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Title: Sulfate sensitivity of aquatic organism in soft freshwaters explored by toxicity tests and species sensitivity distribution

Year: 2023

Version: Published version

Please cite the original version:

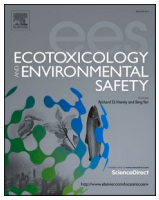
Karjalainen, J., Hu, X., Mäkinen, M., Karjalainen, A., Järvistö, J., Järvenpää, K., Sepponen, M., & Leppänen, M. T. (2023). Sulfate sensitivity of aquatic organism in soft freshwaters explored by toxicity tests and species sensitivity distribution. Ecotoxicology and Environmental Safety, 258, Article 114984. https://doi.org/10.1016/j.ecoenv.2023.114984

Ecotoxicology and Environmental Safety 258 (2023) 114984

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Sulfate sensitivity of aquatic organism in soft freshwaters explored by toxicity tests and species sensitivity distribution

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ARTICLE INFO

Edited by G. Liu

Keywords:
Fish
Hazardous concentration
Invertebrates
Macrophytes
Phytoplankton
Aquatic risk assessment

ABSTRACT

Elevated concentrations of sulfate in waterways are observed due to various anthropogenic activities. Elevated levels of sulfate can have harmful effects on aquatic life in freshwaters: sulfate can cause osmotic stress or specific ion toxicity in aquatic organisms, especially in soft waters where Ca^{2+} and Mg^{2+} concentrations are low. Formerly, chronic toxicity test data in soft water have been scarce. The chronic and acute sulfate toxicity tests conducted with aquatic organisms from 10 families across various trophic levels in this study multiplied the number of tests conducted in soft freshwater conditions and enabled derivation of the species sensitivity distribution (SSD) and sulfate hazardous concentrations for soft freshwaters. The cladoceran *Daphnia longispina* and freshwater snail *Lymnaea stagnalis* were the most sensitive to sulfate among the studied species. Harmful effects on the reproduction of *D. longispina* were observed at 49 mg SO_4 /L while growth of *L. stagnalis* was inhibited at 217 mg SO_4 /L. Most studied organisms tolerated high sulfate concentrations: the median of chronic effective concentrations (EC10 or LC10) was 1008 mg/L for all the species tested in this study. Based on the species sensitivity distribution of the studied species the hazardous concentration for 5 % of aquatic organism (HC5) in soft waters was 117–194 mg SO_4/L. Different data set combinations were used to demonstrate the data variability in SSD-based HC5 estimates. The lowest values were produced from combining biotest results from the present study and earlier literature, while the highest values were calculated from the present study only. The derived chronic no-effect concentrations (PNEC) varied between 39 and 65 mg SO_4/L.

1. Introduction

Elevated concentrations of sulfate anion (SO_4^{2-}) in waterways are observed due to anthropogenic activities such as tilling of soil and fertilization in agriculture, forest management, municipal sewer systems, pulp production, mine drainage and some other industrial processes (Ekholm et al., 2020; Elphick et al., 2011). Hydrological modification in coastal zones, increased atmospheric sulfur (S) and differences in wetting/drying cycles induced by climate change cause also changes in sulfate loads (Johnson et al., 2019). In Finnish surface waters, diffuse sulfate-rich runoff waters from arable lands and timber growing areas dominate the sulfate loads (65 % of total load) whereas waste waters from industry (35 %) may also increase locally the salinity in the recipient waters: the diffuse sulfate loads to rivers from large discharge areas can be over 100 000 t/yr and the maximum loads from the industrial point sources can exceed 20 000 t/yr (Ekholm et al., 2020). Sulfate may cause freshwater salinization (Canedo-Argüelles et al., 2018), affect stratification and biochemical cycles in freshwaters and thus, alter nutrient availability and oxygen resources in lake hypolimnion (Holmer and Storkholm, 2001; Leppänen et al., 2017; Orem et al., 2011). Despite the high loads of sulfate to freshwaters for decades, only recently has attention been paid to local water quality guidelines (WQG) developed for sulfate (Elphick et al., 2011; Sahlin and Ågerstrand, 2018). So far, sulfate has not been included in the regularly monitored species in the environment quality standard guidelines of EU water framework directive (European Commission, 2018).

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https://doi.org/10.1016/j.ecoenv.2023.114984
Received 17 January 2023; Received in revised form 2 May 2023; Accepted 3 May 2023
Available online 10 May 2023

Natural background sulfate concentrations in soft boreal lakes and rivers are low, typically around 3–30 mg/L (Sahlin and Ågerstrand, 2018; Ekholm et al., 2020), but in Finland, anthropogenic activities have created several dozen hotspots, where sulfate concentrations in freshwaters have been measured to be multi-fold varying from 50 mg/L to over 2000 mg/L (Ekholm et al., 2020). Elevated levels of sulfate can have harmful effects on aquatic life in freshwater, and the most sensitive species reported previously was a cladoceran *Ceriodaphnia dubia* with the effective concentration (EC10) of 137 mg/L (Elphick et al., 2011). Sulfate can cause osmotic stress or specific ion toxicity in aquatic organisms, especially in soft waters where Ca^{2+} and Mg^{2+} concentrations are low (Davies and Hall, 2007; Elphick et al., 2011; Griffith, 2017). Because the toxicity of sulfate is dependent on concentrations of other major ions, with a general decrease in toxicity associated with an increase in water hardness (Elphick et al., 2011; Erickson et al., 2022), additional data of sulfate toxicity in soft waters are needed to further develop sulfate WQGs for waters of low hardness (Elphick et al., 2011; Karjalainen et al., 2021; Meays and Nordin, 2013; Sahlin and Ågerstrand, 2018).

Species sensitivity distribution (SSD) is a statistical approach that is used to estimate either the concentration of a chemical that is hazardous to no more than a certain proportion of all species (default value HC5 i.e., hazardous concentration for 5 % of species) or the proportion of species potentially affected by a given concentration of a chemical (Aldenberg and Jaworska, 2000; Fox et al., 2021). An SSD is derived by fitting a selected statistical model or models to compound- and species-specific ecotoxicity data (Posthuma et al., 2019). European Commission (2018) guidance advise using chronic effective concentrations (EC10) and acute EC50 values for the derivation of a long-term (annual average environmental quality standard, AA-EQS) and short-term (maximum acceptable concentration, MAC-EQS) quality standard for the freshwater community, respectively. In addition, at least 10 EC values from different species across at least 8 different taxonomic groups are suggested for SSD analysis. In the risk assessment of local aquatic environments, the SSD-derived HCs have been divided by assessment factors (AF) to estimate the predicted no-effect concentrations (PNEC) of chemicals, metals, and drugs to protect ecosystems from the toxicity of different harmful substances (Lei et al., 2010; Huang et al., 2018; Park and Kim, 2020). The magnitude of AF is both a scientific and a policy-based decision (Belanger and Carr, 2019) and AF should be high if the degree of uncertainty is high (Sorgog and Kamo, 2019). The selection of AF should be based on known sources of uncertainty and variability (Belanger and Carr, 2019).

In this study, ecotoxicity data on 13 different aquatic species from 10 taxonomic groups including teleost fishes, bivalves, gastropod, cladoceran, rotifer, oligochaetan, green algae and plant were obtained following modified standard test procedures to derive the chronic and/or acute effective concentrations and/or lethal concentrations (LC) for SSD modelling. Species from different taxonomic groups and trophic levels were selected to represent boreal aquatic ecosystems and the toxicity tests were carried out in filtered water of the River Kokemäenjoki (Southern Finland) and Lake Southern Konnevesi (Central Finland). PNECs for adverse effects of sulfate with variable assessment factors were derived then based on chronic SSD estimated hazardous concentrations. Furthermore, different combinations of data (ecotoxicity data obtained in this study and from literature) were adopted in modelling to demonstrate the effect of sample size and data variation on the chronic HC5 and PNEC approximation by different AFs. The HC5s of acute SSDs were also estimated to derive acute to chronic ratios of SSD-based HC5s. The acute SSDs can be used to evaluate a short-term quality standard (MAC-EQS) for sulfate in soft freshwaters.

2. Material and methods

2.1. Toxicity tests

Test species, durations and endpoints were selected from 10 families (Salmonidea, Cyprinidea, Unionidea, Lymnaeidae, Daphniidae, Lumbriculidae, Chironomidae, Araceae, Brachionidae, Selenastraceae) to achieve wide assortment of aquatic organisms that meet the guidance of European Commission (2018) for deriving environmental quality standards. Chronic exposures were conducted for all species and some species had also acute test endpoints from different developmental stages such as glochidia tests of bivalves or fertilization tests of fish in the beginning of the chronic tests. Species selected also included endangered organisms from Kokemäenjoki ecosystem (migratory whitefish *Coregonus lavaretus*, sea-migratory brown trout *Salmo trutta* and thick-shelled river mussel *Unio crassus*) as well as species (i.e., cladoceran *Daphnia longispina*) which is potentially sensitive to sulfate even though not typically observed in the Kokemäenjoki ecosystem. Part of the toxicity tests were conducted by the modified standard test procedures, which were originally designed for tests in hard waters only (in detail below and in the Supplementary material S1). Due to the lack of standardized test protocols for several important species included in the present study, we developed new methodology with high quality control following key principles of other similar standard protocols.

Ecotoxicity data on 13 different aquatic species were obtained (Table 1). All tests were done in soft water with water hardness calculated from the Mg and Ca concentrations (SFS-EN ISO 11885:2009a, 2009b): total Ca-Mg hardness of natural, filtered experimental water was 0.14 ± 0.02 mmol/L (mean ± SD) and 0.30 ± 0.02 mmol/L in the Lake Konnevesi (N 62° 36.549′, E26° 24.769′, KV) and River Kokemäenjoki (N 61° 20.102′, E22° 7.075′, KJ) control waters, respectively. Detailed characterisation of the test waters is given in Table S2 (Supplementary material Table S2). Filtered KV and/or KJ water was used as a test water for the exposure-response experiments by adding sodium sulfate (Na_2SO_4, Merc, Supelco, purity ≥ 99 %) to yield needed ranges of exposure concentrations. Mean sulfate (SO_4, SFS-EN ISO 10304–1:2009a, 2009b) concentrations of KV and KJ control water were 4.8 ± 0.8 mg/L and 13.4 ± 3.7 mg/L, respectively. In the *Daphnia longispina* test, the control water was Lake Mekkojärvi (N 61° 13.778′, E 25° 8.576′) water (MJ, hardness 0.15 mmol/L and sulfate concentration 2.8 mg/L) where the *D. longispina* adults originated. Before the tests, test waters were filtered by a tangential flow filtration (Millipore Pellicon & Durapore GVPP 0.22 casette) with 0.22 μm filter pore size.

Quality control of chemical analyses were achieved by sample replication. Analysis uncertainty for sulfate was ± 10 %. Sulfate concentrations in the treatments were analysed in the beginning and end of the experiment as well as multiple times during the experiments depending on the length of the exposure. Measured sulfate concentrations were consistent with nominal values (average relative difference between nominal and measured exposure concentration below 3.5 %) and were stable throughout the experiments (coefficient of variation below 4.5 %). Water temperature, oxygen concentration ja pH were monitored frequently during all tests.

2.1.1. Teleost fish

The effects of sulfate on the reproduction of autumn-spawning brown trout (*Salmo trutta)*, migratory whitefish (*Coregonus lavaretus*) and spring-spawning roach (*Rutilus rutilus*) were studied in chronic toxicity tests from fertilization of eggs to hatching (Table 1) from 2021 to 2022. Eggs and sperm of mature brown trout (6 females, 6 males) were obtained from the Laukaa aquaculture station of the Natural Resource Institute Finland (the Lestijoki stock), while eggs and sperm of mature, wild whitefish were acquired from River Kokemäenjoki (Karjalainen et al., 2021). Egg batches of all females (ca. 50–100 eggs per female) were gently put into a glass beaker and fertilized with 5 μl of sperm from all males. The mixture was then activated with the prepared test

Table 1
Summary of toxicity tests carried out in this study. Stage is developmental stage in the beginning of the experiment. Test type means A acute or C chronic test. Test waters were Lake Konnevesi (KV), River Kokemäenjoki (KJ) and Lake Mekkojärvi (MJ) waters.

Test species	Stage	Test type	Test endpoint	Test duration	Test water
Salmo trutta, brown trout	Eggs	A	Fertilization	Fertilization to eyed stage 37 d,	KV
		C	Mortality	Fertilization to emergence 179 d	KV
Coregonus lavaretus, whitefish	Eggs	A	Fertilization	Fertilization 2–25 d	KV, KJ
		C	Mortality	Fertilization to hatching 165 d	KV, KJ
Coregonus albula, vendace	Eggs	A	Fertilization	Fertilization 2 d	KV
Rutilus rutilus, roach	Eggs	A	Fertilization	A Fertilization to eyed stage 8 d,	KV
		C	Mortality	C Fertilization to hatching 23 d	KV
Daphnia longispina, waterflea	<24 h	C	Reproduction	21 d	MJ
Chironomus riparius, dipteran midge	<24 h	C	Growth, dry mass	10 d	KV
Unio crassus thick-shelled river mussel	glochidia, 3 wk	A, C	Mortality	A 24 h C 7 d	KV
Margaritifera margaritifera, pearl mussel	glochidia, 7 wk	A, C	Mortality, Growth, water content	A 24 h C 7 d	KV
Lymnaea stagnalis, great pond snail	<24 h	C	Growth, dry mass	21 d	KV, KJ
Brachionus calyciflorus, planktonic rotifer	<2 h	C	Reproduction	48 h	KV, KJ
Lumbriculus variegatus, blackworm	Adults	C	Reproduction	28 d	KV
Raphidocelis subcapita, green algae	-	A, C	Growth	72 h	KV, KJ
Lemna minor, common duckweed	-	A, C	Growth	7 d	KV, KJ

solutions respectively. The test solutions for brown trout experiments were prepared adding Na_2SO_4 to KV (range of 7 exposures from 300 to 2400 mg SO_4/L) water while the whitefish experiments were carried out both in the KV and KJ water spiked with sodium sulfate (range of 7 exposures from 300 to 2400 mg SO_4/L). KJ and KV waters were control waters of both species. After 2 days from fertilization, eggs (N = 36) were placed individually into the wells of a 6-well tissue culture microplates (lid-covered VWR 6-well plate) with the corresponding treatment concentration in which they were fertilized. During the experiment the test solutions were changed on a weekly basis and samples were taken to monitor the sulfate levels. Fertilization success and survival in different developmental stages (early and late embryonic stage, during hatching and larval stage) were monitored. The duration of the whole experiment of autumn-spawning species varied from 165 to 179 days. Water temperature simulated the annual temperature in Kokemäenjoki during the incubation period (Karjalainen et al., 2021). The third autumn-spawning species tested was vendace (*Coregonus albula*) with only acute fertilization test (range of 9 exposures from 300 to 2900 mg SO_4/L) being carried out (Table 1). Fertilization (%) of eggs was used as an acute endpoint also for whitefish and brown trout.

A chronic 23-d toxicity test of roach (*R. rutilus*) from fertilization to hatching was carried out in spring 2022. Eggs and sperm were obtained from mature fish (1 female, 3 males) caught from Lake Päijänne during the spawning season. Batches of eggs were fertilized in petri dishes using 1.5 µl sperm from all three males. Three replicates were prepared for each sulfate treatment and the treatment solutions were added to activate the sperm. In the roach test, the test water was KV with 7 sulfate treatments (from 300 to 2400 mg SO_4/L) and control treatments were conducted in both KV and KJ waters. Control treatments were KV and KJ water treatments. Mortality was monitored for two periods: from fertilization to eyed stage (pigmentation in eyes observed) and from fertilization to hatching. Water temperature simulated the natural temperature during spawning and increased gradually from 9.3 ℃ at fertilization to 14.6 ℃ at hatching.

2.1.2. Crustacean

A chronic 21-d toxicity test of *D. longispina* was conducted at 20 ℃ with *D. longispina* caught from Lake Mekkojärvi (MJ) in June 2021. The procedure followed the chronic *Daphnia magna* guidelines (OECD, 2012; ISO, 2000). Test water was natural MJ water, and the endpoint was reproduction. The test set-up consisted of a filtered natural MJ water control with 5 replicates, natural MJ water control with 10 replicates and 8 sulfate concentrations between 27 and 1333 mg SO_4/L with 5 replicates in each treatment. The test organisms were fed daily with algal food prepared from *Rhapidocelis subcapitata* algae: daily amount of the food was 0.1 mg C/replicate for the first 0–7 days and 0.18 mg C for the last 8–21 days. Water was changed three times a week by adding 50 ml of the respective test water into another test vessel and transferring the mother gently with plastic Pasteur pipette into the water. The number of offspring was counted, and the mortality checked daily until the last day of the test.

2.1.3. Insects

A chronic 10-d toxicity test of *Chironomus riparius* midge from SYKE laboratory was conducted according to OECD test guidelines at 20 ℃ (SD± 0.7) (OECD, 2004a; b) in 2022. Less than 24-h-old first instar larvae were selected and distributed randomly to the test beakers. The midges were exposed individually in five replicates. The KV water was spiked with Na_2SO_4 in seven concentrations (range from 1600 to 6500 mg SO_4/L) and used as overlying water in beakers. Two control treatments without sulfate were also established using KV water and OECD standard water. Substrate was prepared by mixing different sizes of quartz sand according to the OECD guideline. The other ingredients of this OECD artificial sediment were not applied to keep the water quality of Lake Konnevesi constant and as close to original as possible (i.e., low water hardness). The larvae were fed *ad libidum* three times per week using Tetramin fish food. The water was not changed but was gently aerated through Pasteur pipettes and evaporated water was replaced with MilliQ water. Water samples were retrieved after the test to measure actual exposure concentrations and hardness. In the end of the 10-d exposure, larvae were sieved out and wet and dry weights were recorded as endpoints.

2.1.4. Molluscs

Mollusca test species were thick-shelled river mussel (*Unio crassus*), freshwater pearl mussel (*Margaritifera margaritifera*) and the freshwater snail (*Lymnaea stagnalis*). A chronic 7-d toxicity test of 3-week-old juvenile *U. crassus* were conducted at 16.5 ℃ (SD±0.2) in lid-covered 6-well tissue culture microplates with 10 ml test water per well in 2022. The test set-up consisted of filtered natural KV water control with 6 replicates and 7 sulfate concentrations in KV water (range from 300–2000 mg SO_4/L) with 6 replicates in each treatment. Prior to the experiment, juveniles were confirmed alive by active foot movement within 5 min of observation (ASTM, 2022). The mussels were kept in a static-renewal exposure test with added algal mix, Nanno 3600 and Shellfish 1800 (Reed Mariculture Inc., USA), and exposure water was changed three times a week. Mortality as the endpoint (ASTM, 2022) was confirmed by open shells which did not close upon disturbance.

A chronic 28-d toxicity test of 7-week-old *M. margaritifera* was conducted at 17.3 ℃ (SD±0.04) in 2021 according to the similar protocol as *U. crassus* juveniles, except that the test set-up included filtered natural KV and KJ water controls and 7 sulfate concentrations in KV water

(range from 300 to 1600 mg SO_4/L) with 12 replicated mussels per each treatment. The endpoint in the chronic *M. margaritifera* test was relative water content of mussels, calculated as (wet mass-dry mass)/wet mass.

An acute 24-hour toxicity test of *U. crassus* glochidia less than 24-h-old were conducted at 16.4 ℃ (SD±0.3) in 2022. Before the experiment, glochidia viability was examined according to standard protocol with a drop of saturated NaCl solution, to which 100 % of the glochidia responded by closing the valves (ASTM, 2022). In the 6-well plates, 10–12 glochidia were placed in each well and 6 replicates in KV control and 9 sulfate treatments (range from 150 to 1500 mg SO_4/L) were used. The endpoint was mortality, which was indicated by glochidia not responding to a drop of saturated NaCl water.

An acute 96-h *M. margaritifera* toxicity test was performed on 7-d-old juveniles at 17.3 ℃ (SD±0.2) where the foot movement within 5 min of observation was the endpoint (ASTM, 2022). Static exposure was carried out with no algal feeding to the mussels, and the test set-up consisted of filtered natural KV and KJ water controls, and 8 sulfate concentrations in KV water (range from 160 to 3350 mg SO_4/L) with 12, 17 or 18 replicated mussels depending on the treatments.

A total of 50 adult *U. crassus* (37 females, 13 males) were collected from Perniönjoki river in Southwest Finland in mid-May 2021 (permission to collect and maintain protected species, VARELY/2507/2021) and the glochidia were collected in mid-May 2022 after rearing the mussels in the Konnevesi Research station for a year. Three-week-old juvenile *U. crassus* used in the 7-d test were originated from a rearing facility at the Mill of Kalborn (Luxembourg). *M. margaritifera* glochidia and juveniles were collected from the artificial breeding program in Konnevesi Research Station (Hyvärinen et al., 2022). Adult mussels were collected from their original rivers, River Ähtävänjoki in Western Finland (permission to collect and maintain protected species, EPOELY/2278/2015) and River Lutto in Northern Finland (permission to collect and maintain protected species, LAPELY/2252/2019).

A 21-d chronic static-renewal toxicity test with *L. stagnalis* originated from KV was conducted to assess the toxicity of sulfate using the growth inhibition endpoint OECD, 2016. Both KV and KJ filtered natural waters were used in the toxicity tests. The experimental set up consisted of 9–10 concentrations of sulfate (range from 55 to 967 mg SO_4/L) in KV or KJ water, a KV or KJ water control and a culture medium control. Six replicates were included in each treatment except in the natural water control, which included ten replicates. The age of the test organisms was less than 24 h at the initiation of the test. The test was performed in a temperature and light controlled room at 20 ℃ (SD ± 1). The water was changed, and test organisms were fed with Tetramin fish flakes three times a week. At the last day of the test, the snails were weighed for the wet weight, dried in oven at 105 °C for overnight and weighed for the dry weight.

2.1.5. Rotifers

A chronic 48-h toxicity test with the rotifer (*Brachionus calyciflorus*) was conducted to assess the toxicity of sulfate following the ISO (2008) standard test protocol with the reproduction as an endpoint. Two successful toxicity tests were conducted: tests were done both for KV and KJ water, and both experimental set-ups consisted of 7 sulfate concentrations (range from 309 to 2534 mg SO_4/L) and a natural filtered KV and KJ water controls with 6 replicates in each treatment. The cysts of the test organism *B. calyciflorus* were obtained from Aboatox Ltd, Finland. Once hatched, the rotifers were fed. The test organisms were less than 2-h-old at the beginning of the test. One rotifer was placed into each test well of a multi-well plate with the respective test water including algae food. The plates were placed into a light- and temperature -controlled plant growth chamber at 24 ℃ for 48 h and after that, the living female rotifers were calculated from each well.

2.1.6. Annelids

A chronic 28-d sediment-water toxicity test of *Lumbriculus variegatus* worms was performed with natural KV sediment in accordance with the modified standard protocol (OECD, 2007). Artificial sediment of standard protocol (OECD, 2007) was not adopted due to its high $CaCO_3$ content, which would increase the water hardness of our target maximum value in the beakers by at least 40 mg/L. Sodium sulfate was spiked into KV water to obtain 9 sulfate concentrations (range from 300 to 3500 mg SO_4/L) of test water but not into the sediment. Five replicate glass beakers were prepared for each sulfate treatments and 8 replicates for the KV control water treatment. For each replicate, a wet weight of 40 g of sediment was added into 250 ml glass beakers (6 cm in diameter) to achieve a layer of 2 cm sediment. Test water was gently decanted onto the sediment, and the sediment to overlying test water height ratio was at 1:4. Then the sediment was given two days to equilibrate with the overlying water before the introduction of worms. Gentle aeration into the overlying water was also started one day before the experiment, via a sterile needle (BD Microlance, 0.8 mm × 50 mm) positioned 2–3 cm above the sediment surface. Ten similar-sized worms were placed into each beaker, and sediment burrowing was immediately observed. The test was performed at 20 °C with 16 L:8D photoperiod (<500 lx). The worms were not fed during the 28 days since the natural sediment had a high organic carbon content (total organic carbon of 7.7 % ± 0.13). Two times a week the overlying test water was renewed with aerated new test water to avoid unexpected changes in pH and hardness in the overlaying water. At the end of the experiment, the sediment was first sieved (250 μm pore size), and the sediment remaining on the sieve was rinsed twice in aerated KV water to find the worms. Endpoint variable of the test was reproduction (worm number) (Table 1).

2.1.7. Algae

Unicellular green algae, *Rhapidocelis subcapitata*, is a standard algal species in the aquatic chronic toxicity tests. According to standard protocols (ISO, 2012; OECD, 2011) *R. subcapitata* strains were cultured for several generations in a defined medium in 7 sulfate concentrations (range from 530 to 9100 mg SO_4/L) prepared by mixing appropriate quantities of growth medium, sodium sulfate dissolved to both KV and KJ water and an inoculum of exponentially growing algal cells. Control treatments were prepared in the filtered natural KV and KJ waters. The test batches were incubated in the glass Erlenmeyer beakers under constant light at 22 ℃ for 72 h and the cell density in each test solution was measured every 24-h. Endpoint variable of the test was specific growth rate.

2.1.8. Higher plants

In the 7-d growth test, individuals of the duckweed *Lemna minor* grew in different sulfate concentrations (range from 291 to 9471 mg SO_4/L) of the KV and KJ test waters. The strain is local from Askola, Southern Finland and cultured in SYKE Helsinki laboratory. The test protocol followed ISO 20079:2005 international standard (ISO, 2005). Seven sodium sulfate concentration were tested both in KV and KJ waters with two controls: test water and MilliQ water. Test vessels (3 replicates per treatment) were hold in climate controlled (20 ℃, 6600–10000 lx and 16 h light:8 h dark) cabin. The plants were photographed after 24, 48 and 72-h exposures and analyzed with LemnaTec Scananalyzer. The inhibition was modelled using growth rate as an endpoint. The specific growth rate (growth rate per day) was calculated from the change in frond ("leaf") number and from the change in frond area.

2.1.9. Concentration-response analysis

Package 'drc' in R was used to analyse the toxicity data (Ritz et al., 2015). EC10/LC10, EC20/LC20 and EC50/LC50 with their 95 % confidence intervals were derived based on the parameter estimates from logistic (L), log-logistic (LL) or weibull (W) models within the 'drc' package. For model selection a comparison by Akaike's information criterion (AIC; Akaike, 1973) was used across different models (among L.4, LL.2, LL.3, LL 0.3 u, LL.4, LL.5, W1.3, W1.4, W2.4 etc., Ritz et al., 2015, letters represent type of model and number is the number of

parameters in the model), and the models with the lowest AIC values which can successfully yield realistic fitting curves and EC/LC values were used for the analysis. Type of the applied model in each toxicity test is given in Tables 2 and 3.

2.2. Species sensitivity distribution modelling

SSD of sulfate for aquatic organisms was modelled by the model averaging method (Fox et al., 2021) with 4-model averaged model (log-logistics (llogis), gamma, log-normal (lnorm), weibull models) included in the ssdtools R package of Thorley and Schwarz (2021). Firstly, all 4 models were fitted to the data separately and then corrected AICc values were used to rank the candidate models using AIC differences. Weights for each assigned model were calculated based on the AIC differences and used in the averaged model (Fox et al., 2021). Model statistics for all models with weights used in the 4-model averaged model are given in Table S3 (Supplementary material Table S3). The model averaging method avoids the problem of choosing only one model, then generating more stable HCs with reduced uncertainty (Fox et al., 2021). The chronic and acute SSDs were modelled and the hazardous concentrations (HC5) of sulfate were estimated from the 5 % percentile of the averaged models. Median, lower, and upper confidence limits of HC5 were obtained. In the chronic SSD models, EC10 or LC10 values from chronic ecotoxicity tests were used. Thus, 10 % of the individuals in the toxicity test had displayed the endpoint effects in this concentration. The acute EC50 or LC50 values were used in the acute SSD models (Table 1, Table S3). EC50 of *Lemna minor* and *Raphidocelis subcapitata* was considered an acute value and EC10 a chronic value as suggested in the OECD guidelines (OECD, 2006; OECD, 2011).

The SSD models were fitted for different data sets compiled from the available data:

1) "All data" sets (N = 22 in chronic SSD and N = 18 in acute SSD) including our ecotoxicity data obtained in this present study in the different test waters separately (Chronic: 11 tests in KV, 5 tests in KJ, 1 test in MJ; Acute: 9 tests in KV, 3 tests in KJ) and data for soft freshwaters (Chronic: 5 tests, Acute: 6 tests, $CaCO_3$ hardness $\leq$40 mg/L, Table S4) obtained from the literature. All data sets include all available information which is used for the estimation of HC5.

2) "Present study data" refers to ecotoxicity data obtained in this present study (Chronic: N = 17, Acute: N = 9) but again the KV and KJ toxicity test results were used separately.

3) "All data, means" data sets (Chronic: N = 17; Acute: N = 13) correspond to the data set 1, but KV and KJ results were averaged in the cases where the same species had the tests in both KV and KJ test waters. Previous studies have recommended summarizing SSD modelling data by using an average response for each species or using the most sensitive response observed (Wheeler et al., 2002). We used here a geometric mean.

4) "Present study means" data sets (Chronic: N = 17, Acute: N = 9) corresponds to the data sets 2, but KV and KJ results were averaged (geometric mean) in the cases where the same species had the tests in both KV and KJ test waters.

The predicted no-effect concentrations (PNEC) were calculated by dividing the chronic HC5 values by assessment factors (AF) of 2, 3 and 5, which fell within the recommended range (AF from 1 to 5) in the SSD-based chronic PNEC evaluation in the EU guidance (European commission, 2018). The chronic PNECs were calculated for all 4 data sets. Furthermore, the calculated PNECs were used to estimate the fraction affected (%) of all species and their lower and upper confidence limits by SSD models. PNECs from the acute SSDs were calculated by dividing the HC5 by the AF of 2, 5 and 10. The selection of AF 10 for the acute data was justified by the EU giuidance (European commission, 2018), and AF 5 was the acute-to-chronic ratio calculated from this present study.

3. Results

3.1. Toxicity tests

According to the chronic toxicity tests in this study, the cladoceran *Daphnia longispina* and freshwater snail *Lymnaea stagnalis* were the most sensitive to sulfate among the studied species (Table 2). The effective concentration (EC10) of the reproduction of *D. longispina* was observed in sulfate concentration of 49 mg/L while growth of *L. stagnalis* was inhibited at sulfate concentration of 217 mg/L. Most studied organisms seem to tolerate quite high sulfate concentrations: the median of all chronic effective concentrations (EC10) was 926 mg SO_4/L and 1387 mg SO_4/L in the KV (hardness 0.14 mmol/L or 14 mg/L) and KJ water (hardness 0.30 mmol/L or 30 mg/L), respectively. In the acute toxicity tests (Table 3), the median of the effective concentration (EC50 or LC50) for KV water was 1312 mg SO_4/L while the most sensitive species was thick shelled river mussel *Unio crassus* in the acute glochidia test (857 mg SO_4/L) and freshwater pearl mussel *Margaritifera margaritifera* in the 96-h juvenile test (904 mg SO_4/L) (Table 3). In KJ water, an acute test was conducted: in *C. lavaretus* fertilization test LC50 was 2504 mg SO_4/L.

3.2. Species sensitivity distribution

Based on the SSD of studied species from both the present study and literature data ("All data" model), chronic AA hazardous concentration

Table 2
EC10/LC10, EC20/LC20 and EC50/LC50 (95 % confidence intervals in parentheses) of sulfate chronic toxicity tests (mg SO_4/L) conducted in soft freshwaters (KV: L. Konnevesi water, KJ: R. Kokemäenjoki water and MJ: L. Mekkojärvi water) in this study. Type of each model function is given according to Ritz et al. (2015). [1]Recalculated from data of Karjalainen et al. (2021). NA lower limit not calculable.

Test species	Endpoint	Model function	EC10 or LC10	EC20 or LC20	EC50 or LC50	Test water
Salmo trutta, fertilization to emergence	Reproduction	LL.3u	1139 (1017–1261)	1213 (1111–1316)	1351 (1276–1427)	KV
Coregonus lavaretus, fertilization to 5-d larvae	Reproduction	LL.3u	1008 (686–1330)	1193 (907–1479)	1591 (1362–1820)	KV[1]
Coregonus lavaretus, fertilization to 5-d larvae	Reproduction	LL.3u	1800 (1402–2199)	2028 (1752–2303)	2485 (2175–2796)	KJ
Rutilus rutilus, fertilization to 5-d larvae	Reproduction	L.4	1062 (865–1260)	1283 (1139–1427)	1661 (1523–1799)	KV
Daphnia longispina	Reproduction	LL.3	49 (31–66)	69 (50–87)	125 (105–145)	MJ
Chironomus riparius	Growth	W1.3	779 (392–1166)	1225 (799–1650)	2425 (2064–2786)	KV
Unio crassus, juveniles	Mortality	LL.3u	844 (NA–2171)	862 (NA–2181)	894 (NA–2223)	KV
Margaritifera margaritifera, juveniles	Growth	LL.3	426 (264–589)	543 (391–696)	822 (692–952)	KV
Lymnaea stagnalis, juveniles	Growth	LL.5	303 (260–345)	331 (268–394)	461 (161–761)	KV
Lymnaea stagnalis, juveniles	Growth	W1.3	217 (142–292)	345 (266–424)	695 (628–762)	KJ
Brachionus calyciflorus	Reproduction	LL.3	654 (536–772)	711 (612–810)	821 (736–905)	KV
Brachionus calyciflorus	Reproduction	LL.3	867 (548–1187)	1009 (729–1289)	1308 (1096–1520)	KJ
Lumbriculus variegatus	Reproduction	W1.3	1815 (1186–2444)	2064 (1625–2503)	2508 (2108–2909)	KV
Raphidocelis subcapita	Growth	LL.4	1294 (1033–1555)	1655 (1429–1881)	2521 (2291–2715)	KV
Raphidocelis subcapita	Growth	LL.4	1937 (1665–2211)	2741 (2413–3069)	4959 (3912–6006)	KJ
Lemna minor	Growth	LL.4	1195 (998–1391)	1570 (1397–1744)	2374 (2265–2483)	KV
Lemna minor	Growth	W2.4	1387 (1186–1588)	1711 (1516–1906)	2802 (2297–3308)	KJ

Table 3
EC10/LC10, EC20/LC20 and EC50/LC50 (95 % confidence intervals in parentheses) of sulfate acute toxicity tests conducted in soft freshwaters (KV: L. Konnevesi water, KJ: R. Kokemäenjoki water and MJ: L. Mekkojärvi water) in this study. Type of each model function is given according to Ritz et al. (2015).[1]Recalculated from data of Karjalainen et al. (2021).

Test species	Test type	Model function	EC10 or LC10	EC20 or LC20	EC50 or LC50	Test water
Salmo trutta, fertilization to eyed stage	Reproduction	LL.3u	1147 (1041–1252)	1219 (1130–1308)	1353 (1284–1423)	KV
Coregonus lavaretus, sea-migratory, fertilization	Reproduction	LL.3u	1039 (767–1311)	1219 (980–1459)	1601 (1408–1795)	KV[1]
Coregonus lavaretus, sea-migratory, fertilization	Reproduction	LL.3u	1696 (1298–2093)	1958 (1681–2235)	2504 (2131–2877)	KJ
Coregonus lavaretus, lake-spawning, fertilization	Reproduction	LL.2	942 (873–1011)	1039 (977–1011)	1230 (1176–1284)	KV
Coregonus albula, fertilization	Reproduction	LL.2	676 (605–746)	811 (744–878)	1107 (1044–1169)	KV
Rutilus rutilus, fertilization	Reproduction	W2.4	1187 (1081–1291)	1235 (1130–1340)	1312 (1111–1514)	KV
Unio crassus, glochidia	Reproduction	LL.3u	732 (690–774)	776 (742–810)	857 (833–881)	KV
Margaritifera margaritifera, juvenile	Foot movement	LL.3u	600 (383–818)	698 (500–897)	904 (749–1060)	KV

(HC5) of sulfate for aquatic organism in soft waters was 132 mg/L (confidence interval 62–290) i.e., if the sulfate concentrations are below HC5, < 5 % of the species studied may experience harmful effects (Table 4, Fig. 1). HC5 of sulfate for the "Present study data" set was higher (194 mg/L) than the "All data" HC5, while the lowest estimated HC5 (117 mg/L) was obtained based on "All data means" data set with the average EC values for species tested in both KV and KJ as well as chronic data pooled from literature. (Fig. 2).

When the HC5-values of the chronic "All data" SSD model (132 mg/L) were divided by the assessment factors 2, 3 and 5, the annual average PNECs were 66, 44 and 26 mg SO_4/L, respectively (Table 4). Corresponding estimates for fractions of species affected were 1.6 %, 0.9 % and 0.4 % for AF 2, 3 and 5, respectively. The chronic HC5 of the "All data means" was 117 mg SO_4/L and PNECs using AF 2, 3 and 5 were 59, 39 and 23 mg SO_4/L with corresponding percentages of species affected being 1.5 %, 0.7 % and < 0.1 %, respectively.

The HC5s of the acute SSDs varied between 514 and 678 mg SO_4/L (Table 5) and acute HC5 was 3.5–4.8 times higher than the HC5 of the chronic SSDs. The confidence intervals of acute HC5s were narrower than the confidence intervals of chronic HC5s. The acute PNECs of sulfate using AF of 10 (Guidance of European commission, 2018) were from 51 to 68 mg SO_4/L according to the data available in our study (Table 5). The upper confidence limit of the fraction affected (%) of the acute PNECs derived by AF 5 and even by AF 2 remained well below 5 % (Table 5). In Fig. 3, "left tails" of the models of all four data sets with HC5s as well as HC1 were represented for both chronic and acute SSD models.

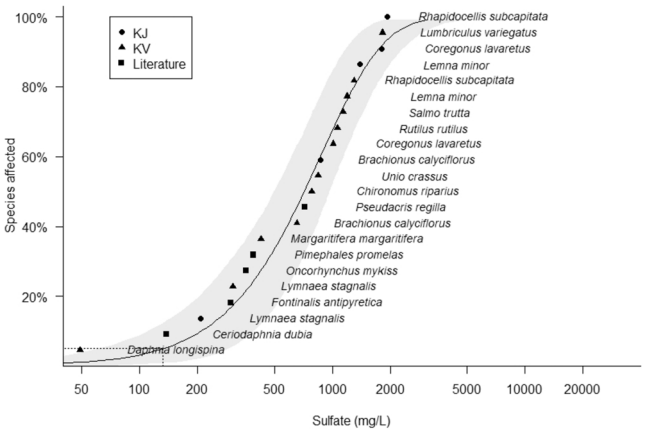

Fig. 1. Species sensitivity distribution for sulfate based on chronic data of this study and data obtained from literature for estimation a hazardous concentration (chronic HC5). Solid curve is a 4-model averaging fit to data with 95 % confidence interval (grey zone). Dotted line indicates the HC5.

4. Discussion

The set of the chronic sulfate toxicity tests conducted in this study multiplied the number of the tests available in soft freshwater conditions (water hardness ≤ 30 mg/L) for SSD analysis. Formerly, the number of available chronic toxicity data in soft water (water hardness ≤ 40 mg/L) was much lower than what is required to derive the species sensitivity distribution and subsequent probabilistic derivation of the hazardous concentrations for sulfate (Sahlin and Ågerstrand, 2018). Carr and Belanger (2019) stated that "the starting point for sample size guidance" would be a minimum of 13 values in derivation of SSD and our data sets included 9–22 data points which was a typical sample size compared to SSDs of different chemicals (Belanger and Carr, 2019). Major part of the species tested in this study had high tolerance to sulfate. On the contrary, the cladocerans (as here *D. longispina* and *C. dubai*) seem to be the most sensitive group to sulfate. Due to their enhanced vulnerability, more chronic toxicity tests of freshwater cladocerans as well as other crustaceans such as isopods, amphipods and copepods are strongly recommended. Usually, the most sensitive species are the most influential to evaluate HC5 but in some cases it can be also the most tolerant species (Belanger and Carr, 2019). Although new toxicity data within the range of already identified chemical-specific toxicity will generally not drive HC5 lower, the addition of data points can lend benefits to data sets that are small (Belanger and Carr, 2019; Kamo et al., 2022). In small data sets, the additional tolerant species may also improve the protective significance of the SSD.

In this study, chronic EC10 and acute EC50 values were used for the derivation of a long-term (chronic tests) and short-term (acute tests) HC5, respectively (European Commission, 2018). Chronic freshwater tests generally refer to long-term tests that are conducted at lower exposure levels with the objective to cover either the whole life span of the organisms or at least critical stages such as early development or

Table 4
Hazardous concentration of sulfate (mg/L, HC5, 95 % confidence intervals in parentheses) based on the species sensitivity distribution of chronic ecotoxicity tests (EC10/LC10) and modelled by the 4-model averaging method (llogis, gamma, lnorm, weibull models included). $PNEC_{AF2}$, $PNEC_{AF3}$ and $PNEC_{AF5}$ are calculated by dividing HC5 by three assessment factors (AF2, AF3, AF5). % of species affected for the PNECs (confidence intervals in parenthesis) are given separately. N is the number of toxicity tests in the SSDs. Detailed model statistics and data taken from literature are given in Table S3 and S4 respectively.

Data sets	HC5	N	$PNEC_{AF2}$	% affected	$PNEC_{AF3}$	% affected	$PNEC_{AF5}$	% affected
All data	132 (62–290)	22	66	1.7 (0.1–5.4)	44	0.9 (0.1–3.4)	26	0.4 (<0.1–1.8)
Present study	194 (85–410)	17	97	1.5 (0.1–5.1)	65	0.7 (0.2–3.4)	39	0.3 (<0.1–1.6)
All data, means	117 (46–284)	17	59	1.7 (0.1–6.3)	39	0.9 (<0.1–4.0)	23	0.4 (<0.1–2.2)
Present study, means	176 (70–441)	12	88	1.5 (<0.1–6.5)	59	0.7 (<0.1–4.1)	35	0.3 (<0.1–2.2)

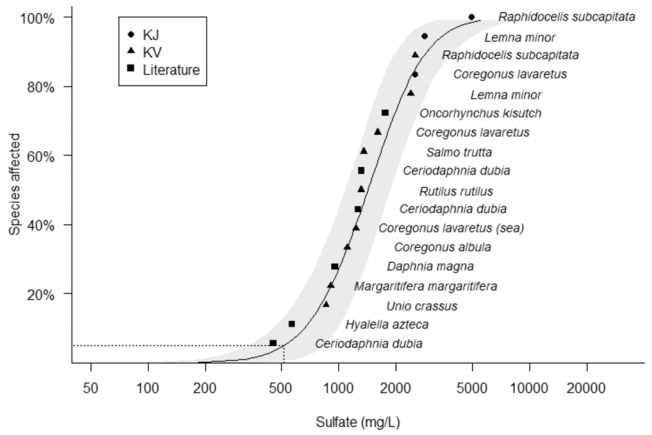

Fig. 2. Species sensitivity distribution for sulfate based on EC50 or LC50 data of acute toxicity tests in this study and data obtained from literature for estimation a hazardous concentration (acute HC5). Solid curve is a 4-model averaging fit to data with 95 % confidence interval (grey zone). Dotted line indicates the HC5.

reproduction (Cooney, 1995). The duration of our tests varied according to species and followed the standard protocols.

In aquatic risk assessment, the SSD-derived hazardous concentrations (HC_p) have been used to estimate the predicted no-effect concentrations (PNEC) of harmful substances to protect aquatic ecosystems (Lei et al., 2010; Huang et al., 2018; Park and Kim, 2020). Default value for SSD-based risk assessment is the "arbitrarily determined" fifth percentile, HC5, below which no-observed-effect concentrations can be gained (Aldenberg and Jaworska, 2000). Often, the data sets available for SSD of a certain substance or compound are small, and it is important to provide uncertainty estimates of the HC and fraction affected (Aldenberg and Jaworska, 2000). Therefore, statistical estimation of median HC5 with lower and upper confidence limits is required in SSD modelling (Aldenberg and Jaworska, 2000; Fox et al., 2021; Posthuma et al., 2019; Thorley and Schwarz, 2021). In this study, four different data sets (All data, Present study data, All data means and Present study data means) with different number of cases (N varies from 9 to 22) were used to provide HC5 and PNEC values. Furthermore, the chronic and acute SSDs were carried out separately. As expected, if there were more cases in the SSD models the confidence interval was slightly narrower, but in all SSDs, the HC5 estimates had rather high uncertainty in the left tails of the models, and the confidence intervals overlapped largely (Fig. 3). When toxicity data acquired from tests conducted both in KV and KJ was included, even though the number of cases was higher, more variation was still observed due to the difference in water hardness in KV and KJ water. In 3 of 4 cases where the species was tested in both KV and KJ water, the harder KJ water (water hardness 30 mg/L) had, on average, 30 % higher EC10 of sulfate than the softer KV water (water hardness 14 mg/L). Only *L. stagnalis* had lower EC10 in KJ water than in KV water. Differences in water hardness influence sulfate toxicity even when the water is very soft (Elphick et al., 2011; Mount et al., 2016). Effect of water hardness on the toxicity of sulfate and its actual effective mechanism varies between different organism groups, but generally the modifying factors are other major ion concentrations such as calcium, magnesium and chloride, their interactions, and the overall ionic strength, which affects the osmotic stress in the organisms (Davies and Hall, 2007; Elphick et al., 2011; Mount et al., 2016; Sahlin and Ågerstrand, 2018; Soucek and Kennedy, 2005). Mechanisms of major ion toxicity to aquatic organisms are due to osmotic stress, specific ion toxicity or imbalance of the ionic composition, while the toxicity of sodium sulfate was primarily linked to osmotic stress influenced by the presence of the anion (Davies and Hall, 2007; Elphick et al., 2011; Erickson et al., 2017; Griffith, 2017; Mount et al., 2016). Different mechanisms of sulfate uptake may also affect species sensitivity to sulfate, and e.g., algae accumulate sulfate by active transport while sulfate uptake of fish occurs likely by passive diffusion through ion channels

Table 5
Hazardous concentration of sulfate (mg/L, HC5, 95 % confidence intervals in parentheses) based on the species sensitivity distribution of acute ecotoxicity tests (EC50/LC50) and modelled by the 4-model averaging method (llogis, gamma, lnorm, weibull models included). $PNEC_{AF2}$, $PNEC_{AF5}$ and $PNEC_{AF10}$ are calculated by dividing HC5 by three assessment factors (AF2, AF5, AF10). % of species affected for the PNECs (confidence intervals in parenthesis) are given separately. N is the number of toxicity tests in the SSDs. Detailed model statistics and data taken from literature are given in Table S3 and S4, respectively.

Data sets	HC5	N	$PNEC_{AF2}$	% affected	$PNEC_{AF5}$	% affected	$PNEC_{AF10}$	% affected
All data	514 (327–838)	18	257	0.1 (<0.1–2.9)	103	0.1 (<0.1–0.5)	51	<0.1 (<0.1–0.1)
Present study	678 (419–1167)	12	339	0.6 (0.1–3.4)	136	0.1 (<0.1–0.6)	68	0.1 (<0.1–0.6)
All data, means	557 (368–878)	13	279	0.4 (<0.1–2.6)	111	<0.1 (<0.1–0.3)	56	<0.1 (<0.1–0.3)
Present study, means	646 (403–1123)	9	323	0.9 (<0.1–3.23)	129	0.2 (<0.1–0.5)	65	0.2 (<0.1–0.5)

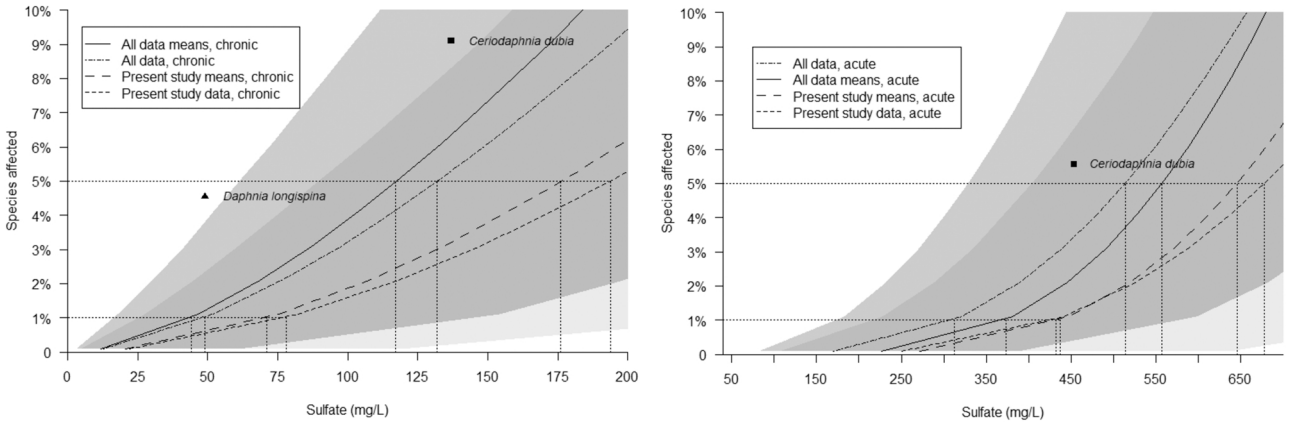

Fig. 3. The "left tail" curves of the SSD models generated from four different data sets by the model averaging method for annual average chronic (left) and acute (right) SSDs. Dotted lines indicate the 1 % and 5 % percentiles of SSD models. Confidence bands for "All data set" (dark) and "Present study data set" (light) are given separately. The darkest grey indicates the area where these two confidence bands overlapped.

(Elphick et al., 2011). In general, sulfate transport and regulation in freshwater animals has not been well studied compared with other ions (Griffith, 2017).

In SSD model fitting, the model averaging method was used in this study. Many different potential statistical models may fit toxicity data and the choice of model can influence the value of the endpoint calculated from ecotoxicity studies (Meyes and Nordin, 2013). The calculation of the endpoints in the left tail of the distribution is particularly sensitive and subject to remarkable variation, especially when extrapolating from a small sample size (Fox et al., 2021). The average of several weighted estimates will reduce such variance and uncertainty in the HC5 estimates (Fox et al., 2021). The model-averaged method is integrated in several SSD tool packages, and the weighting method based on the Akaike's information criterion (AICc in Table S3) in ssdtools (Thorley and Schwarz, 2021, Table S3) was used in our study. Environmental quality standard guidelines of European Union (European Commission, 2018) suggested using the EXT-computer program (Van Vlaardingen et al., 2004) for the calculation of HC5 and the confidence intervals by log normal distribution. In the Table S3, the lnorm models correspond to the models used in the EXT-program.

With the SSD method, a HC5 value is estimated from the ecotoxicity data available and then divided by an assessment factor (AF) to derive the PNEC (European Commission, 2018; Sorgog and Kamo, 2019). AFs from 1 to 5 have been recommended when deriving chronic PNEC by SSD (European Commission, 2018). Criteria to use different AFs are contextual and the ambiguity of AF selection often leads to the application of the highest AF value by environmental managers aiming to ensure effective protection of ecosystems. Theoretically, the AFs were designed to cover potentially untested sensitive species and address both uncertainty and variability (Belanger and Carr, 2019), and thereby guaranteeing a PNEC lower than a HC5 with 95 % probability (Kamo et al., 2022; Sorgog and Kamo, 2019). After calculating $PNEC_{AF}$ values by dividing HC5s by AF 2, 3 and 5, we also generated the fractions affected (%) and their confidence limits for each $PNEC_{AF}$ values by SSD models. The upper limit of fraction affected of different PNECs can be used to examine how the "PNEC lower than HC5″ target will be fulfilled. In all our data sets, the upper confidence limit of the fraction affected of chronic $PNEC_{AF3}$ remained below 5 % (i.e., PNEC was lower than HC5 with 95 % accuracy, Table 4). By contrast, $PNEC_{AF5}$ seemed to generate an excessively protective situation for sulfate. Therefore, AF 3 were concluded to be the most appropriate to apply to our data, which produces a chronic $PNEC_{AF3}$ close to the 1 % percentiles (HC1) of our different SSD models (dotted line in Fig. 3). The use of AF3 in our study can also find justification in the statistical approaches suggested by EFSA (2013) and Kamo et al. (2022). Kamo et al. (2022) cited that the advice of EFSA (2013), "if the lower confidence limit of HC5 is less than 1/3 of the median HC5, a higher AF from the range proposed (i.e., 3–6) may be warranted", is parallel to their statistical approach to connect AF, sample size and variation in species sensitivity. In our data sets, the lower limits of HC5 were clearly higher than 1/3 of the HC5 suggesting use of lower AFs.

The ratio between chronic and acute HC5s of sulfate in our study was lower than observed ratios, on average, in earlier studies with several different harmful substances (Duboudin et al., 2004; Hiki and Iwasaki, 2020). Hiki and Iwasaki (2020) observed that in the analysis of 150 chemicals, the HC5 of acute SSDs was, on average, 10 times higher than the chronic SSD HC5 and that acute HC5 can be used to obtain a first initial approximation of the chronic HC5 by dividing acute HC5 by 10. This preliminary conversion is often useful due to higher number of acute toxicity tests available than chronic tests, which are generally more laborious. In our study, the acute to chronic ratio is approximately 5, suggesting that in the case of sulfate, the initial approximation based on an acute-to-chronic-ratio of 10 could be overly protective. More accurate estimations of chronic HC5s, thus, depend on empirical data with a large sample size covering various representative species.

In all, chronic $PNEC_{AF3}$ varied from 39 to 65 mg SO_4/L depending on the data sets used in this study. The acute PNECs (51–68 mg SO_4/L) using the AF 10 overlapped the chronic PNECs. These are the levels of PNECs, which can be recommended to apply to the boreal soft freshwaters and provide a suitable degree of protection even for the most sensitive species represented in our data set (*D. longispina*). Elphick et al. (2011), using a smaller sample size for SSD, suggested a higher chronic WQG for sulfate i.e., 129 mg SO_4/L in soft waters using AF1 (water hardness <40 mg/L). By the safety factor method, they suggested a WQG of 75 mg SO_4/L (Elphick et al., 2011). In addition, Meays and Nordin (2013) suggested that the approved 30-day average WQG of sulfate to protect aquatic life in very soft water (water hardness <30 mg/L) should be 128 mg SO_4/L. Furthermore, Sahlin and Ågerstrand (2018) reviewed the data available for soft water from the literature and found that it was not at all possible to derive chronic AA-QS for sulfate in soft waters due to scarce data, but they proposed the acute MAC-QS of 59.6 mg SO_4/L for sulfate in soft waters (AF 10, water hardness 25–40 mg/L) which also corresponds to the acute PNECs calculated in this present study using AF 10. Extrapolation of the experimental information obtained from laboratory conditions to ecosystem level is always challenging and Belanger and Carr (2019) essentially reviewed that mesocosm and field data have demonstrated that HC5s of SSDs tend to be conservative and lead to low PNEC values, further justifying the use of lower AFs in high-quality SSDs with representative assortment of species from a particular ecosystem. In the end, scientific and policy-based interpretation and finally, the decision in the local protective context by local environmental authorities is needed to determine an appropriate AF.

5. Conclusions

This study contributed to the development of sulfate environmental risk assessment and examined sulfate toxicity to 13 aquatic species across different trophic levels and produced predicted no-effect concentration by species sensitivity distribution modelling based on combinations of different data sets. Our toxicity tests greatly expanded the data available on sulfate toxicity to aquatic life in soft waters. Most of the studied species exhibited high tolerance for sulfate even in very soft boreal waters tested. However, more toxicity tests are still recommended, especially on crustacean species such as isopods, amphipods, and copepods, which could be potentially sensitive to sulfate. The comparison between four different data sets showed the effect of data averaging and sample size on the hazardous concentration with lower sample size yielding wider confidence limits of the hazardous concentrations. The confidence intervals overlapped highly between all data sets indicating the uncertainty and variability in the species sensitivity distribution modelling. New approaches to select the proper assessment factor for determining the no-effect concentrations were applied and assessment factor 3 was suggested to be applicable to our data.

CRediT authorship contribution statement

Juha Karjalainen: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Visualization. **Xiaoxuan Hu**: Methodology, Investigation, Formal analysis, Writing – review & editing, Visualization. **Mikko Mäkinen**: Methodology, Investigation, Writing – review & editing. **Anna Karjalainen**: Methodology, Supervising, Writing – review & editing. **Johanna Järvistö, Kaisa Järvenpää, Minna Sepponen**: Implementation of tests, Writing. **Matti T. Leppänen**: Methodology, Validation, Investigation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juha Karjalainen reports financial support was provided by BASF.

Data Availability

Data will be made available on request.

Acknowledgements

We thank Janne Koskinen, Risto Latvanen, Hanna Suonia, Jouni Taskinen, Saana Tepsa, Siiri Keurulainen and Kimmo Puosi their advice and support to the implementation of this study, local fishers for the delivery of fish samples, Mervi Koistinen, Emma Pajunen, Nina Honkanen, Rauni Kauppinen, and Anne Palmgren for skilful technical assistance, and KVVY and SYKE Oulu labs for laboratory analyses. This study was partly financed by BASF Finland.

Supplementary material

Detailed description of toxicity tests carried out in this study (S1).

The test water characteristics during the Na_2SO_4 toxicity tests (Table S2).

Hazardous concentration of sulfate (HC5) derived by four models (llogis, gamma, lnorm, weibull models) included in the ssdtools R package of Thorley and Schwarz (2021) with model statistics (Table S3).

Ecotoxicity data in soft freshwaters obtained from literature (Table S4).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.114984.

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