fig. 1B). The number of endpoints assessed per study were commonly < 4 (Fig. 1C). In the determined studies, the most used mercury compound in the exposure experiments was $HgCl_2$ (23

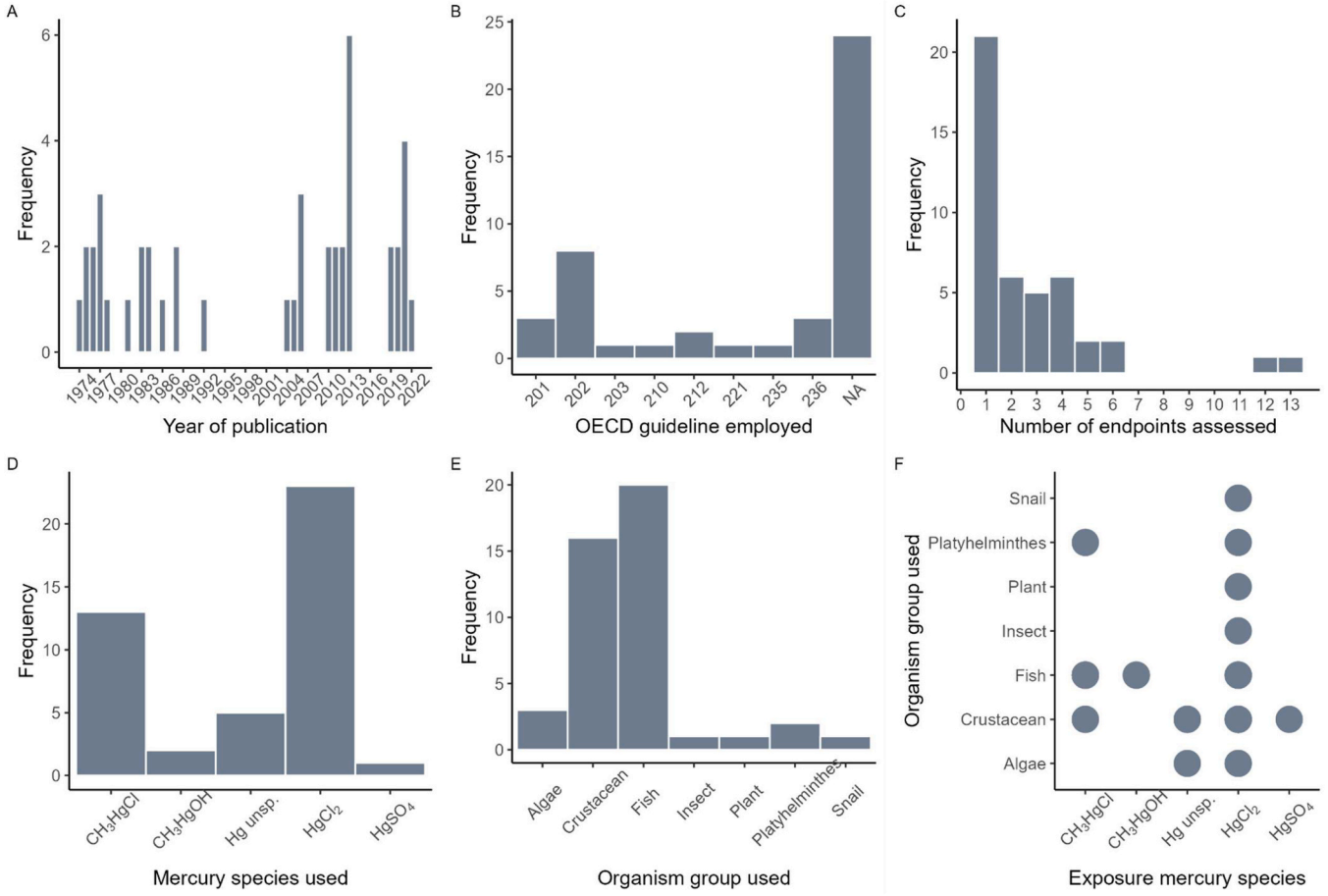

Fig. 1. Summary graphs for all publicly accessible ecotoxicology studies for various mercury species. See A.1 for details.

tests), followed by CH_3HgCl (13 tests), undefined Hg species (5 tests), CH_3HgOH (2 tests), and $HgSO_4$ (1 test) (Fig. 1D). The most assessed organism group were fish (20 times) and crustaceans (16 times), followed by algae (3 times), platyhelminths (twice), and insects, snails, and plants (once) (Fig. 1E). Lastly, only $HgCl_2$ was assessed in all organism groups, while CH_3HgCl was assessed in 3 groups, an unspecified mercury species in 2 groups, and CH_3HgOH and $HgSO_4$ in one group each (Fig. 1F).

3.2. Speciation modelling

Only a total of six experimental publications provided enough detail for the PHREEQc modelling approach of this work (Fig. 2). These publications only used $HgCl_2$ and MeHgCl as exposure compounds, and a total of 5 different exposure media were used. The chemical modelling carried out using the PHREEQC software predicted a different speciation behaviour for $HgCl^2$ in the three media: MBL and Steinberg medium, as well as artificial water. The results suggest that majority of $HgCl_2$ in artificial water is $HgCl_2$ (64%) and HgClOH (30%), with various other species forming to negligible fractions, summarised as 'others' in Fig. 2. In MBL medium, $HgCl_2$ speciates to the tetrachlorido-mercury ($HgCl_4^{-2}$, $>$ 99 %). The prominent mercury species formed in the Steinberg medium were modelled to be the hydroxide ($HgOH_2$, $\sim$ 45%) and ethylenediaminetetraacetic acid (EDTA) chelates (Hg-$EDTA^{-2}$, $\sim$ 50 %). MeHg speciation did not vary between artificial water and the M7 Elendt medium, with C_2H_6Hg comprising 100 % of all formed species. See A.2 for more details on the speciation modelling results and the species summarised as 'others'.

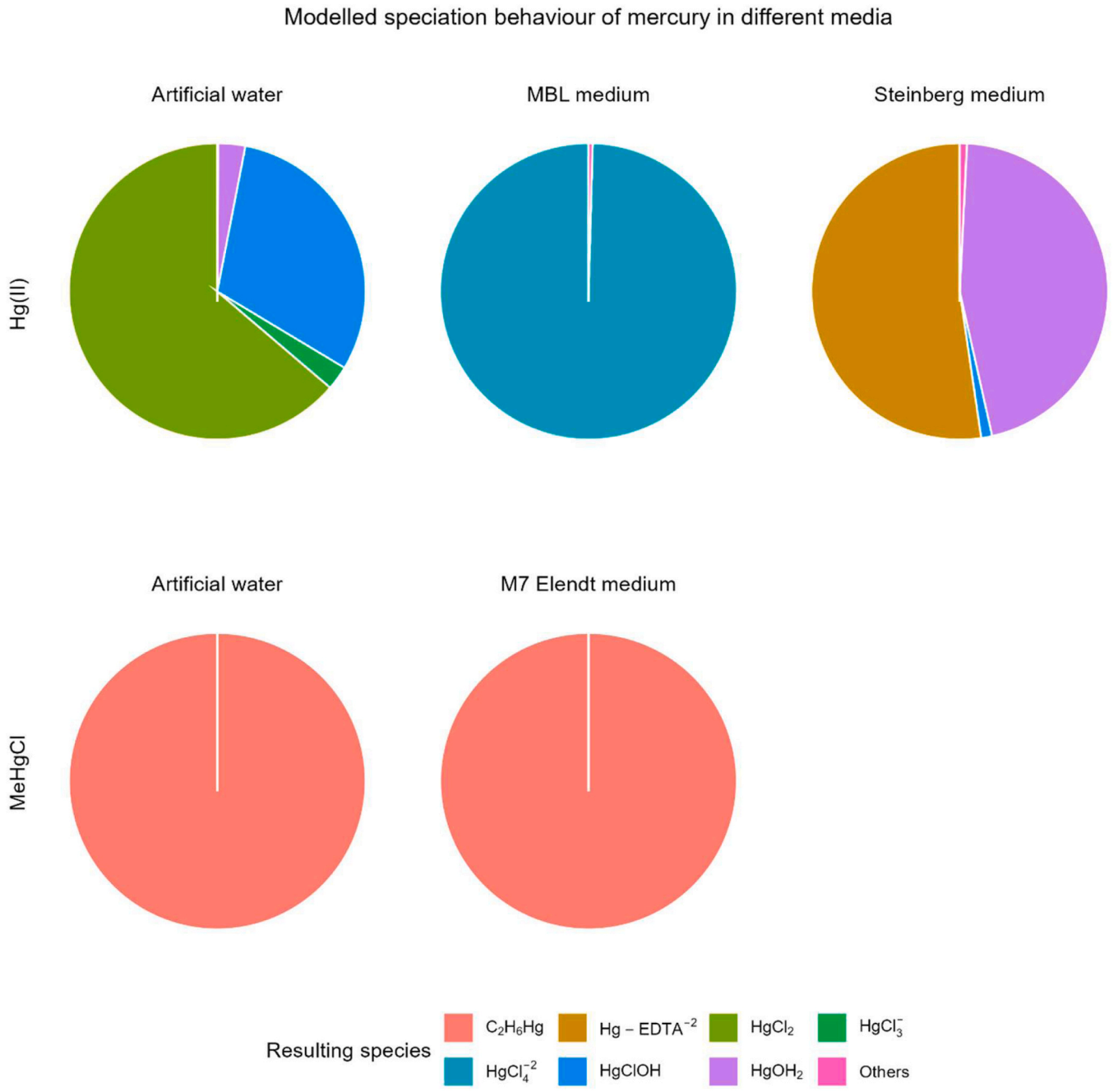

Fig. 2. Summary graphs speciation modelling using PHREEQc, for $HgCl_2$ and MeHgCl exposure in artificial water, MBL, Steinberg, and M7 Elendt medium. See A.2 for details.

3.3. Descriptors determination

No shape file could be obtained for the following species: $HgOH_3^-$, $HgHCO_3^+$, $Hg(CO_3)_2^{-2}$, Hg-$EDTA^{-2}$, Hg-$(EDTA_2)^{+2}$, $CH_3HgCO_3^-$, CH_3HgHCO_3, $CH_3HgSO_4^-$, CH_3HgOH, $(CH_3Hg)_2OH^+$, $((CH_3Hg)_2OH)^+$, and $(CH_3HgHPO_4)^-$. For the remaining species, shape files could be obtained and were entered into ChemDes. A total of 30 constitution, 35 topology, 44 connectivity, 7 kappa, and 6 molecular indices descriptors were provided by ChemDes. For Hg(II), HgClOH, $HgOH_2$, $HgCO_3$, $HgOH^+$, $HgSO_4$, Hg_2^{+2}, $Hg(NO_3)_2$, $Hg(NO_3)^+$, and CH_3HgCl, no topological descriptors could be determined. Additionally, some descriptors provided a value of zero for all species, two descriptors returned "nan" for some of the species (the total information index on distance equality (IDET), and the mean Randic connectivity index (mChi1)) and one descriptor returned "-inf" for two species (the mean information index on distance equality (IDE)) (See A.3).

4. Discussion

A plethora of publications allude to the devastating impacts of methylmercury exposure to humans and other animals outside of the ecotoxicological context (Aschner and Syversen, 2005; Farina et al., 2011; Grajewska et al., 2019; Lee and Freeman, 2014; NRC, 2000; US EPA, 2002). However, as part of the present work, only 31 publicly accessible publications under the above-described search criteria for ecotoxicological data could be determined (Fig. 1), providing a total of 116 datapoints. Of these 31 publications, not all were determined suitable for QSAR modelling, as not all datapoints are in line with best practices (Piir et al., 2018). This includes detailed reporting on the characterisation of the chemicals and their molecular structure used in the model development (such as chemical name, CAS numbers, InChi code, or other identifiers). Here, only 6 publications provided the compounds' CAS number (Devlin, 2006; Huang et al., 2011, 2010; Simão et al., 2021; von Hellfeld et al., 2020; Yu et al., 2019), while various others provided no further information about the compounds other than a generic compound name (Barbieri et al., 2005; Reeve et al., 1977; Roales and Perlmutter, 1974; Törökné, 2004; Umarani et al., 2012; Wobeser, 1975; Zamani-Ahmadmahmoodi et al., 2020). In their publication proposing QSAR modelling best practices, Piir et al., (2018) further recommend that the chemical structure, pre-treatment manipulations, as well as information about the chemical properties, or additional experimental data should be published. This includes the presentation and source of experimental data itself, the definition of measured endpoints, and the nature of the data. Additionally, homogeneity of acute toxicity data for QSAR modelling is vital (Tropsha, 2010), which requires that all gathered data is filtered by species (to gather data for at least one species for each trophic level) and defined time of exposure (to ensure the same exposure time/duration), as well as ensuring comparability of endpoints and measured effects (Gramatica et al., 2016). To further support the reliability of the data and the accuracy of the resulting QSAR predictions, data should only be considered if the determined EC or LC values do not exceed the range of tested concentrations, i.e., extrapolated data (Tropsha, 2010).

Considering these best practices and the model requirements, a total of 76 datapoints would have to be excluded from the gathered dataset (see A.1). This would include the removal of all datapoints for platyhelminths and snails, two of the three datapoints gathered for algal species (Rodrigues et al., 2013; Zamani-Ahmadmahmoodi et al., 2020), one of three datapoints for plant species (Rodrigues et al., 2013), 15 of the 22 crustacean datapoints, and 55 of the 83 datapoints for fish (see A.1). The excluded datapoints include the lowest recorded toxicity values for many species, and further limit the suitability of the dataset. As per best practices, the EC and LC values used in QSAR modelling should be derived from the same species and experimental procedure (Gramatica et al., 2016; Tropsha, 2010). However, when removing the datapoints highlighted as unsuitable in the present work, no complete dataset (i.e., LC and EC values) remain for a given organism. Thus, no QSAR modelling with the determined dataset could be conducted after applying the best practices guidelines.

It should further be considered that most test organisms in the 'QSAR suitable' dataset are freshwater species, with exception of the olive flounder (*Paralichthys olivaceus*), read seabream (*Pagus major*), turbot (*Psetta maxima* or *Scophthalmus maximus*), and yellow croaker (*Larimichthys crocea*). This significantly limits the ability for such data to predict the toxicity of mercury to marine species. This is relevant, as mercury speciation is impacted by e.g., the presence of chloride. Moreover, mercury is known to be associated with e.g., hydrocarbon reservoirs and their extraction or activity around these may lead to increased risk for marine organisms (Gissi et al., 2022; Kho et al., 2022). Interestingly, the OSPAR Commission for the protection of the marine environment suggests the use of marine species such as turbot *S. maximus* and the sheephead minnow *Cyprinodon variegatus*, in the determination of acute fish toxicity for compounds that may enter the marine environment, such as those used in the offshore industry (OSPAR, 2000). However, only one publication examined in the process of this study utilised *S. maximus* (Mhadhbi et al., 2010), while no data for *C. variegatus* could be determined. Such data would have significant benefits, not only for ecotoxicological QSAR modelling, but also for e.g., environmental impact assessments in offshore decommissioning proposals (Gissi et al., 2022; Kho et al., 2022).

4.1. Chemical speciation modelling

The chemicals speciation modelling conducted here was limited by the fact that only a total of 31 studies could be determined, and only in 10 of these experiments, enough detail about the exposure medium was available for speciation modelling: Elendt M7 medium (Elendt, 1990), Artificial water (ISO, 1996), MBL medium (Nichols, 1973), and Steinberg medium (ISO, 2005). In MBL medium, the use of $HgCl_2$ leads to a formation of a total of 18 different mercury species, of which $HgCl_4^{-2}$ accounted for almost 100 % of all species. The formation of the tetrachlorido-mercury complex is mostly likely due to the presence of chloride ions in the medium, in the absence of other complexing agents such as organic matter (OM) (Lee et al., 2019). In Steinberg medium, a total of 15 species were formed, the majority of Hg which where $HgOH_2$ (46 %) and Hg-EDTA-2 (52 %). Hg(II) hydroxide has been observed in e.g., solid neon and argon (Wang and Andrews, 2005), but is not known to form under natural conditions. More information was available for EDTA-complexed mercury, however. Considering the widespread incidence of EDTA in the environment, formation of metal complexes is thought to promote the bio-uptake of e.g., Hg(II) (Thomas and Gaillard, 2015). In the medium, only limited amounts of chloride present, which may account for the formation of non-chloride complexed mercury species. In artificial water, 14 species were formed when using $HgCl_2$, with $HgCl_2$ and HgClOH representing to 64 % and 31 % of formed species. As previously described for MBL medium, the presence of chloride ions in the medium explains the formation of chloride complexation of mercury species. When using CH_3Hg^+ in artificial water, a total of 9 species are formed, with C_2H_6Hg accounting for almost 100 % of formed species. Similarly, when using CH_3Hg^+ in Elendt 7 medium, C_2H_6Hg accounts for almost 100 % of the 7 formed species. Dimethylmercury, although more toxic than monomethylmercury, is also a less stable mercury species in the natural environment and will convert to monomethylmercury at a pH-dependent rate (Black et al., 2009).

Modelling suites such as PHREEQc are highly adapted to their purpose and are capable of accurately representing the potential speciation formation in various media. However, such approaches are dependent on e.g., the accuracy of the input data and the reliability of underlying calculations (Parkhurst and Appelo, 2013), while further limitations include the difficulty to include e.g., photodegradation (West et al., 2022). With test methods such as the OECD TG 236 having a strictly

regulated light-dark cycle for optimal zebrafish embryo development (OECD, 2013a), it can be hypothesised that such parameters may impact the speciation of organic mercury in aqueous media. It should also be noted that whilst chemical speciation modelling can provide insight into the species to be expected, as well as the proportions that would occur under static conditions, factors such as e.g., adsorption to glass/plastic exposure vessel, photodegradation, or metabolic alterations are not taken into consideration in the present approach but should be included in future works. Such limitations can only be addressed by quantifying all mercury species at the beginning and end of the experiment (or pre and post medium renewal in e.g., the FET test).

The limited number of media that were suitable for chemical speciation modelling were due to the inclusion of naturally occurring or added dissolved organic matter (DOM) without further definition in many of the remaining media (or lack of sufficient detail for others). A review assessing the interactions between mercury and DOM in aqueous solutions determined that the presence of DOM affected the speciation, solubility, mobility, and toxicity of mercury (Ravichandran, 2004). DOM can in- or decreased the formation of methylmercury, as the complexation of Hg(II) with DOM overall limits its availability for methylation (Barkay et al., 1997), while the fulvic and humic acid fractions in DOM can reduce ionic mercury the more volatile elemental mercury (Alberts et al., 1974). Stability constants for mercury-DOM complexes span over 25 orders of magnitude, thus forming a large uncertainty in speciation modelling (Ravichandran, 2004). This is complicated further by the impact of DOM on speciation being dependent on other environmental parameters (Benoit et al., 1999).

4.2. Chemical descriptor determination

The results of the present work highlight the limitation of not only sufficient ecotoxicological data for mercury species of environmental relevance, but also the chemical information of formed species. Of the determined 28 mercury species, entries for only ~60 % could be found in the open chemistry database of the National Institutes of Health. This significantly limited the amount of information that could further be extracted for each modelled species from the ChemDes platform. Additional limitations for the availability of topological descriptors for 10 mercury species for which other descriptors could be obtained also impeded the work. Moreover, whilst certain descriptors could be obtained, these may not be the most suitable when addressing heavy metals in ecotoxicological contexts. A recent publication on metal-organic networks analysis determined the most suitable molecular descriptors for their purpose to being the harmonic index, the reciprocal randic index, the modified version of the forgotten index, and various redefined or modified versions of the Zagreb topological indices (Zaman et al., 2023). Of these, two variations of the harmonic index, one variation of the randic index, and four Zagreb indices were determined through ChemDes for the present mercury species. No values for the forgotten index were provided. In addition, definitions for the some of the descriptors derived from the platform could not be obtained, limiting the interpretation of the data obtained.

Taking the nature of the chemical in question into account influences the choice of suitable descriptors (Bertoni et al., 2021). However, it has also been suggested that special attention here was paid to descriptors which consider the context of the aquatic environment and the metal's effect path, alongside classic chemical descriptors (Lepădatu et al., 2009). For example, the molecular target for metal ions can be both the active site of biological receptors and a ligand (McCloskey et al., 1996). However, limited discussion on this matter can currently be found in published literature, in the context of aquatic ecotoxicology QSAR modelling of heavy metals. The majority of the over 70 publications addressing heavy metal QSAR modelling, focused on terrestrial systems, and thus did not face the limitation of compound speciation during dosing, as this rarely includes aqueous media (Wang et al., 2018). Of those assessing aquatic datasets, one study utilised the criteria maximum concentrations (CMCs) of 10 metals (US EPA, 2009) to determine the CMCs for a further 25 metals using quantitative ion character-activity relationship (QICAR) modelling (Wu et al., 2013). Although successfully determining the most significant descriptors for metals and metalloids, the authors acknowledged that metal speciation and complexation, as well as environmental interactions post a significant challenge to correlating metal toxicity with physical or chemical properties. The importance of accounting for speciation to determine the proportion of biologically active heavy metals has long been accepted in e.g., the water quality criteria development. To determine the effect of copper on organisms, for example, it was determined that organic complexes were non-bioavailable, whilst the amount of free copper ions were imperative for determining the metals toxicity (Allen and Hansen, 1996).

4.3. Data suitability for ecotoxicological QSAR modelling

Although ecotoxicological data for 4 mercury species ($HgCl_2$, $HgSO_4$, CH_3HgCl, and CH_3HgOH) could be obtained from published works, toxicity data for the remaining species is missing. Moreover, whilst toxicity data was published for 4 mercury species, the results for only two of these were published in a manner that were sufficient for the use of QSAR modelling approaches, in accordance with best practices (Piir et al., 2018). In addition, the remaining EC and LC values for $HgCl_2$ and CH_3HgCl were not derived for the same timepoints and organisms, thus further hindering its application to QSAR modelling. However, it should be kept in mind that the chemical speciation work conducted here showed that not just the initial exposure species are likely present in the medium, but also various other mercury species. Thus, the published EC and LC values are potentially a result of the exposure to the mixture of mercury species during the experiment. To address such shortcomings, methods otherwise applied to e.g., physiologically based pharmacokinetic (PBPK) modelling, where compound concentrations are measured in the exposure medium and all vital compartments of the exposed organism, as well as taking e.g., partitioning coefficients with the exposure vessel and surrounding air into consideration (Siméon et al., 2020). The previously listed QSAR heavy metal modelling limitations were supported by others, indicating that investigating the toxicity of metals and metal mixtures is complicated by the fact that several metals are also vital elements for organism health and that there are organism-dependent active uptake mechanisms, along with metal-species dependent bioavailability and absorption rates (Khan et al., 2020; Norwood et al., 2003; Sauvé et al., 2000).

5. Conclusion

This study set out to determine the current state of available ecotoxicological data for mercury for QSAR modelling. An extensive search for data regarding acute and chronic toxicity of mercury species commonly used in laboratory exposure experiments was conducted and a limited number of suitable studies was determined. Many studies either lacked the necessary details regarding the exposure compound or medium or were conducted in set-ups with were not in line with currently accepted guidelines. Where the exposure medium was described in sufficient detail, the chemical speciation of mercury was modelled, and all available chemical and physical parameters concerning these species were gathered. The speciation modelling highlighted that, in the absence of effective chelating compounds, hydroxide species commonly formed in freshwater media, whilst chloride complexes were dominant in saline solutions. Although descriptor information for some of the modelled mercury species could be obtained, no QSAR modelling was conducted, due to the lack of toxicity data outlined above.

This study has highlighted that more detailed studies are needed, before ecotoxicological QSAR modelling for heavy metals can be conducted. This includes quantifying the speciation and uptake rates, which were determined as the limiting factor for the present work. Heavy

metals are released into the environment through natural and anthropogenic processes. Due to their bioaccumulation and biomagnification potential, it is vital to augment the data landscape for future QSAR modelling approaches. This would significantly improve our understanding of the potential risk for not just some, but all environmentally present heavy metal species for any ecosystem of interest.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Astley Hastings reports financial support was provided by The UK Energy Research Centre.

Data Availability

Data will be made available on request.

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Appendix (A) material list – All can be found in the attached Excel document

A.1. Published ecotoxicological data for mercury and methylmercury in various aquatic species.

A.2. PHREEQc chemical speciation modelling output.

A.3. Chemical descriptors.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tox.2023.153661.

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