INTELLIGENT ECOTOXICOLOGY OF CHEMICALS IN UK RIVERS – DEVELOPMENT OF A RISK-RANKING METHODOLOGY

A Thesis submitted for the degree of Doctor of Philosophy

By

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1. DEVELOPMENT OF A RISK-RANKING METHODOLOGY

1.1. ABSTRACT

Given the presence of 1000s' of chemicals discharged by the human population to water, regulators and environmental scientists have to decide where best to focus their efforts. As a test case, this study used an identical protocol to rank 12 metals, 21 pesticides, 15 persistent organic pollutants (POPs), 13 pharmaceuticals, 10 surfactants/others and 2 nanoparticles (total of 73) of concern against one another by comparing their reported UK river water and published ecotoxicological effect concentrations. The chemicals were compared initially on the basis of the proximity of the median effect and median environmental concentrations. The closer the two median values are to each other, the greater the perceived risk. Further refinements to the risk-ranking were then introduced, including incorporating bioconcentration factor, using only recent water measurements and excluding either lethal or sub-lethal effects. The management of these data led to each chemical being ranked in terms of risk against every other. The top 10 chemicals which emerged as having the highest risk for UK surface waters using all the ecotoxicity data were copper, linear alkylbenzene sulfonate (LAS), zinc, aluminium, ethinylestradiol (EE2), triclosan, manganese, iron, methomyl and chlorpyrifos. In the majority of cases it is unknown if any of these chemicals are actually harming wildlife in rivers, but the implication is that reductions in water concentrations of these chemicals would be the most beneficial for UK aquatic wildlife. This approach revealed big differences in relative risk; for example, zinc presented a million times greater risk then metoprolol and LAS 10,000 times greater risk than nanosilver. With the exception of EE2, most pharmaceuticals were ranked as having a relatively low risk. The relatively high risk of EE2 suggests we should be most concerned about pharmaceuticals that could act as hormones. Some of the chemicals identified as of high risk to aquatic wildlife, such as LAS, are not regulated whilst many of the lower risk-ranked chemicals examined are.

1.2. DECLARATION

The work submitted in this thesis was conducted between 2012 and 2016 at Brunel University London and the Centre for Ecology and Hydrology. This work was carried out independently and has not been submitted for any other degree.

Rachel Donnachie

1.3. ACKNOWLEDGEMENTS

Firstly, I wish to thank my supervisors, Professor Andrew Johnson and Professor John Sumpter, who have both been incredible people to work with and learn from. Their support and mentoring has ensured that I have gained as much as possible out of this experience, and I am extremely grateful to them both.

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To my friends and family, thank you, you have supported me during my PhD and encouraged me through this process and helped keep me smiling, I have really appreciated it.

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1.7. MOTIVATION

1.7.1. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates

Although the acute toxicity test has been rightly criticized for a variety of technical reasons that are beyond the scope of the present discussion, the principal criticism probably stems from inferential uses of acute toxicity data beyond their limitations, and out of context from other measures necessary for hazard evaluation. Unfortunately, that is the way things are in the real world, because acute toxicity measurements may be the only effects data available for many chemicals, and then for only a fraction of the thousands of chemicals that have been identified as having potential for escape into the environment. Ideally, evaluators of potential chemical hazards to the environment would prefer a plethora of additional measurements concerning possible effects on growth, reproduction, pathology, biochemistry, populations of aquatic organisms, and ecological relationships. Frankly, the U.S. scientific community does not have the time, research facilities, trained personnel, experimental animals, nor financial resources to provide the additional data needed for "comfortable" predictions of the possible environmental effects of a broad spectrum of chemical contaminants. What is needed is a strategy for concentrating limited scientific resources on those chemicals most likely to have adverse impacts on aquatic systems. Similarly, a chemical-analytical strategy is needed for a more comprehensive approach to the detection, identification, and analysis of a broader spectrum of chemicals in selected environmental compartments. Such strategies would probably not be foolproof and would be different for aquatic ecosystems than for terrestrial ecosystems.

Schoettger RA. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. US Dept of the Interior, Fish and Wildlife Service, Washington, DC.

1.7.2. The Invisible World

Lakes, rivers, chalk streams, brooks, waterfalls, burns, ponds, the UK has a wide range of freshwater environments, some are vast and flow for hundreds of miles, some no bigger than a garden pond. They're a crucial factor when considering the health of our natural world. For where there's water there's life. These freshwater environments are home to an abundance of wildlife, some flourish above the surface, or in the margins, others stay beneath in an almost invisible world. But whether above or beneath the surface they all serve a crucial role in these complex and delicate ecosystems. As well as being a haven for wildlife, there is a far tamer creature who relies on these watery underworlds equally as much. Since the being of mankind, we have been fascinated by water, we worship it, we have created Gods around it. Our freshwater environments inspire art, music and poetry. They are places we can actually touch nature, places we can escape to, or even just somewhere to go to think. It might be somewhere where you exercise or work, or may even just be a place where your dog goes for a dip on a hot day. In one way or another we are all connected to these environments, right down to the very water we drink. But while the list of benefits from having healthy functioning ecosystems is endless, the sad reality is around only ¼ of our freshwater systems are classed as being healthy. England contains over 85% of the worlds chalk streams. These gin clear natural wonders are an extremely important part of our natural heritage, and again 75% of our chalk streams fail to make good status in terms of their ecological health. The list of threats to our freshwater environments is also a very long one, from pollution and water abstraction to the lack of trees present in our countryside, and in a country where rain can seem relentless, it can often feel like water is an infinite resource. But as our demands for water increase, more and more water is taken from our natural environment.

Film by Andrew O'Donnell

Salmon & Trout Conservation UK

(https://www.youtube.com/watch?v=aAM3X1Fr_AI)

2. INTRODUCTION

2.1. THE FOCUS

Freshwater is an essential resource for humans, animals and plants alike, and freshwater ecosystems are an integral part of the earth's make up. For centuries, natural and anthropogenic influences have impacted this resource via numerous activities and stresses. Now, more than ever, it is a resource which needs to be protected and man's impacts on it understood and controlled. Chemicals may be playing an important role in the health of freshwater ecosystems. In the European Union there are over 100,000 chemicals which aquatic organisms are potentially exposed to in freshwater environments [1]. There is a concern that many are having damaging effects on wildlife [2]. However, we do not know which of these chemicals represents the greatest risk. Which of these 100,000 chemicals is of the greatest concern to wildlife and which the least?

2.2. FRESHWATER CHALLENGES

The earth consists of 71% water and 29% land, with approximately 96.5% of that water found in the oceans (saltwater), 2.5% is freshwater and 1% is other saline water. The actual amount of liquid freshwater available is minimal compared to the 2.5% of freshwater on the planet. Approximately 68.7 % of the freshwater on earth is stored in the icecaps, glaciers and as permanent snow. Of the remaining 31.3%, can be found as ground water, actual surface freshwater makes up only 1.2% of the world's freshwater resource, which is 0.03% of the world's total water [3]. Yet this liquid freshwater is considered one of the most essential natural resources. It is a valuable natural resource, in economic, cultural, aesthetic, scientific and educational terms. Due to its importance, freshwater has been used extensively and now there is a crucial demand for innovative approaches to conserve it [4].

Freshwater water bodies include rivers, lakes, ponds and wetlands. As with any ecosystem, freshwater ecosystems are made up of a network of species which are dependent on each other and on the freshwater environment. Organisms which inhabit the freshwater environment include bacteria, phytoplankton, algae, plants, zooplankton, invertebrates and fish and amphibians. Other organism such as mammals and birds are reliant on freshwater environments as a food and water source.

Freshwater systems such as rivers are very dynamic, their flows will change throughout the season and human inputs vary along their length. Humans rely on and use a large percentage of freshwater (and saltwater) ecosystems for a range of purposes ranging from drinking water sources, leisure activities to industrial demands. As the human population has grown and society has developed, so the need for freshwater has increased [5]. In the UK the value of freshwater ecosystems, as natural capital, has been calculated, emphasising their value (importance) in monetary terms. Based on the methods described in the Office for National Statistics report, the services provided by UK freshwaters can be categorised as provisioning services, regulating services and cultural services [6]. These categories can be further broken down and valued (Table 2-1). The combined value of these services, based on the report for the year 2012, was £37 billion.

Table 2-1 Asset values of UK freshwaters (2012)

Service		**Asset value**
Provisioning service	Fish extraction	£0.9 billion
	Water abstraction	£23.9 billion
	Peat extraction	£0.2 billion
Cultural service	Recreational visits	£14.5 billion
	Educational visits	£0.9 million

The anthropogenic influences which have impacted freshwater ecosystems range from habitat degradation, introduction of alien species, disease, climate change, chemicals and industrialisation. The threats that freshwater ecosystems face can be categorised into five categories, namely overexploitation, water pollution, flow modification, destruction or degradation of habitat and invasion of exotic species [7].

Humans effects on aquatic biota have been assessed and reported for over a century [8] (Rimet, 2012, citing a report by Kokwitz and Marson 1908). Globally the biodiversity which occupies freshwater ecosystems consist of >126,000 species [9]. Reports state that freshwater biodiversity is in a state of crisis [10], and that 65% (globally) of the freshwater aquatic habitat is under moderate to high threat due to anthropogenic stresses [10]. Freshwater ecosystems are some of the most endangered ecosystems in the world [7].

It is likely that the pressure from the various anthropogenic sources is not going to reduce in the near future. Water pollution via chemicals is just one aspect of concern to freshwater ecosystems, and may not even be the biggest threat. But it is important to have an understanding of which chemicals are of the greatest concern, so that they can be put into context with other stressors [11].

2.3. CHEMICALS IN FRESHWATER

The use of chemicals has bought benefits to human society as well as risk to both human and environment health. Humans rely on chemicals, they are used every day, often without full consideration of the life cycle of that chemical, its source, production, use and after life. Chemicals are used in agriculture, industry, housing, transport, textiles, and health. As with many trends in history, as the human population has increased, so has their demand for resources, and the demand for chemicals is no different. As the population increases so does the per capita demand on energy, resources and consumer goods. This is often associated with standard of living, and our desire for improved standards of living [12]. This relationship between humans, chemicals and impacts has been classed as the Chemical Age, which started in the 1930's. It then escalated and the consequences of it have been seen since the 1950's. The key question that needs to be answered: what is the use of chemicals and humans reliance on them doing to the environment? There is a conflict between the desire to have development and improvement with the desire to have a healthy environment: can these two go hand in hand [13]?

The fate of chemicals in the environment is dictated by their chemical properties and how they interact with the ambient environment [14]. Chemicals with even a short half-life can now be considered persistent due to their continual release into the environment via treated sewage. This factor has been termed 'pseudo-persistence'.

2.3.1. Sources and groups of chemicals

There are some 100,000 chemicals reportedly in use in the European Union today [12]. This number is made up of a variety of classes and subclasses of chemicals, all with different purposes, structures and properties. It includes both naturally occurring and man-made substances such as metals, pesticides, pharmaceuticals, surfactants, nanoparticles and many more. A large percentage of these chemicals can make their way into the terrestrial and the aquatic environments, through their production, use and disposal, either deliberately or accidently (Figure 2-1, Table 2-2) [15]. Entry can be related to both human and natural process. The simplest classification of water pollution sources are a) point source and b) non-point source. Point source usually involves the direct release of chemicals from a discharge source in to the environment, where as non-point source is usually a less direct route, often involving run-off from adjacent land. Point source contamination can be spatially limited due to the manner of the input in the environment, where as non-point source can be very diffuse in nature due to the contamination occurring over a broad geographical area [16].

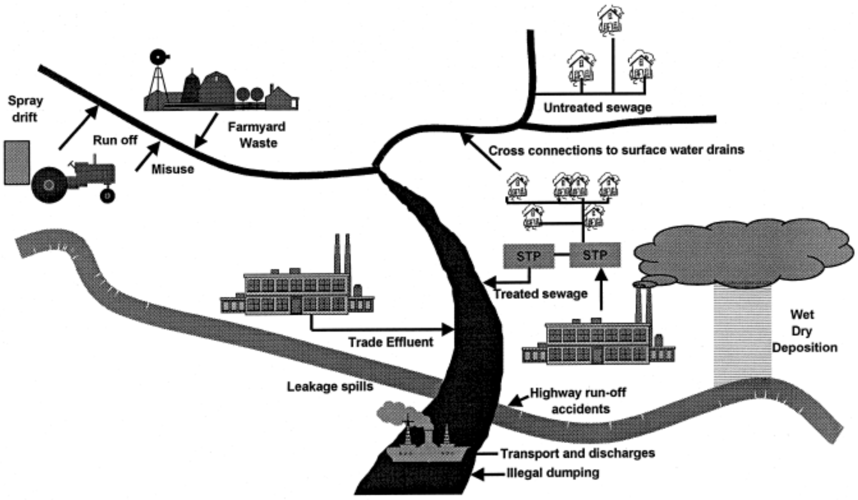


Fig. 1. Major routes of entry into surface waters.

Figure 2-1 Sources of chemicals into surface water [12]

2.3.2. Metals

Metals are natural substances, they are generally defined as elements which are good conductors of electricity and heat. Metals were previously categorised as ‘heavy metals’ or ‘light metals’, although these terms are generally now considered outdated. Some metals are essential for human and animal life i.e. iron, zinc, copper, manganese, chromium, molybdenum and selenium. They are components of enzymes involved in metabolic or biochemical process [17]. Metals have been used by humans since 6000BC, although the repertoire of their use has increased alongside industrialisation and technological developments, and they are now used to create jewellery, form electrical connections and wires, create transport and structural building materials, to name only a few of their numerous uses.

Their entry into the aquatic environment can be from natural and man-made sources. Their classification as pollutants is usually related to their use in human activities. They can naturally enter the environment via weathering of igneous and metamorphic rocks and soils. Metals may contaminate water following atmospheric deposition, such as mercury from combustion [18]. Entry into the environment due to human influence includes corrosion of metal pipes, direct discharge via contaminated waste (industrial and domestic), disturbance of drain basins as well as contamination via mining.

Metals cannot be created or destroyed. Both essential and non-essential metals are naturally bioaccumulated and concentrations are internally regulated by organisms. The toxicity of metals will be influenced by bioavailability according to the chemistry of the local water [19].

The discharge of metals from industry and domestic sources has drastically increased the input and release of metals into the aquatic ecosystem [17]. However, with the reduction of the heavy metal industry and the banning of lead in petrol, it might be expected that concentrations of at least some metals in freshwater will decline.

2.3.3. Pharmaceuticals

Pharmaceuticals are a class of chemicals which have been developed to address the medical needs of humans and animals. It is a diverse group of chemicals with an array of chemical properties. They can be used to treat a vast range of aliments and diseases i.e. the use of analgesics (painkillers) such as paracetamol to treat common aches and pains, antibiotics (i.e. penicillin) to fight infections, antidepressants (i.e. fluoxetine) to treat depression, and cancer fighting drugs such cyclophosphamide, which is used in chemotherapy treatment.

Human pharmaceuticals enter the aquatic environment following human consumption of the drug, excretion of the parent molecule and then a proportion enters sewage treatment plants (STPs). The release of pharmaceuticals into the environment following use in veterinary medicine is harder to quantify, but as with humans, the drug will pass through the animal and be released via faeces and urine. The drug may then be leached through the soil into ground and surface water. The spreading of manure off-site is one way that veterinary pharmaceuticals could cause diffuse pollution. In the 1990's the occurrence of pharmaceuticals in the environment became of interest to the scientific and non-scientific community, due to developments in the ability to measure them in the environment. This interest has continued and thus this class of chemicals has been of particular interest to science and policy over the last 15 years.

Unlike some of the other chemicals studied in this project, environmental exposure is likely to be strongly linked with point sources (sewage effluent) and likely to grow rather than diminish.

2.3.4. Persistent Organic Pollutants

Persistent organic pollutants (POPs) are a group of organic compounds which to a varying degree resist photolytic, biological and chemical degradation. They are characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues of a wide variety of organisms. They are also semi-volatile, enabling them to move long distances in the atmosphere before deposition occurs. They include halogenated legacy contaminants such as polychlorinated biphenyls (PCBs) and DDT, as well as emerging contaminants such as polybrominated diphenyl ethers (PBDEs). Most POPs are man-made, and they are, or were, used in a variety of ways including agriculture, for disease control, and in manufacturing and industrial processes. POPs are now ubiquitous in the environment, they degrade at slow rates, and thus they can persist in the environment.

Sources of POPs into the environment includes direct entry (i.e. via agricultural runoff following use as a pesticide), or via transfer to almost everywhere via atmospheric transport.

The use of many POPs is now restricted or banned entirely, and hence their presence in water is likely to very slowly diminish. In most cases we could view them as providing low level but continual exposure for wildlife.

2.3.5. Pesticides

Pesticides are a group of chemicals which have been designed to kill, repel or control pests such as weeds and insects. Within the pesticides group there are herbicides (weeds), insecticides (insects), fungicides (fungi), nematocides (nematodes), rodenticides (vertebrate poisons) and molluscicides (snails). Pesticides are used both on an industrial and domestic scale i.e. they are used in agriculture to protect crops and animals, in both urban and industrial sites to control weeds, and in the home to control pests.

Pesticides entry into the aquatic environment will be depend on their purpose. Application will be related to the crop growing cycle or period of peak emergence of the pest to be controlled. In other words, contamination will be dominated by seasonal spikes.

2.3.6. Metal nanoparticles

Nanoparticles are classed as particles between 1-100 nanometres in size. The term metal nanoparticle is used to describe nanosized metals within this nano size range (length, width or thickness). The application of nanoparticles relevant to the aquatic environment includes silver nanoparticles in fabrics and medical applications and nano zinc oxide in cosmetics and sunscreens [20].

As with pharmaceuticals, thanks to their use in personal care products they can enter surface water through domestic sewage. It is possible that exposure from these compounds will increase with time.

2.3.7. Surfactants

Surfactants (surface active agent) are organic chemicals which lower the surface tension between two liquids and between a liquid and a solid. They contain both hydrophobic and hydrophilic groups, thus they have a water soluble and water insoluble component. Surfactants function by breaking down the interface between water and oils and/or dirt. They also hold these oils and dirt in suspension, and so allow their removal. They are used as detergents, wetting agents, foaming agents and dispersants. Common application of surfactants includes soaps, laundry detergents, dishwashing liquids and shampoos. They are added to detergents to remove dirt from skin, clothes and household articles.

Surfactants are produced and used in extremely high amounts, in 2008 the global production was estimated to be 13 million tonnes [21]. Surfactants are classed as down the drain chemicals as their main route into the aquatic environment is following their use both in industry and domestically, from where they are released into the sewage treatment plant. Surfactants are also used in agriculture in pesticides, diluents and dispersants.

As with the pharmaceuticals and nanoparticles, we might expect their major association would be a point source contaminant. Due to environment concerns, some surfactants have fallen from favour whilst others remain popular.

Table 2-2 Sources of chemicals in surface water

Source		Chemical
Point	**Industrial**	
	This includes process effluents from pulp and paper mills, chemical manufacturers, steel and metal product manufacturers, textile manufacturers, food processing plants	Organochlorines, metals, dyes, BOD
	Waste water treatment plants	
	Wastewater treatment plants that may receive indirect discharges from industrial facilities or businesses	Metals, pharmaceuticals, antimicrobials, nutrients
	Storm tanks and sewer overflows	
	Wastewater treatment facilities or single facilities that treat both storm water and sanitary sewage, which may become overloaded during storm events and discharge untreated wastes into surface waters	Pathogens, metals, polycyclic aromatic hydrocarbons (PAHs), sediment

	Resource extraction	
	Mining, petroleum drilling, runoff from mine tailing sites	Metals, PAHs, acidity
	Land disposal	
	Leachate or discharge from septic tanks, landfills, industrial impoundments, and hazardous waste sites	Pathogens, nitrates, hazardous chemicals
Non-Point	**Agricultural**	
	Crop production, pastures, rangeland, feedlots, animal operations	Pesticides, nutrients, pathogens, sediment
	Storm sewers/urban runoff	
	Runoff from impervious surfaces including streets, parking lots, buildings, roof, and other paved areas	PAHs, sediments, pesticides, pathogens, metals
	Silvicultural/forestry	
	Forest, crop, and pest management, tree harvesting, logging, road construction	Pesticides, sedimentation
	Atmospheric deposition	
	Emissions from industrial stacks and municipal incinerators, pesticide applications	Persistent organic and polar pollutants (POPs and PPOPs), metals

Table taken from [16] and edited based on [12, 22].

Table 2-3 Quantities of chemicals produced or used that have the potential to enter surface water

Chemical	Quantities produced or used
Metals	Total world production (individually) of Cu, Zn, Cr is greater than $1x10^7$ t/year. Total world production (individually) of Pb, Ni, As, Ag, Cd, Hg is between 1,000 - $1x10^6$ t/year.
Pharmaceuticals	Usage and sales data UK 2000 Based on top 25 pharmaceuticals used in England – 1,431,596 kg/year Sweden 2002 Based on a selection of the top pharmaceuticals used in Sweden – 697,508 kg/year Denmark 1997 Estimated annual consumption (kg) based on the data for the main ATC groups in Denmark. 414,108 kg/year
Pesticides	EU sales data Sales of pesticides in 2014 - 400,000 tonnes With fungicides and bactericides being the greatest proportion, followed by herbicides.

	In the UK – 22,662.7 tonnes in 2014 (5.7% of EU total)
POPs	Following the Stockholm Convention (2004) there has been legislation in place to support participating countries to reduce the use and production of POPs. In an EU report for 2007-2009; one case of suspected production (<1,000 kg/year) of hexachlorobenzene (banned substance) was reported in France.
Nanoparticles	Predicted major markets for nanoparticles by volume (2002): automotive catalysts (11,500 tonnes), chemical mechanical planarisation slurry (9,400 tonnes), magnetic recording media (3,100 tonnes) and sunscreens (1,500 tonnes). A recent survey found that more than 2300 products containing nanoparticles are available to European consumers. No exact production quantities are currently publicly available. Production estimates: TiO2 (550– 5500 t/year), SiO2 (55–55000 t/year), AlOx (55– 5000 t/year), ZnO (55–550 t/year), carbon nanotubes (CNTs; 55–550 t/year), FeOx (5.5–5500 /tyear), and CeOx and Ag (both 5.5–550 t/year).
Surfactants	Worldwide production in 2006 - 12.5 million tonnes (synthetic surfactants). 2007 – in Europe – 3 million tonnes.

	In Europe, the amount of LAS reportedly used in 2005 – 430ktonnes.
Chemicals & pharmaceuticals	Based on a report by the Chemical Industries Association (CIA 2015), detailing the world's top global producers of chemicals and pharmaceuticals the UK ranks in the top ten with an estimated sales in 2015 of 54 billion euros.
References	
www.europa.eu, [23], [24], [25], [26], [27], [28], [29], [30], [31].	

2.4. WHAT'S BEING DONE ABOUT CHEMICALS IN THE ENVIRONMENT?

2.4.1. Research

Of these 100,000 chemicals perhaps only a quarter may have been adequately assessed for potential hazard [32]. As the chemical and pharmaceutical industry has developed, this has led to an increase in freshwater contamination by chemicals over time [33]. There are serious questions to ask over whether we will ever be able to obtain sufficient information to evaluate the safety of all of these chemicals in the environment [34]. The issue of thousands of pharmaceuticals, and more recently also nanoparticles, appears to overwhelm our capacity to assess the risk to wildlife from exposure to chemicals, especially if we proceed on a chemical by chemical basis. Nevertheless, we are not short of information; in 2012 Chemical Abstracts Service reported nearly one million articles, out of which nearly half covered research at the interface of chemistry and biology. This indicates that there is a wealth of knowledge available in the subject area of chemical and biological science to help us assess risk [35]. There is an abundance of literature which is publicly available that details both the exposure and the hazard aspects of the impacts of chemicals in the environment.

(Figure 2-2, Figure 2-3, Figure 2-4). This literature is an indication of the time and resources that have been spent on researching/studying chemicals. It has allowed developments and understanding of chemicals and the role they play in the aquatic environment to be explored. The message to take away from this is that a lot of research has been completed.

However, given the inevitably modest budgets available for environmental study, which chemicals should we examine further, or regulate, in order to best protect our aquatic environment? Environmental research funding is not necessarily a rational or objective process as funding organisations (and their reviewers) are influenced by fashion, novelty or political imperatives. This subjective process could leave us with considerable knowledge on some chemicals whilst others remain unstudied [36, 37]. But if fish, as an example of aquatic wildlife, could vote, which chemical would they indicate as their greatest concern?

Globally it has been recognised that there is a need to develop a better understanding and management strategy with regards the risk posed by chemicals to human health and the environment [36]. Deciding which chemicals are of most concern is a global challenge and has been highlighted as one of the top research questions needing to be answered by the Society of Environmental Toxicology and Chemistry (SETAC) [38]. The safeguarding of freshwater ecosystems is an increasing challenge as human and industrial demands on water resources grow and we continue to be in an era of water scarcity [39], with the potential for extreme low flow events, which may occur more frequently as a consequence of climate change.

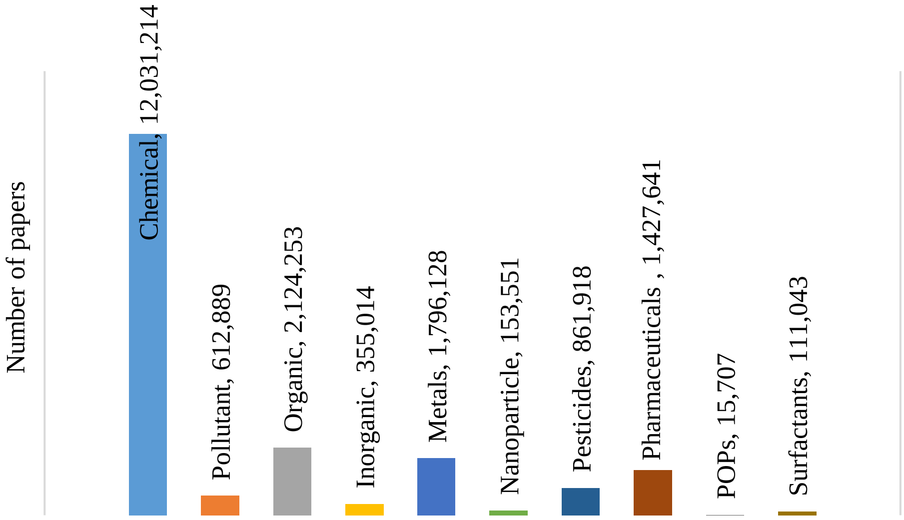


Figure 2-2 Number of papers per chemical term, obtained from the Web of Knowledge (WoK) on 10th July 2015, (based on all years reported in WoK).

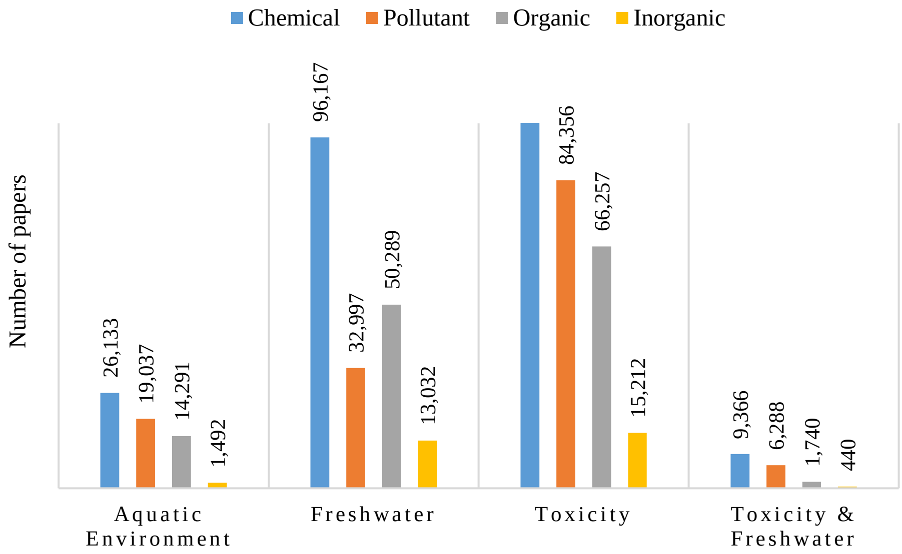


Figure 2-3 Number of papers per chemical term, obtained from the Web of Knowledge (WoK) on 10th July 2015, (based on all years reported in WoK), using then search terms aquatic environment, toxicity, and freshwater.

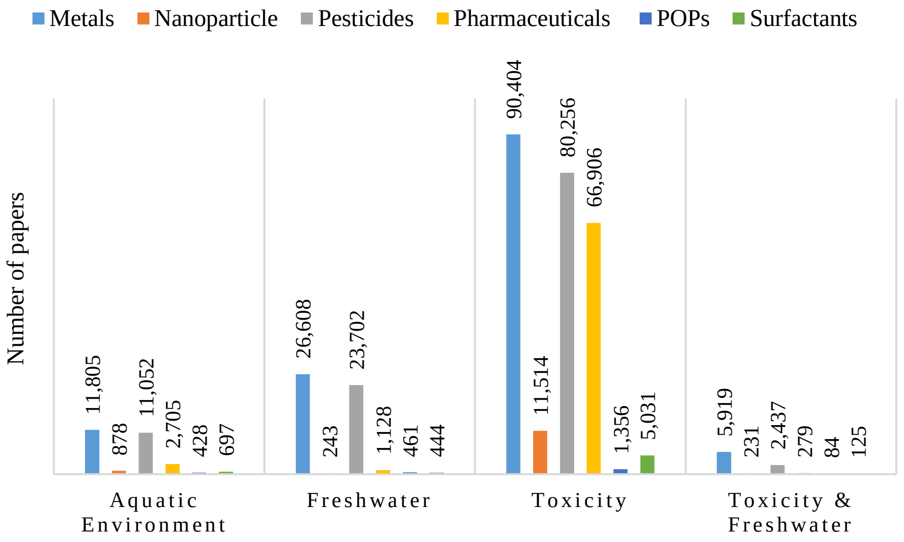


Figure 2-4 Number of papers per chemical class, obtained from the Web of Knowledge (WoK) on 10th July 2015, (based on all years reported in WoK), using then search terms aquatic environment, toxicity, and freshwater.

2.4.2. Evidence of chemicals having harmful effects

Ecotoxicity as a field of research was stimulated by widespread concern following Rachel Carlson's book Silent Spring [13], which brought the danger of pesticides to the attention of scientists and policy makers. She suggested that the continued release of chemicals would result in a world with reduced diversity as well as weak, sick animals. This led to greater efforts to control chemicals through regulation.

The earliest reports of chemicals being a problem in the aquatic environment dates back to the 1960's [40]. Chemicals can affect organisms from a subcellular level through to the community and ecosystem level. Common end-points measured to help establish an understanding of the effects of chemicals include their impact on survival, growth, reproduction and mortality of organisms.

Examples of high profile cases of chemicals having harmful effects on wildlife are detailed in Table 2-4. Scientists and policy makers are not the only people interested in the effects of chemicals on aquatic wildlife, the media regularly report on the effects of chemicals on humans, wildlife and the environment (Figure 2-5). It is the knowledge, that severe harmful effects can occur due to the presence of chemicals in the aquatic environment and the vested interest of multiple parties, which reinforces the need for a robust understanding of chemical impacts in the environment and their relative risk compared to other environmental stressors.

Table 2-4 Historical chemical impacts on wildlife

Chemical and Impact
Aluminium (Al) is a metal which has no biological function for aquatic organisms. Its fluxes in the environment can have a negative effect on aquatic organisms. In freshwater, Al becomes more soluble in acidic (<pH 6) and alkaline (>pH 8) conditions, its toxicity increases dramatically [41]. In the 1970's, due to an increase in the burning of fossil fuels, acid precipitation occurred (acid rain). The sulphur in coal was the reason for the acid rain (H_2SO_4), and the effects were often dramatic, and widespread. Acid-rain from the UK had major adverse effects in Scandinavia [42]. This increase in the acidic levels of freshwater resulted in an increase of soluble Al. The result of this was a loss of aquatic life in freshwater bodies affected by 'acid rain' [43].
DDT (dichlorodiphenyltrichloroethane) is an organochlorine insecticide, its uses included the control of malaria, typhus, body lice and bubonic plague. DDT accumulates in body tissues of aquatic organisms. It was proven to have adverse impacts throughout the food web. It biomagnified up the food chain, and hence species at the top of food chains had the highest body burdens, and showed the most severe effects. Birds of prey, specifically the peregrine falcon and the sparrowhawk, were affected resulting in the thinning of the eggshells causing hatching difficulties [44, 45]. The effects of DDT on bird populations were

devastating. Due to its adverse effects DDT was banned from use in the 1980's, however due to its ubiquity and persistence it is still classed as a concern today.

Tributyltin (TBT) is an organotin and for approximately 40 years TBT was used as a biocide in anti-fouling paints, to prevent the growth of algae and barnacles on ship hulls not only in the UK but worldwide.

However, the use of TBT has resulted in significant adverse effects on aquatic organisms, especially mollusc, such as induced imposex in snails and shell malformations in oysters and mussels [46]. This led to their disappearance from some bays in the UK and elsewhere across the world [47].

Since its ban, the effects of TBT on aquatic organisms has been reduced. The reported levels of imposex within populations has decreased and the recovery of species populations has been seen within the UK, as well as globally **[**48**,** 49**].**

The synthetic steroid **17-a-ethinylestradiol (EE2)** is one of the active ingredients in the contraceptive pill **[**50**]**. Its release into the aquatic environment via sewage treatment plants has led to low but continuous concentrations occurring in freshwater bodies, especially rivers. It was identified as playing a major role in endocrine disruption of fish. This could lead to reduced fertility in male fish **[**51**]**.

Diclofenac is an anti-inflammatory treatment used in both human and veterinary medicine.

In India in the 1990's there was a mass decline in the number of vultures, a crucial species that acts as a natural animal-disposal system. Diclofenac was used to treat cattle in India for inflammation and fever, etc. When the cattle died (not from diclofenac), vultures fed off the carcass. It was the exposure to diclofenac via the cattle carcases that caused mass declines in vulture populations in India **[**52**]**.

NATURE | NEWS

Europe sounds alarm over freshwater pollution

Environment agency review also charts gloomy news on biodiversity.

You are in: Health

Monday, 12 August, 2002, 10:26 GMT 11:26 UK

Fears over 'gender-bending' chemicals

There are fears that chemicals could damage health

A global report has failed to rule out risks humans and wildlife from "endocrine disrupting" chemicals in the environment

Experts at the World Health Organisation International Programme on Chemical Sa say that research linking this type of poll to health problems is patchy.

You are in: Science/Nature

Thursday, 26 September, 2002, 09:41 GMT 10:41 UK

Chemicals spark Arctic alert

By Alex Kirby

BBC News Online environment correspondent

Chemicals used to make household products fire-resistant are being discovered in several Arctic species.

The chemicals, brominated flame retardants,

Study links insecticide use to invertebrate die-offs

Wake up to the danger of slug pesticides

What happens to the excreted drugs you flush down the toilet?

Doctors should take into account the 'downstream' effects on the environment when they prescribe drugs, suggests a scientist at the US Environmental Protection Agency

Study shows dangers of BPA chemical used in plastic packaging

Figure 2-5 Media acknowledgment of the presence of chemicals in the environment and some of the adverse effects they can have on wildlife.

2.4.3. Prioritisation

Prioritisation of chemicals and their impacts on the environment and human health has become of increasing importance, as our ability to assess and control has become outstripped by the sheer number of chemicals we produce. The purpose and need for regulation is driven by society's ethical responsibility, the need to maintain a healthy aquatic environment and the need to protect fish and other organisms, and consumers of those organisms, from the adverse effects of chemicals. There is a need to prioritise research based on the chemicals which are of greatest concern, not those which are most fashionable [53]. Environment pollution goes beyond effects on aquatic organisms. It is important to note that the contamination of freshwater and the effects of chemicals on freshwater organisms is what is under study here, but there is a clear link between the negative effects of chemicals on freshwater ecosystems and the potential for human health threats. Hence, there is still a need for improved and adequate risk management for this purpose also [54].

2.4.4. Approaches in the literature to assessing the risk posed by chemicals

Since the global concern both to human and environmental health posed by chemicals became apparent, studies have been published based on different methods to prioritises and rank chemicals of concern, and establish risk assessments in order to gain a better understanding of the effects of chemicals in the environment [1, 55-57]. A chemical risk assessment is usually based on a risk quotient/ratio, calculated using a combination a either measured (MEC) or predicted (PEC) environmental concentrations which is compared to ecotoxicity data (i.e. LC50) or a risk threshold which is derived from experimental ecotoxicity data or modelled data to establish a predicted no effect concentration (PNEC); a value greater than one is considered to be of concern [58]. Other examples of approaches in the literature used to prioritise or rank chemicals are based on: usage and sales data [59], exposure/occurrence data [23], biological data [60], risk ratio/quotient based on a combination of environmental and ecotoxicity data [2, 61-63], multiple variables schemes and tiered approaches [64-68], species sensitivity distribution [69], and read across theory, [70-72].

These methods range from simple assessments to complex processes. There is currently no scientific consensus on the best method to assess hazardous chemicals; there are advantages and disadvantages in most approaches [73].

There is not (to my knowledge) an approach that considers all chemicals, regardless of their class, all aquatic species and all end-points. No current approach takes all the data available in the literature and uses it to consider the relative risk of chemicals to create an unbiased risk-ranking.

2.4.5. EU policy

The relevant EU the legislation which governs hazardous, or priority, substances is the Water Framework Directive (WFD). It was established in 2000 with the aim of achieving good ecological and good chemical status of surface waters by 2015. The main objective of the Priority Substances Directive of the EU [74] is protecting wildlife and humans from harmful effects of chemicals identified as priority substances in surface waters, and to monitor trends in the concentrations of these chemicals. It does this through setting environmental quality standards (EQS) for a number of chemical pollutants, below which no harmful effects are expected to wildlife, or humans. Environmental quality standards are defined as the "concentrations of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment". Initially there were 33 priority substances, but now, through three different 'campaigns', this number has increased to 45. The priority substances list includes metals, pesticides, polycyclic aromatic hydrocarbons and organotin compounds (Table 10-2), and it is now set that this list will be reviewed every six years. Priority substances are defined as "individual pollutants or groups of pollutants presenting a significant risk to or via the aquatic environment, including such risks to waters used for the abstraction of drinking water", while priority hazardous substances are defined as "substances or groups of substances that are toxic, persistent and liable to bio-accumulate; and other substances or groups of substances which give rise to an equivalent level of concern". There is an option for member states to set biota, or sediment, standards which offer "at least the same level of protection" as the water standards. This applies to chemicals for which water standards are not sufficient [74]. The methodology and process that generated the list of chemicals has fluctuated since 2000, and there is now documentation discussing an outline protocol to be used to determine which chemicals should be of concern and which should go forward onto the priority substance list. As stated before, the typical risk assessment approach considers the environmental concentration either the measured environmental concentration (MEC) or a predicted environmental concentration (PEC) and a derived ecological safety threshold known as a predicted no-effect concentration (PNEC). These values are used to calculate a risk ratio (PEC/PNEC), a risk ratio of greater than one is considered to be a concern and based on the current EU legislation would require an EQS to be developed for that chemical (*see section 10.1.1 for further details on regulations and derivation of EQS).* There are other approaches/ schemes to risk assessment and ranking as detailed by Guillen et al 2012 [55].

Due to the presence of chemicals in water and the implementation of legislation to meet regulations (EC 2000, 2008, 2015), means of controlling chemical concentrations in the environment have needed to be developed. Some control measures are: source control measures, enhanced treatment of wastewater, alternative options to assessing compliance i.e. taking into account bioavailability of the chemical. Source control is a means of preventing the release of chemicals prior to them reaching the environment and causing a problem. That is, stopping them reaching the environment rather than trying to take it out, i.e. restrictions within industry and agriculture on the amount of chemicals which are used.

2.4.6. Data reliability

The reliability of data is something that has been a concern within the ecotoxicity field [75]. The quality and the reliability of the data used to produce a risk assessment has been well documented, and schemes have been devised to assess the quality of the data used, to determine if data should be included or excluded. Perhaps the most widely used approach has been the Klimish approach [76], but others have been developed [76-78]. These methods generally assess whether the laboratory experiments are well documented and were conducted under standardised conditions. Problems that arise from the use of these quality control measures are that they rely on the information provided (something that is changing with a drive in science towards greater transparency, open access and an increased use of supplementary information when publishing work), the actual process is time consuming, and the process can be very subjective.

2.5. MATTHEW EFFECT & BIAS IN SCIENCE DECISIONS

2.5.1. The Matthew Effect

Ideally, science should reach an objective conclusion in answer to a scientific question. However, bias can enter into science both consciously and unconsciously at the conceptual as well as experimental stages. At the conceptual stage, before any science has been conducted, there is the opportunity of bias and external influence to enter into the decision making. Scientific topics can be ‘hot topics’ and thus can be more popular than others with scientists, funders and policy makers. At the experimental stage, scientists can bring in bias starting with the hypothesis development and experimental set up and then leading through to the analysis of the results and conclusions, with subjectivity being brought into the decisions. It has been reported that it is impossible to have truly objective, value-free scientific research [79]. The bias that is introduced in environmental science has a knock-on effect on the policies which are generated from the results of that research. One aspect of the bias that can be introduced is the idea that topics, or in this case chemicals, can become perpetually popular and thus the body of research and the funding available for a particular chemical increases. This concept is known as the Matthew Effect, the idea of the "rich get richer and the poor get poorer"; it originates from a verse in the Gospel of Matthew; *For unto every one that hath shall be given, and he shall have abundance: but from him that hath not shall be taken even that which he hath* (Matthew 25:29, King James Version) [80]. The influences of the Matthew Effect on different aspects of science such as communication, reward systems and resources have been discussed, suggesting that it can be both a conscious and unconscious action within the scientific community [80, 81].

2.5.2. Risk or fashion?

The concern with regards to risk-ranking chemicals and doing so by using scientific literature as a source of data is thus tainted by the notion that the risk of a chemical can be accelerated by politics, media, fashion, funding and a scientists own subjectivity. The attention placed on certain chemicals due to these factors may be justified, but it can also be misleading. The reporting of one finding (i.e. chemical X affects end-point Y in species Z) can thus influence the funding streams and thus the research which is conducted and published. Pharmaceuticals, specifically diclofenac and EE2 are examples of this, with funding and concern around both these drugs spiking following initial concern, especially compared to other chemicals. With regards to chemicals and the risks they present to aquatic organisms, this can lead to a chemical's prominence in the literature being due to the Matthew Effect, rather than due to actual risk. Grandjean et al (2011) went as far to say that the impacts of the Matthew Effect can actually limit innovation and discovery in research and science [37].

2.5.3. Chemical X did what to Species A! – Ecotoxicity advances

The ability to study unique sub-lethal end-points has improved considerably over the last 50 years. These advances have given us a better understanding of the impact of chemicals on organisms and also an increased number of sub-lethal end-points to consider when gauging the potential threat of a chemical. Providing further detail helps answer the questions; is a chemical going to be harmful to aquatic organisms, is its presence in the aquatic environment going to have detrimental effects at the individual level, community level or population level, and on one species or multiple species'? This increased knowledge has opened up questions beyond the original one of does the chemical cause mortality to a species? However, the influence of improved scientific techniques can introduce possibly misplaced concern surrounding chemicals. Understanding of the effects of chemicals on species may be increasing, but there is no systematic monitoring of the normal biochemical or the physiological changes that occur in fish, for example, and thus there is no baseline. Changes in populations of aquatic species are extremely difficult to detect and monitor. Mescoms studies can be used to replicate a specific environment, (i.e. taking a study further than a single species laboratory test), but even in such studies it is very difficult to identify specific effects caused by a chemical and monitor population changes which are a consequence of that effect.

2.5.4. How low can you go? Analytical improvements

Chemicals present in the aquatic environment are generally found in the ng/L to µg/L range. Chemicals being present at these lower levels is positive in terms of "pollution" levels, but with many chemicals present in the environment in the ng/L range, this presents its own challenges with regards to accurately determining what concentrations are in the environment. There is a requirement for analytical techniques to be developed to measure chemicals at that level, as well as to understand the consequence of the concentrations of that chemical. This is a difficult task, partly because river water will contain a complex mixture of chemicals [82].

As analytical techniques have improved, the ability to measure chemicals in the environment at lower and lower levels has improved scientific knowledge with regards to the vast infiltration that chemicals have in both freshwater and saltwater. Thus, the limit of detection recorded for a chemical i.e. in long term monitoring, changes as technical improvements are made. Mercury is a clear example of this (Figure 2-6) where the level of detection has improved significantly, since monitoring began. The LOD data available for dissolved mercury from the Environment Agency WIMS database dates from 1974 – 2012 is plotted in Figure 2-6. Based on this data the LOD has included 30 µg/L, 10 µg/L, 5 µg/L, 1 µg/L, 0.5 µg/L, 0.3 µg/L, 0.2 µg/L, 0.1 µg/L, 0.05 µg/L, 0.03 µg/L, 0.01 µg/L, 0.008 µg/L. This variation impacts the value used when incorporating below LOD records into a database, using the ½ LOD will not be consistent due to variation in LOD values over time. However, there is evidence of a misguided sense of risk related to chemicals which scientists could not previously measure, but can now, but which would undoubtedly have been present in our aquatic environment for years. Pharmaceuticals are an example of this; as analytical techniques have developed, so have the concerns based on the information from them, but is this concern justified? [83].

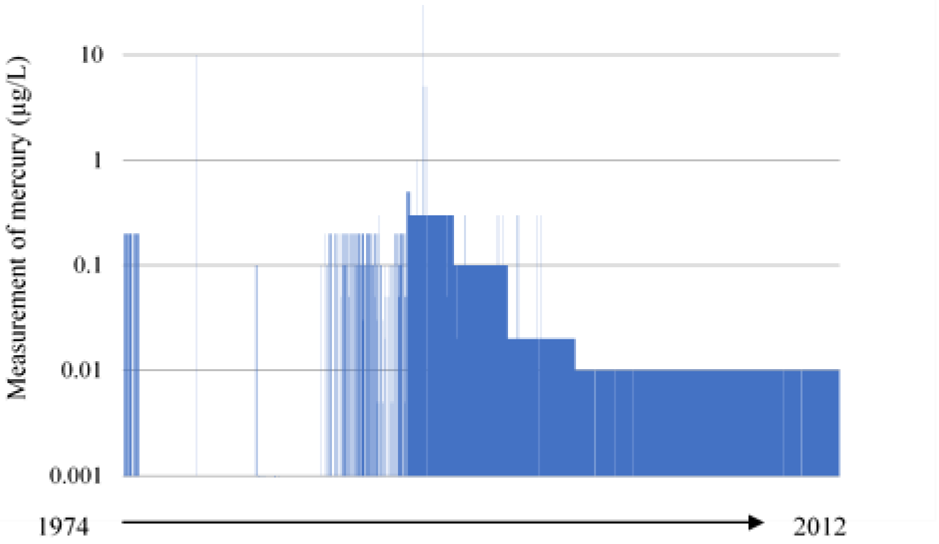


Figure 2-6 Mercury LOD only values to show change in LOD over time (1974-2012)

2.6. SUMMARY

The aim of environmental science is often to ultimately influence policy to improve and conserve the natural environment. Humans have a responsibility to protect and conserve the natural environment. This is where science and policy come together to implement actions to ensure that the environment is protected.

As research continues, and the field of ecotoxicity develops, it is fair to assume that the amount of information available on the effects of chemicals in the aquatic environment will increase. Alongside this growth in knowledge is a realisation that the questions that maybe should be asked or are already being asked, are:

- When chemicals enter the aquatic environment are aquatic species adversely affected by the presence of those chemicals?
- Are vulnerable species being affected by the presence of that chemical?

- Of the chemicals which have entered the aquatic environment, which one is of the greatest risk to aquatic organisms?

If these questions aren't being considered and answered, the potential worst case scenario would be the loss of various aquatic populations, purely due to the presence of an aquatic contaminant or a mixture of these chemicals.

Following the work that has been done on understanding the impacts of chemicals in aquatic systems, this issue is still considered to be a challenge going forward, which requires improved analytical and modelling tools to probe the distribution of chemicals and better understand the bioavailability, as well as improve our knowledge of the biological effects of single compounds and of chemical mixtures [33]. Thus, going forward it is important to have an understanding of where the bench mark of concern should be for 'chemical risk'? Should the level and impact of the effect matter? Should freshwater ecosystems be returned to the pristine environments they once were? Is this true conservation? Is this realistic? Is it needed? These may seem like philosophical questions, but actually they are very practical questions.

2.7. RESEARCH QUESTION

Chemicals enter the environment, they can cause harmful effects, there are multiple ways to complete risk assessment and there is bias in risk assessment. There is an overwhelming number of chemicals out there and there isn't the time or the resources to study them all. The UK represents an environment which has the potential to be greatly affected by chemicals, especially down the drain chemicals. Humans have a responsibility to look after and protect the environment. This study aims to use current expertise and resources to put the presence of chemicals in the aquatic environment into context. Risk-ranking chemicals using this approach is a means to check if regulations are appropriate. Currently scientists and policy makers alike don’t know which chemical is of greatest concern: can an unbiased approach be developed to shed light on this problem?

The aim of this project was not to complete a risk assessment. Instead, it was/is an attempt to compare one chemical against another using an unbiased risk-ranking approach. Enabling chemicals from different groups to be compared to better understand the effects chemicals could potentially have on UK rivers systems would be a significant advance.

It would be almost impossible and unrealistic to assess all chemicals produced as time and resources do not allow for it. Instead, the aim of the work herein was to develop a methodology to risk-rank chemicals, ultimately to answer the question: which chemical or group of chemicals is of the greatest concern to freshwater organisms in the UK? The thesis is split into 4 principle data chapters (chapters 4-7) and involves the analysis and comparison of ecotoxicity data and environmental data for a range of chemicals.

3. METHODOLOGY

3.1. INTRODUCTION

Freshwater aquatic organisms face the challenge of being exposed to a multitude of chemicals discharged by the human population. The objective of this study was to rank chemicals according to the threat they pose to aquatic organisms. The initial method involved the development of an uncritical data collection process and the comparison of information on ecotoxicological thresholds with measured and predicted chemical concentrations in rivers. The data are then refined to understand if the chemicals which appear to be of the greatest concern remained at the top of the risk-ranking following moderation of the approach and data.

The objective of this study was to rank chemicals on the basis of risk to aquatic wildlife. But the novel aspect to the approach is to try and use an unbiased method to risk-rank the chemicals, regardless of their class. The aim was to develop a rational approach to risk-ranking which is not influenced by subjectivity, preference, politics, fashion, etc.

This methodology chapter details:

- The chemicals chosen to be included in the project and why
- The data collection process
- The initial risk-ranking approach
- The second risk-ranking approach

3.2. WHAT CHEMICALS HAVE BEEN STUDIED

It would be impossible to investigate all chemicals with regards their threat to freshwater organisms in the UK in one PhD project. So this study can be seen as an example of how the topic could be addressed chemical by chemical. The classes of chemicals which have been included in the ranking exercise are metals, pharmaceuticals, persistent organic pollutants (POP's), pesticides, surfactants, nanoparticles and others (Figure 3-1). For each class, key representatives were studied in the expectations that they would act as an indication of the risk of that group as a whole (Table 3-1). In total, 73 chemicals were investigated in this study. The chemical selection was not random, the key representatives were chosen based on trying to identify chemicals which are prominent in the literature and that are considered by the scientific community to be a potential concern to freshwater ecosystems. Thus, comparing the potential worst case chemicals from each group to each other. For the chemicals examined here, this selection was something of a subjective process based on the wider project groups experience. However, in the case of the pharmaceuticals group, the selection process used an objective review of the literature.

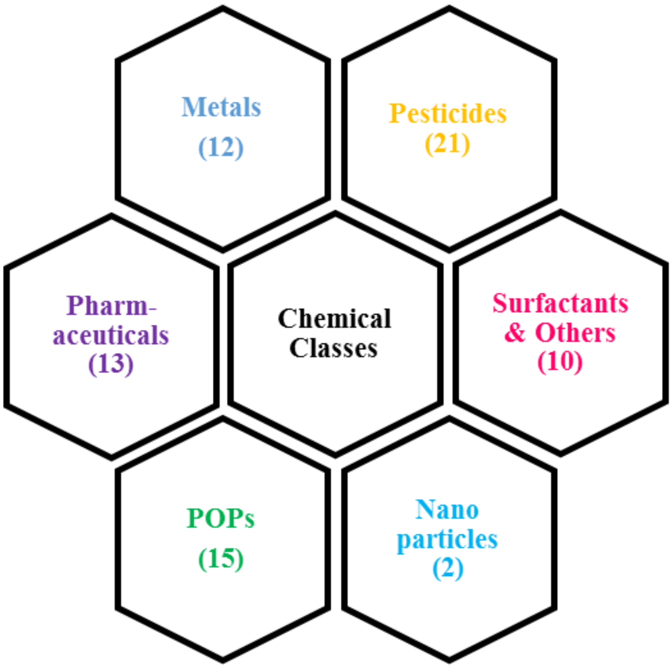


Figure 3-1 Classes of chemicals considered

*The coloured text represents the colour each class will be depicted in graphs throughout the thesis.

The groups of chemicals chosen and their selection is briefly described here:

3.2.1. *Metals selection*

Metal are natural elements; some are required for optimal functioning of biological and biochemical processes. The role of metals within living organisms includes stabilisation of protein structure, facilitation of electron transfer reactions and as catalyst in enzymatic reactions. Non-essential metals, which have no biological function, exert their toxicity by competing with essential metals for active enzyme or membrane protein sites. Essential metals, when present at sufficiently high concentrations, are toxic too [84].

Their entry into the aquatic environment can be from natural and man-made sources. Naturally they enter the environment via weathering of rocks, their entry through human activity includes corrosion of metals pipes and mining.

Twelve metals, representing both essential and the non-essential metals, were selected as representative of the metals class. The metals studied for this project were: aluminium, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, silver and zinc. These metals were selected in consultation with colleagues at CEH, the aim to include a range of metals some of which are regulated, some classed as a potential concern as well as others which historically have been of concern. As with all the classes of chemicals, there are numerous options, and only a small selection were selected to represent the class in this study.

3.2.2. *Nanoparticles selection*

Nanoparticles are classed as particles between 1-100 nanometres in size. The term metal nanoparticle is used to describe nanosized metals within this nano size range (length, width or thickness). Nano Ag and nano ZnO are the nanometals which have been well studied, and due to their extensive use are expected to be emitted into surface waters in Europe [20]. Nano Ag is used in personal care products while nano ZnO is used in paints and sunscreens.

3.2.3. Pharmaceutical selection

Pharmaceuticals are chemicals designed to treat a range of ailments and illnesses in both humans and animals. They enter the aquatic environment following human and animal consumption of the drugs. Following human consumption, drugs are excreted and enter waste water treatment plants, while following animal consumption the drugs are released directly into the environment.

Thirteen out of the potential 3,000 pharmaceuticals were selected as representatives of the pharmaceutical class. There are many different pharmaceuticals prioritisation methods available in the literature [56]. Each approach has its merits and weaknesses. From the 22 papers reporting prioritisation methods for pharmaceuticals, their lists of pharmaceuticals of potential concern were collated. The frequency of pharmaceuticals appearing on these lists was used to select the top 13. This approach was chosen with the aim of providing as unbiased a selection of 13 pharmaceuticals as possible. Thus, the top 13 ranked pharmaceuticals selected reflected the community opinion on which pharmaceuticals were most likely to cause environmental harm. However, it is important to remember that scientists working in the field have still only examined perhaps 100 out of 3,000 different pharmaceuticals (See section 10.1.5, Table 10-10 – Pharmecutical Paper for full deatil). The reason this approach was chosen for the selection of the pharmaceuticals was due to the recent/current concern around their use, and thus the multiple papers reporting prioritisation available in the literature.

3.2.4. POPs selection

Persistent organic pollutants (POPs) are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. They are characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. They are also semi-volatile, enabling them to move long distances in the atmosphere before deposition occurs. Their entry into the environment is dependent on their specific use, for example direct entry into the environment occurs following the use of pesticides, whereas PCBs enter the atmosphere following combustion. The POPs included in this study were chosen through consultation with expert colleagues at the Centre for Ecology and Hydrology (CEH) in Lancaster. Key representatives from different sub-classes within the POPs class were seen as sensible representatives of this diverse class of chemicals. The sub-classes within the POPs class include pesticides, combustion products, industrial chemicals, brominated flame retardants and polyfluorinated compounds. Identifying chemicals which are or have been of concern and which are likely to be found in the environment.

Since this project began, a Europe-wide study, conducted by the Helmholtz Institute in Germany, was published. This study was based on 223 organic chemicals [2]. The implications of that study were that organics, particularly pesticides, pose a clear and present danger to aquatic wildlife. A selection of these chemicals which were not initially considered were reviewed and included in the project.

This class of chemicals as with other classes includes a wide range of compounds which could have been studied. It was not possible to study all POPs therefore a selection were chosen as key representatives, and potential worse case scenarios.

3.2.5. Pesticides selection

A pesticide is a substance which has been designed to kill, repel, or control either a plant or animal which is considered as a pest, both at an industrial and a domestic level. The method of application of the pesticide will dictate how it enters the aquatic environment, i.e. following application onto crops a pesticide can leach through the soil and enter the aquatic environment. Pesticides make up the largest group considered within the project. Originally a section of 12 pesticides was chosen, inclusive of insecticides, herbicides, a biocide and molluscicide. They were chosen based on either their current use or their reputation as legacy pollutants. As with the POPs, additional pesticides were considered based on the publication of the Malaj et al (2014) study.

3.2.6. Surfactants & others selection

This class of chemicals includes surfactants and other individual chemicals which make up the final class of chemical to be considered. They are classed as down the drain chemicals as their main route into the aquatic environment is following their use both in industry and domestically. Surfactants lower the surface tension between two liquids and between a liquid and a solid, are a wide range of compounds. There are four different types: anionic, non-ionic, cationic and amphoteric. They all contain a hydrophilic and a hydrophobic end, it is the electrical charge on the hydrophilic end which distinguishes the different types of surfactants from each other. Their charge and characteristics makes the different types more appropriate to certain applications. Anionic have a negative charge on the hydrophilic end, non-ionic have no charge on their hydrophilic end, cationic have a positive charge on their hydrophilic end and amphoteric have both a positive and a negative charge on their hydrophilic end, their charge will change with pH. They can be anionic, non-ionic, or cationic depending on pH. Anionic have been the most widely used surfactants, especially LAS, thus they were deemed good representatives of the surfactant class. As well as being detergents and soaps, surfactants are also used in agricultural pesticides as diluents and dispersants.

The class includes not only surfactants, but also plasticizers, chemicals used to produce plasticity and flexibility in products, as well as an antimicrobial, an artificial sweetener and a corrosion inhibitor. With many of the chemicals investigated within the project, there is potential for them to be relevant to one or even several of the named chemical classes. The chemicals were grouped for the benefit of sub-dividing the total of 73 chemicals considered into manageable discussion classes. In their use and function some of the individual chemicals are not exclusive to just one class.

Table 3-1 List of chemicals (divided into chemical classes) investigated in this study

Metals	Pharmaceuticals	Persistent Organic Pollutant's
Aluminium	Aspirin	Benzo [a] pyrene (B[a]P)
Arsenic	Atenolol	Decabromodiphenyl ether (BDE 209)
Cadmium	Carbamazepine	Dichlorobenzene (DCB)
Chromium	Diclofenac	Dichlorodiphenyldichloroethylene (DDE)
Copper	Ethinyl estradiol (EE2)	Dibutlytin
Iron	Fluoxetine	Fluoranthene
Lead	Ibuprofen	Hexachlorobutadiene (HCBD)
Manganese	Metoprolol	Lindane
Mercury	Naproxen	Polychlorinated biphenyl 153 (PCB 153)
Nickel	Ofloaxcin	Polychlorinated biphenyl 180 (PCB 180)
Silver	Paracetamol	Polychlorinated biphenyl 194 (PCB 194)
Zinc	Propranolol	Polychlorinated biphenyl 52 (PCB 52)
	Sulfamethoxazole	Perfluorooctanesulfonate (PFOS)
		Trichlorobenzene (TCB)
		Trichloromethane (TCM)

Pesticides (1)	Pesticides (2)	Surfactants & others
Bentazone	Pendimethalin	Alcohol ethoxysulphate (AES)
Beta –hexachlorocyclohexane (Beta- HCH)	Permethrin	Alkyl sulfonate (AS)
Carbofuran	Pirimicarb	Benzotriazole
Chlorpyrifos	Simazine	Bisphenol A
Diazinon	Terbuthylazine	Bis(2-ethylhexyl) phthalate (DEHP)
Glyphosate	Tributyltin (TBT)	Linear alkylbenzene sulfonate (LAS)
Imidacloprid		Nonylphenol
Lenacil		Octylphenol
Linuron		Sucralose
Malathion		Triclosan
MCPA		
Mecoprop (MCPP)	**Nano particles**	
Metaldehyde		
Metolachlor	Nano Silver	
Methomyl	Nano Zinc oxide	

3.3. DATA COLLECTION METHODS

The Web of Knowledge was used as a source of published literature. The aim was to use literature which is publicly available. By using key search terms and then looking at citations and reviews and reports, data and opinions on each chemical were collated.

Using the information gained from the literature search a Summary Toxicity Report was compiled for each chemical which summarises the; toxicity data, environmental concentrations, sources of the chemical and uses. For all chemicals, publications were searched for based on a series of key words (Table 3-2). The two main categories of information required from the literature search were the effects of a chemical to aquatic organisms and the concentration in the aquatic environment of a chemical in the UK.

Table 3-2 List of key search terms used to search literature

Chemical name
Chemical name and aquatic environment
Chemical name and water
Chemical name and toxicity
Chemical name and fish
Chemical name and fish and toxicity
Chemical name and fish and toxicity and laboratory
Chemical name and fish and toxicity and laboratory and freshwater
Chemical name and species sensitivity distribution
Chemical name and toxicity and water
Chemical name and toxicity and freshwater
Chemical name and toxicity and OECD
Chemical name and toxicity and water and laboratory

As different groups of chemicals were considered through the project it was clear that the abundance of information per chemical varies drastically between chemical classes and individual chemical (Figure 3-2, Figure 3-3). This demonstrates the varying range of information per chemicals, with >600,000 papers on copper compared to 30,000 on metoprolol and <1,000 on imidacloprid. Thus, for some chemicals there is an abundance of effect data and river water data. With metals, for example, the luxury of information also meant that it was not possible to read every paper. Therefore, for metals only a selection of the 1,000's of papers were used in the study, as it would be near impossible to read every paper written on one metal, let alone all 12 metals considered in this study. Whereas for chemicals, where there is much less literature available, this resulted in it being possible to examine potentially all data in the literature, as there are a manageable number of papers. This had benefits and drawbacks. The main benefit was that it is possible to record all data which has been published on that chemical, but for a lot of chemicals this also means there is very limited ecotoxicology data, some of which is of questionable quality and that there is probably no environmental monitoring data.

By trying to limit influences of bias on the initial data collection, this project wanted to compare chemicals on an even playing field and look purely at the data without taking into account the species, effect, end-point or quality of that data. The aim was to use the raw data to understand if a simple approach to risk ranking echoed that of more complex analysis. This preliminary approach will be referred to in this thesis as tier one ranking. The more complex analysis of the data will be referred to as tier two.

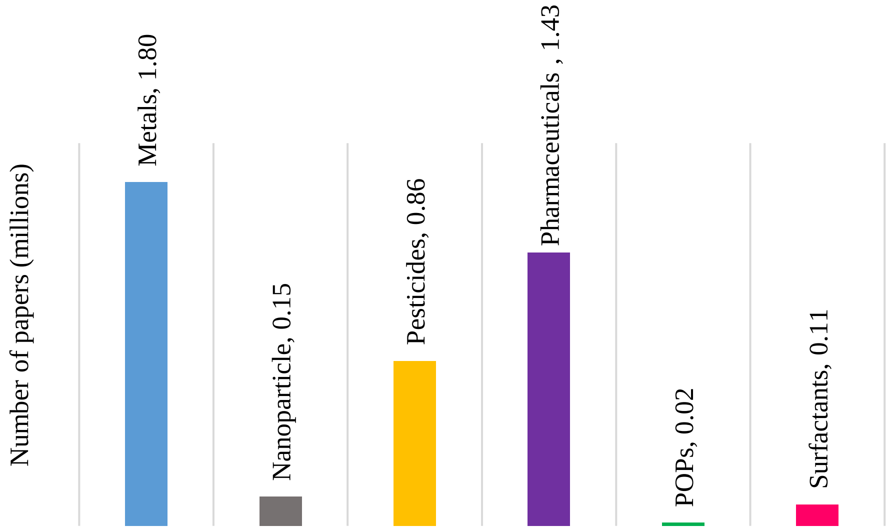


Figure 3-2 Number of papers identified following a Web of Knowledge search (10th July 2015) using chemical class names as search terms

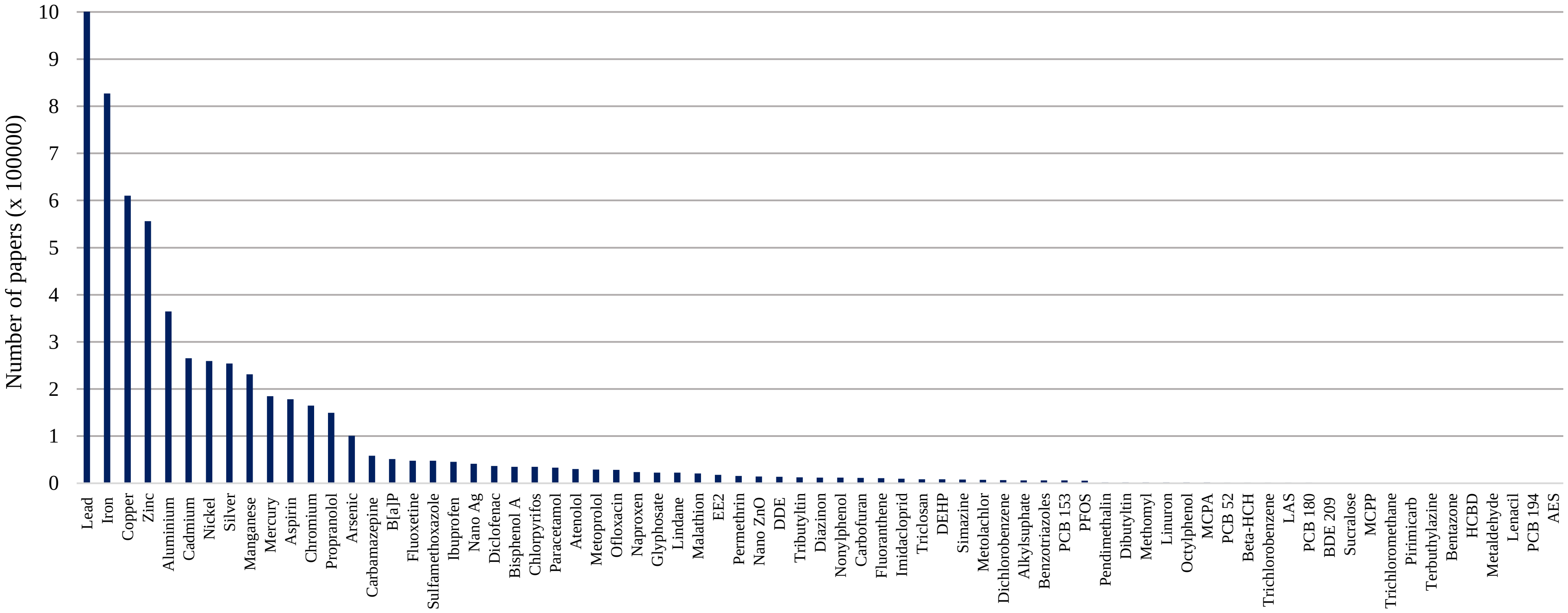


Figure 3-3 Number of papers identified following a Web of Knowledge search (10th July 2015), using the 73 chemicals individual names

3.3.1. Environmental toxicity information gathering

The intention here was two-fold, firstly to fairly reflect the range of impacts, species and end-point that could be attributed to a chemical, and secondly to be precautionary and ensure that sensitive species/effects were captured. The selection of the ecotoxicity data reported in the literature which were included in this study tried to be all encompassing. The preferences were as follows:

- European species were preferred
- Representations from fish, invertebrates, plants and algae were included
- End-points preferred were LC50, EC50, LCx, ECx and LOEC
- Different end-points were included, including any which may be considered disruptive in some way
- Aim to collect 50 – 100 separate ecotoxicity entries per chemical

With chemicals where it was possible (i.e. metals), the use of only measured concentrations, rather than nominal values, was preferred as a means to filter the abundance of data.

For each chemical the references were reviewed. The references per chemical were found using the key search terms, as well as reviews, cross-referencing. If possible, all data available in the literature was included in the database (i.e. for pharmaceuticals), or data were added until the median value didn’t change markedly with the addition of new data (i.e. metals).

Data from reviews and databases were also used, although the primary literature was traced back as far as possible. The data inclusion exercise tried to be unbiased. It has been highlighted by others that the presence of bias in risk assessment can occur with the inclusion of certain data points and studies. Oehlmann et al (2008) highlight this concern based on their discussions of the risk from bisphenol A and phthalates, where the most sensitive end-point was not considered for derivation of a PNEC value for Bisphenol A [85].

3.3.2. Environmental concentration information gathering

To understand the concentrations the aquatic organisms are exposed to in the environment, data on concentrations found in the environment were collated. The focus was on the UK data, but if data were not available for the UK, then concentrations from Europe were considered. The aim was to collate data which represented the widespread UK aquatic environment, rather than concentrations from unusual hot spots or concentrations due to accidental spills.

3.3.2.1. *Measured water concentrations*

For each chemical, if possible only measurements of concentrations made in the UK were used. Measured river water concentrations obtained from Environment Agency WIMS databases were used [86], as well as data reported in the open literature.

For metals, there is an abundance of UK monitoring data which were used as a source of data. This included using data from the Forum of European Geological Surveys (FOREGS, now EuroGeoSurveys) [87, 88].

For some chemicals there was limited or no UK measured environmental data. In those situations data from European rivers were included, obtained either from the scientific literature or from the Waterbase Database [89].

For many chemicals there are limited environmental monitoring data, and thus for a lot of ranking/prioritisation methods that have been reported measured environmental data are not used.

3.3.2.2. *Predicted water concentrations*

For some chemicals there is currently either none or limited measured river concentrations; this is especially true for pharmaceuticals. However, for pharmaceuticals, measured or predicted sewage effluent concentrations are much more readily available. Predicted river concentrations have been calculated based on either reported UK effluent concentrations or consumption (UK)/excretion/sewage removal. Using the work done by CEH, information about the amount of dilution available in the different England/Wales regions is known [62]. A range of dilution values for the R. Thames at Reading and R. Soar at Leicester is also available [90]. By using 90th percentile low flow dilutions for the Thames and Soar, it is possible to predict the exposure concentrations for fish that might be expected to be at the higher end for UK rivers. Predicted concentrations have been used for the pharmaceuticals class in addition to measured river concentrations (see Appendix Table 10-4 for detailed calculations of pharmaceutical predicted concentrations). For nanoparticles only predicted concentrations were available for nano Ag and nano ZnO [20]. For metals and pesticides there was sufficient measured data, thus only measured values or ½ LOD values were used for these chemicals. Within this thesis the combination of measured and predicted data for chemicals is referred to as river water concentration/environmental concentrations.

3.3.1. How to choose papers?

The aim of the data collection was to get an understanding from the publicly available literature of what effects have been reported for a range of species due to their exposure to a specific chemical. Metals were the first class of chemicals to be investigated, and it soon became apparent from conducting searches on the Web of Knowledge that thousands of papers existed for metals (Figure 3-2, Figure 3-3). Looking at the very high number of papers available for the metals it was clear that not every paper on the 12 metals included in this study could be read. The challenge was to understand if the search method developed for the project was sufficient or whether it was biased or not? The search method used a selection of key words to filter through the literature. Papers which reported effect data or environmental concentrations were recorded and the information/data included in the summary toxicity report. It was found that the best method of expanding the search was to look at the reference list of ‘good’ (relevant) papers and pull further information from them. This approach included using review papers to make use of the previous effort of others to pull toxicity data together.

3.3.1.1. *Exploring other approaches to derive an ecotoxicity median value for a chemical*

To test the method employed and compare it with other potentially ‘faster methods’, copper and the search terms ‘copper + toxicity + water + laboratory’ were used. This search produced 1,414 papers. From this 1,414 the search was refined to: a) most recent, b) most cited - based on citations reported in Web of Knowledge; papers and data found were recorded. The aim was to use the papers generated from these searches to gather toxicity data which would be comparable to the data already collected. The first 20 papers for each refined search were looked at (Table 10-1). In each of the searches there were interesting papers which were relevant to the copper toxicity report, as well as to the overall project. However, few papers provided the level of detail needed for the effect concentration analysis and the effect data.

Based on the 20 papers found through using the “most recent” papers as a filter only one paper provided the details which were needed for the report this was entitled; *Toxicity of Copper to Early-life Stage Kootenai River White Sturgeon, Columbia River White Sturgeon, and Rainbow Trout* [91]. The paper provides the LC50 for White sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*). Tests were conducted on fish which were 30 days posthatch (dph) and 123dph. The results show that early stage white sturgeons are highly sensitive to copper, with the LC50 being between 4.1-6.8 µg/l, whereas 4-5 month old white sturgeons are less sensitive to copper with a higher LC50 of 103.7-267.9 µg/l. The rainbow trout produced very different results, with the early stage (30dph) LC50 being 36.5 µg/l copper and the 123dph LC50 being 30.9 µg/l copper. There is not a large difference between these two concentrations. These data show that at the later stage the trout is more sensitive to copper than it was in its early life stage. The paper emphasises the importance of understanding the impact of chemicals to a variety of species, as the threshold levels for one fish species maybe be above or below that of another (i.e. using the rainbow trout as a water quality marker would not adequately protect the early life stage white sturgeon). Using the older (123dph) white sturgeon as a water quality marker would not protect the rainbow trout.

It was decided that using the key search terms to minimise the number of papers selected was a reasonable method to cut down the number of papers, but human effort in terms of filtering through the detail and gathering data was required to gather data.

Following this test, it was deemed that the approach developed for data collection was suitable. Where there is an abundance of data, as with the metals, data should be gathered until there is little change to the median value. Data should also be added to ensure sufficient data on a range of species. Where there is limited data, as much data as available should be collated to the best ability.

When considering the data, the most reliable sources of data were found to be the original citations of valuable experimental data in the reviewed scientific literature. Particularly reliable were those papers which contained a critical review of data from a number of sources as well as independent determinations, calculated or correlated values are viewed as being less reliable. The aim of this work was to gather sufficient experimental data, list the sources of those data, then analyse these data to produce likely (typical) median effect and exposure concentrations. Thus the quality of the data has not been considered; this was deliberate in order not to bring our own bias into the analysis. As what one person's judgment of a good, high quality is, is different to someone else's opinion on quality. By not considering the quality of the data (i.e. like its robustness), the aim was to avoid the problem of one person's judgement of good, high quality being different to someone else's opinion.

3.3.2. Limitations

It is important to understand the limitations of the approach prior to analysis of the results.

3.3.2.1. *Abundance or lack of data*

There are pros and cons to both an abundance of data and limited data. The problem with abundance of data is knowing whether a sufficient amount has been used to capture a snapshot of the science. The problems with limited amounts of data include the question of robustness, and whether or not a wide range of species and end-points has been included. For some chemicals, there was an abundance of literature (i.e. bisphenol A there are >30,000 papers), but for other there was minimal data (i.e. metaldehyde there are <400) (Figure 3-3).

3.3.2.2. *Type of data*

For each chemical there was a range of ecotoxicity data, although there can also be a dominance in the literature for a specific end-point or species, depending on the nature of the chemical and the target at which the research has been directed. For example, there are a lot of studies for aluminium which focus on the extremely acidic environment, as this is where this chemical is more toxic, whereas for others there is a heavier trend towards sub-lethal effects rather than lethal effects, or a specific species due to it being the most sensitive.

It has been highlighted by many scientists that it is desirable to have an abundance of both acute and chronic ecotoxicity data for a range of species, in order to get a robust understanding of the impacts of a chemical. Unfortunately, it has also been documented by many that there is a severe lack of chronic toxicity data for many chemicals. Therefore, it is not uncommon for only acute data to be available and used for a chemical in a risk assessment. One limitation with the effect data is that it is hard to publish uninteresting effects, or no effects of a chemical, which produces an automatic bias in our data source.

3.4. THE TIERED APPROACH

The aim of this project was to use the ecotoxicity data and environmental data collated to risk-rank chemicals in order to compare the relative risk of one chemical to another, regardless of class. A two-tiered approach which has been developed.

- Tier One – Uses the data in its raw state to produce a provisional risk ranking
- Tier Two – Looks at the role of moderating factors (such as bioavailability) to understand if the risk ranking changes once moderating factors are incorporated.

3.5. TIER ONE RISK ANALYSIS (CHAPTERS 4 & 5)

Using the ecotoxicity and environmental data, each chemical was initially analysed on an individual basis. The chemicals were considered individually and within their class (Chapter 4), then all 73 chemicals were considered and risk ranked (Chapter5).

3.5.1. Individual chemical analysis

As part of the initial gathering of information on each chemical, the individual effect concentrations and river water concentrations for each chemical were plotted. The method used created two parallel sets of data, the effect data (inclusive of any endpoint or species) and the river water data. It is the proximity of these two data sets which indicates the degree of concern posed by a chemical, Figure 3-4 is an example of how the data were plotted at this stage of the analysis. Each data point for the chemical was plotted, (Figure 3-4): each diamond is an effect concentration at which an effect on a particular organism was reported. At this stage the end-point or specific species was not considered. This method of presenting the data enables the range of the effect data available for each chemical to be visualised. For the environmental concentrations, each square is either a reported concentration or one half of the LOD for records which were reported as <LOD. This gives an indication of the spread of the environmental data and the possible concentrations that organisms are exposed to in the UK aquatic environment. The method of displaying

the information reveals the range of data and shows any degree of overlap between the two data sets for each chemical.

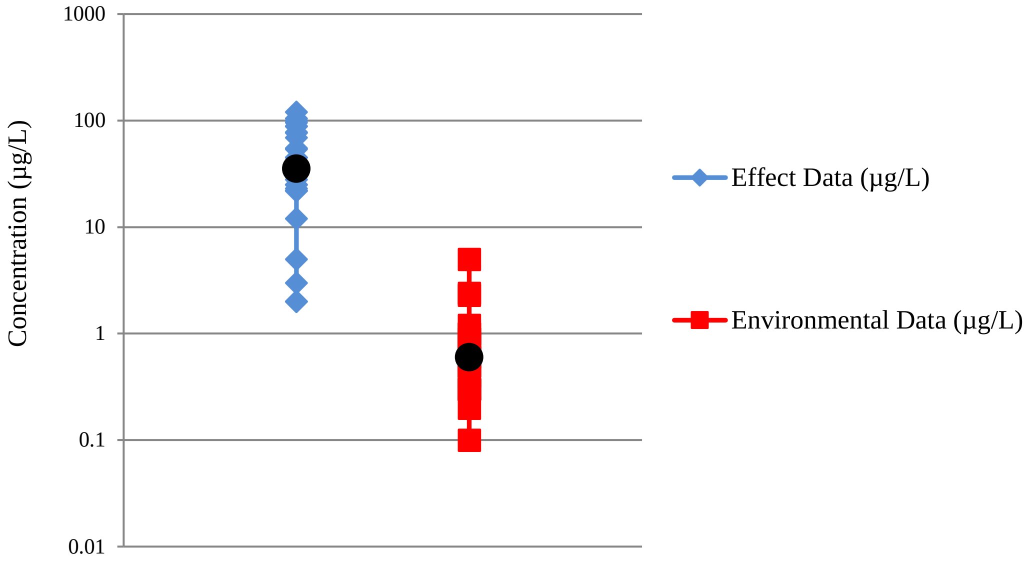


Figure 3-4 Example of Cross-over Graph, in which both the effect data and the concentration data are displayed.

*this is an example data set for a chemical only

The data can also be considered using a cumulative frequency graph to determine if there is any overlap between the effect data and the concentration data, as well as determine what percentage of the data is over-lapping, and thus suggesting a concern or not (Figure 3-5). These two graphs make up the initial analysis of the chemical on an individual basis.

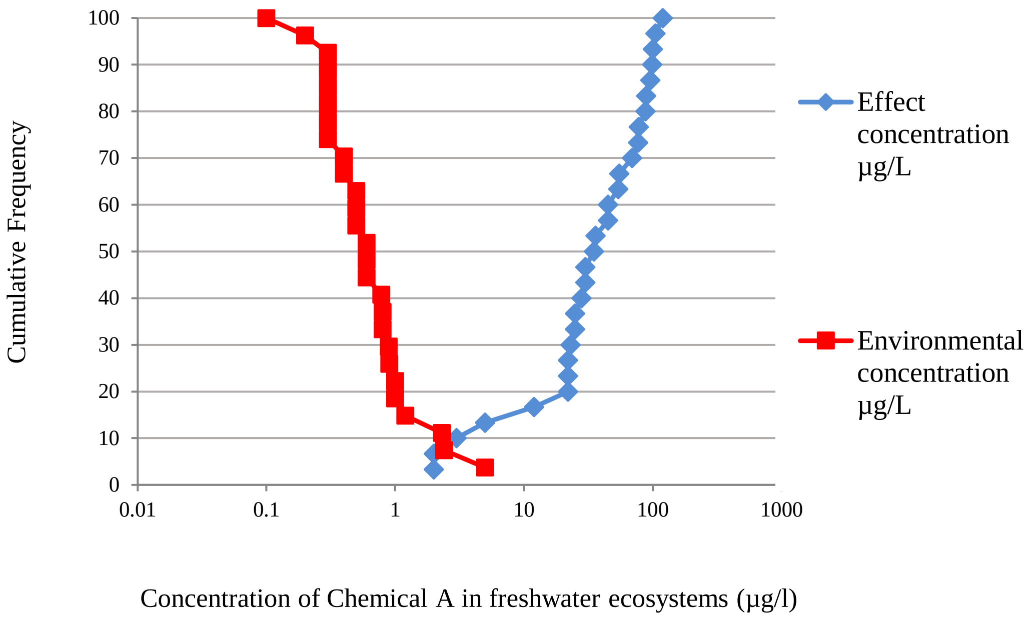


Figure 3-5 Example of Cumulative Frequency graph, in which both the effect data and the concentration data are displayed.

3.5.2. Risk-ranking

3.5.2.1. *Ranking of chemicals based on exposure via the water column*

The data collated for the chemicals individually underpins the risk-ranking for the rest of the project. Following the individual analysis, the chemicals were ranked in relation to other chemicals in the same class (Chapter 4) and secondly ranked altogether regardless of class (Chapter 5).

The possibility exists that some values from ecotoxicity and river water concentration studies or datasets are wrong and cannot be repeated. Thus, the degree of separation between the median effect concentration and the median river water concentration was considered the most stable method for ranking chemicals on the basis of risk (equation 1). It is important to note that the project is trying to find a robust method to compare chemical rather than create a new form of chemical risk assessment. The median of all the effect data used in the study (including all species and all end-points) and the median of all the river data was calculated as an initial comparison.

$$\text{Risk} = \frac{m\boldsymbol{W}}{m\boldsymbol{T}} \qquad \text{(Equation 1)}$$

Where mW is the median river water concentration (µg/L) and mT is the median effect concentration (µg/L).

This value can be described as a risk ratio, which can be used to rank concern; the larger the value, the greater the concern. The chemicals were ranked according to this risk ratio.

3.5.2.2. *Ranking of chemicals based on exposure via the water column: an alternative precautionary approach*

A precautionary approach was to use the 5th percentile ecotoxicity concentration and compare this with the median river water concentration (equation 2). This second ranking approach uses the same risk ranking principle, but in this case it compares the distance between the 5th percentile (low) effect concentration and the median river water concentration to assess whether a chemical is of potential concern or not.

$$\text{Risk} = \frac{m\boldsymbol{W}}{5\%ile\boldsymbol{T}} \qquad \text{(Equation 2)}$$

Where mW is the median river water concentration (µg/L) and 5%ileT is the 5th percentile effect concentration (µg/L).

This approach gives more weight to the potentially vulnerable species and end-points, as well as the more questionable results (very low effect concentrations or very high water concentrations).

3.5.2.3. *Ranking of chemicals based on exposure via the water column: a species perspective*

To take the analysis of the data further, and to understand if any chemical or class of chemicals were of particular concern to certain species group, the effect data were split into three categories, namely fish, invertebrates and algae & aquatic plants. Although data from other species, such as bacteria, was collated and included in the main comparison, fish, invertebrates and algae & aquatic plants were the specific species groups considered here. A risk ratio was calculated based on the median river water concentration and either the median effect concentration or the 5th percentile effect concentration for each species category.

3.5.3. Conclusion

The aim of the tier one analysis and risk-ranking was to use the data collated in an unbiased manner, without further refinement, to rank chemicals by risk.

3.6. TIER TWO RISK ANALYSIS (CHAPTER 6)

The tier two analysis asked the question, "does employing various factors of realism to the chemistry or toxicity data change the outcome of the risk-ranking from the all-inclusive tier one analysis?" Thus, investigating if a more complex approach changes the result in a significant way. Not every possible moderating factor or refinement could be investigated. Is there one moderating factor which drastically changes the chemicals identified as of concern? The tier two risk analysis used the same data as used in tier one, but has made refinements to the data, which could be classed as bringing in bias and subjectivity to the overall risk ranking. The question being asked here is: would any moderating factors drastically change our tier one based ranking? What chemical class is of main concern after further refinement of the risk ranking process? If we complete a more sophisticated analysis, would you get a different result?

3.6.1. Is there an overlap in the toxicity and environmental data?

A first consideration which could be made with regards the data is whether or not there is an actually overlap in the two datasets. A first filter of the data would be to discard any chemical where there is no overlap in the two datasets, judging it to be of no concern (Figure 3-6). This approach eliminates chemicals based solely on whether or not there is an overlap in the two datasets. As described previously and as demonstrated in Figure 3-4 and Figure 3-5, ecotoxicity and environmental data are collated independently for each chemical.

This is not a moderation of actual data but a simple yes or no question, based on the data collated.

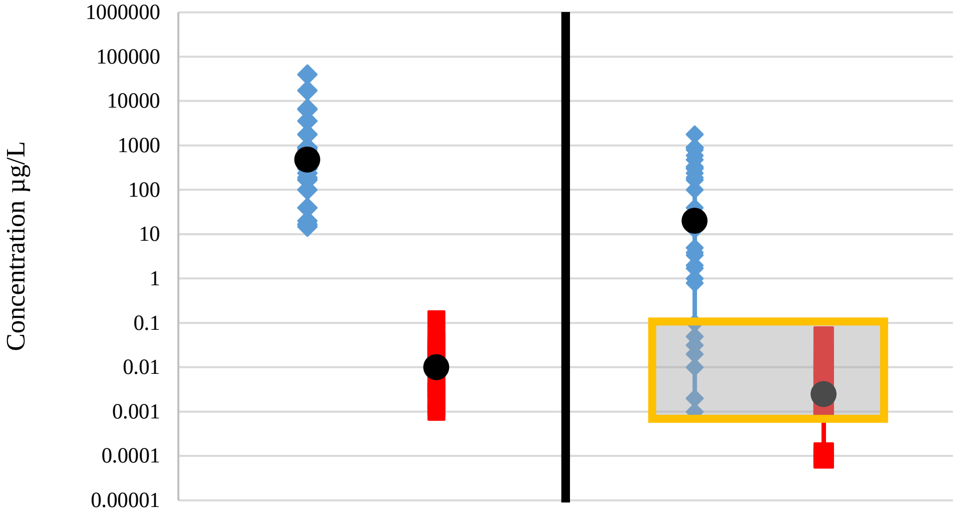


Figure 3-6 Overlap in data? On the left-hand side, there is no overlap between the effect data and the environmental data, whereas on the right-hand side the two datasets overlap (the overlap is within the box).

3.6.2. Refinement of the data

The tier two analysis used the ecotoxicity data and environmental datasets and applied various moderating factors to generate a risk-ranking based on a refined analysis. The refinement/moderations made to the data are not all the possible refinements but a small selection of them (Table 3-3, Figure 3-8, Figure 3-9). These refinements were used to filter the two datasets for each chemical to generate a new risk-ranking. The details of the refinement methods are discussed in the following section.

Table 3-3 The various approaches used to refine the data

Refinement	Detail
Ecotoxicity Data	Lethal only & Sub-lethal only
Environment Data	For all chemicals consider: -UK only environmental data -Measured UK data from 2000 – present -Predicted modelled UK data was included for the pharmaceuticals and nanoparticles as these were considered to be ‘current’
Ecotoxicity & Environment Data	For metals only use dissolved metal concentrations rather than total concentrations For metals include only ecotoxicity data from studies carried out at neutral pH (6.5-8.5)

3.6.2.1. *Refinement of effect data – use of lethal/sub-lethal data only*

There is an argument that only acutely toxic chemicals need concern us. That in reality, wildlife can cope or adapt to sub-lethal effects. Mortality is an end-point where there is definite evidence of harm occurring to a percentage of the individuals being studied. The most common means to report this is as the lethal concentration to affect 50% of the population (LC50). Therefore, one way to refine the ecotoxicity data is to only include data which have reported mortality.

As ecotoxicity has become more sophisticated and scientific techniques have developed, the ability to measure sub-lethal effects has become possible. Sub-lethal include biochemical, physiological, reproductive and behavioural effects on organisms. Sub-lethal effects can be reported as LOEC, EC50, etc. These sub-lethal effects occur at lower concentrations than are required to kill the organisms, thus it is a more precautionary approach. Therefore, another way to risk ranking is to only consider sub-lethal effects in the analysis.

Thus, when calculating a risk ratio, all-inclusive ecotoxicity data can be replaced with either the median effect concentration based only on lethal data or the median effect concentration based only on sub-lethal data.

3.6.2.2. *Refinement of environment data*

Environmental data were collated for the UK and Europe. As a tier two moderation, only data from the UK were used. The data were also modified by date of collection, with only data from 2000 to the present being included in the analysis. The aim of these two refinements was to make the environmental data more relevant, to represent, hopefully, the chemicals which are currently present, or have recently been present in UK freshwaters.

Data from the last decade were included as the inclusion of legacy data can be misleading, (i.e. higher concentrations were present which are now not common due to implemented legislation, such as banning of a chemical). However, the importance of the legacy data is to show how high concentrations can occur in the environment and how the conditions of UK freshwater are perhaps the best they have been in recent times [90].

Monitoring data are not available for every chemical. Unless the chemical has been of concern it is unlikely that there will be monitoring data available for a specific chemical. Thus predicted concentrations or concentrations from out with the UK were used as an alternative. To tailor the analysis to just the UK, only UK-based measurements were considered at the tier two analysis.

Thus when calculating a risk ratio, the all-inclusive environmental data can be replaced with the median environmental concentration based on recent UK data.

3.6.2.3. *Bioavailable concentration of metals*

The definition of the bioavailability of a chemical is '*the extent to which a toxic contaminant is available for biologically mediated transformation and/or biological actions in an aquatic environment'* [92]. The bioavailability of a chemical will determine its ability to be toxic to an aquatic organism, as it is the amount of the chemical which is free for uptake by the organism.

The bioavailable fraction of the metals were considered by looking at the total and dissolved fractions of the metal. Using the Environment Agency WIMS data it is possible to look at total and dissolved concentrations of the metals. Thus as a tier two filter, only the dissolved measurements were used, rather than the total metal concentrations. The dissolved measurement is a more accurate measure of the bioavailable concentration of the metal available to organisms.

The ecotoxicity data were also filtered, by including only ecotoxicity studies carried out at neutral pH. Although there will be environmental conditions where there is a naturally higher pH level, these are not the conditions which freshwater organisms in the UK are typically exposed to. Based on the Environment Agency WIMS 2016 pH records for river / running surface water, 96% of pH records reported are between 6.5-8.5. Suggesting that the majority of freshwater in the UK is within this pH range (Figure 3-7) This is in agreement with the pH map of Europe produced by the FOREGS project (Figure 10-3).

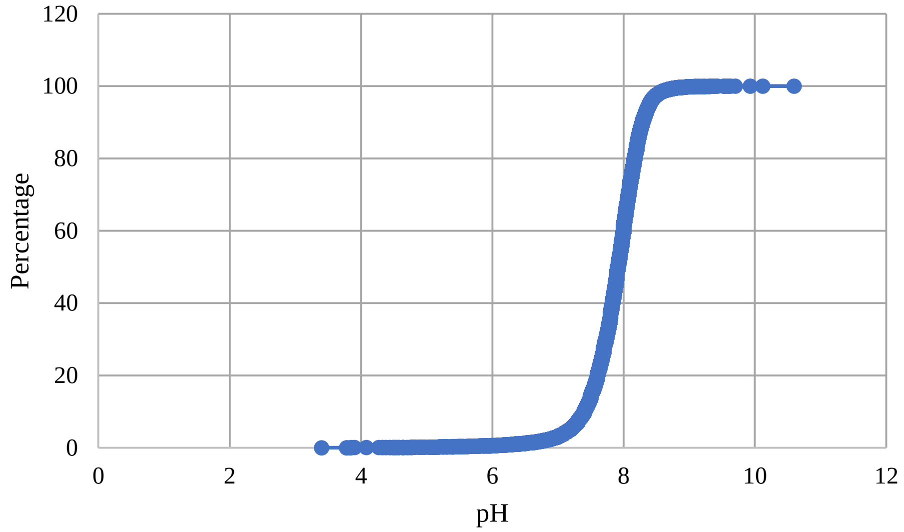


Figure 3-7 pH frequency based on reported pH data via the WIMS database for 2016. Demonstrating the majority of records reported at a neutral pH.

Often in ecotoxicity tests natural water chemistry is not considered, standard test solutions are used. Standard tests allow for reliable and repeatable test conditions. However, the impact of variations in natural water characteristics on the bioavailability of a chemical and the potential toxicity of a chemical aren’t considered. The effect of pH on metals was considered in this study, however the effect of water characteristic could have been taken further by applying this consideration to all chemicals. Variations in pH can impact the bioavailability and therefore toxicity of i.e. pharmaceuticals. For example, pH was shown to the effect the toxicity of triclosan to *Gammerus pluex*. The lower the pH the greater the toxic potential. Therefore, the risk of triclosan to aquatic organism based on standard ecotoxicity tests could overestimate the risk/toxicity of triclosan when pH isn’t considered [93].

3.6.3. Conclusion

Following this first stage of moderation the following was considered (Figure 3-8):

- Overlap in the effect and environmental data.
- Risk-ranking based on all effect data for organics, pH neutral effect data for metals and recent, dissolved (metals), UK environmental data.
- Risk-ranking based on lethal effect data, pH neutral effect data for metals and recent, dissolved (metals), UK environmental data.
- Risk-ranking based on sub-lethal effect data, pH neutral effect data for metals and recent, dissolved (metals), UK environmental data.

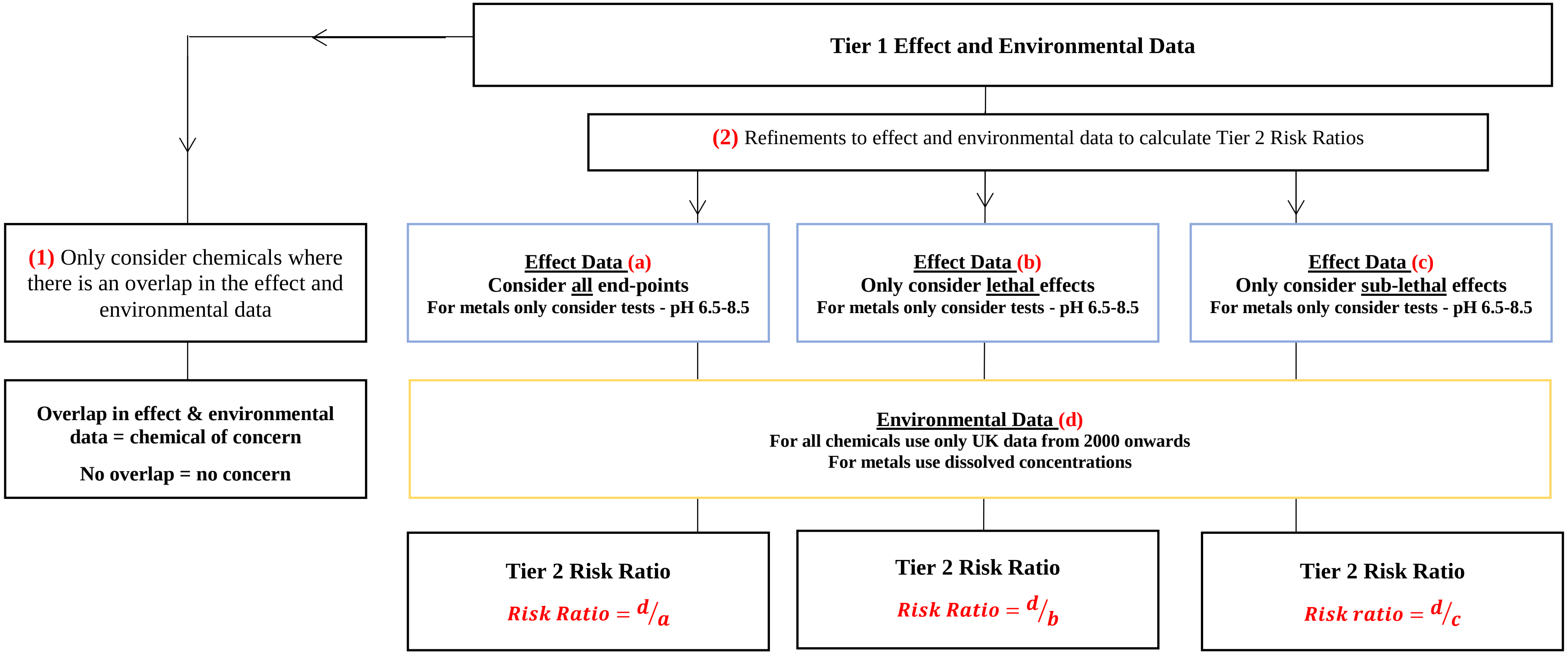


Figure 3-8 Schematic of part one of tier two: (1) is there an overlap in the effect and environment data for each chemical, (2) refinements made to the tier one data to calculate alternative risk ratios based on moderated effect data and environmental data.

3.6.4. Ranking of chemicals based on bioconcentration (BCF)

Up to this point the data used to risk rank chemicals have been based on water column concentrations only, to introduce and give weight to chemicals which bioaccumulate. The chemicals were also ranked based on their BCF factor. BCF values for any species, but predominately fish (both measured and predicted) were collated from the literature for each chemical. If no data were found the database the Hazardous Substances Data Bank (HSDB) and the ‘Handbook of physical-chemical properties and environmental fate for organic chemicals’ or the previous version ‘Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals’ were used as a source of information [14, 94, 95]. Based on the values a median BCF value for each chemical was established (Table 10-9). The BCF is a unitless value calculated by dividing "steady-state" wet tissue concentration by "steady-state" water concentration of a particular substance [96]. By using only the BCF as a ranking tool, without any reference to toxic concentrations, a different ranking order with regard to the threat posed by chemicals to aquatic wildlife can be produced. The BCF is an established ratio, thus values were collected from the literature and the median values used as a comparison between chemicals. The greater the BCF, the greater the concern based on this ranking methodology. Thus, firstly, chemicals have been ranked based on their BCF alone. The top 25 highest ranked chemicals based on their BCF were then ranked again based on their refined data as a means of taking the chemicals of greatest concern based on bioconcentration and greatest concern based on toxicity (Figure 3-9). Thus, this final ranking incorporates both the toxicity and potential bioaccumulation of a chemical.

Persistence has not been considered as a separate moderating factor due to the occurrence of pseudo-persistent chemicals which are chemicals which, although they are not persistent by nature (their properties), they are persistent due to their continual release, their emission into the environment exceeding their half-life.

3.6.5. Conclusion

Following this second stage of moderation the following were considered:

- Risk-ranking based on BCF values.
- Risk-ranking of chemicals with the highest BCF values based on all effect data, pH neutral effect data for metals and recent, dissolved (metals), UK environmental data.
- Risk-ranking of chemicals with the highest BCF values based on lethal effect data, pH neutral effect data for metals and recent, dissolved (metals), UK environmental data.
- Risk-ranking of chemicals with the highest BCF values based on sub-lethal effect, data pH neutral effect data for metals and recent, dissolved (metals), UK environmental

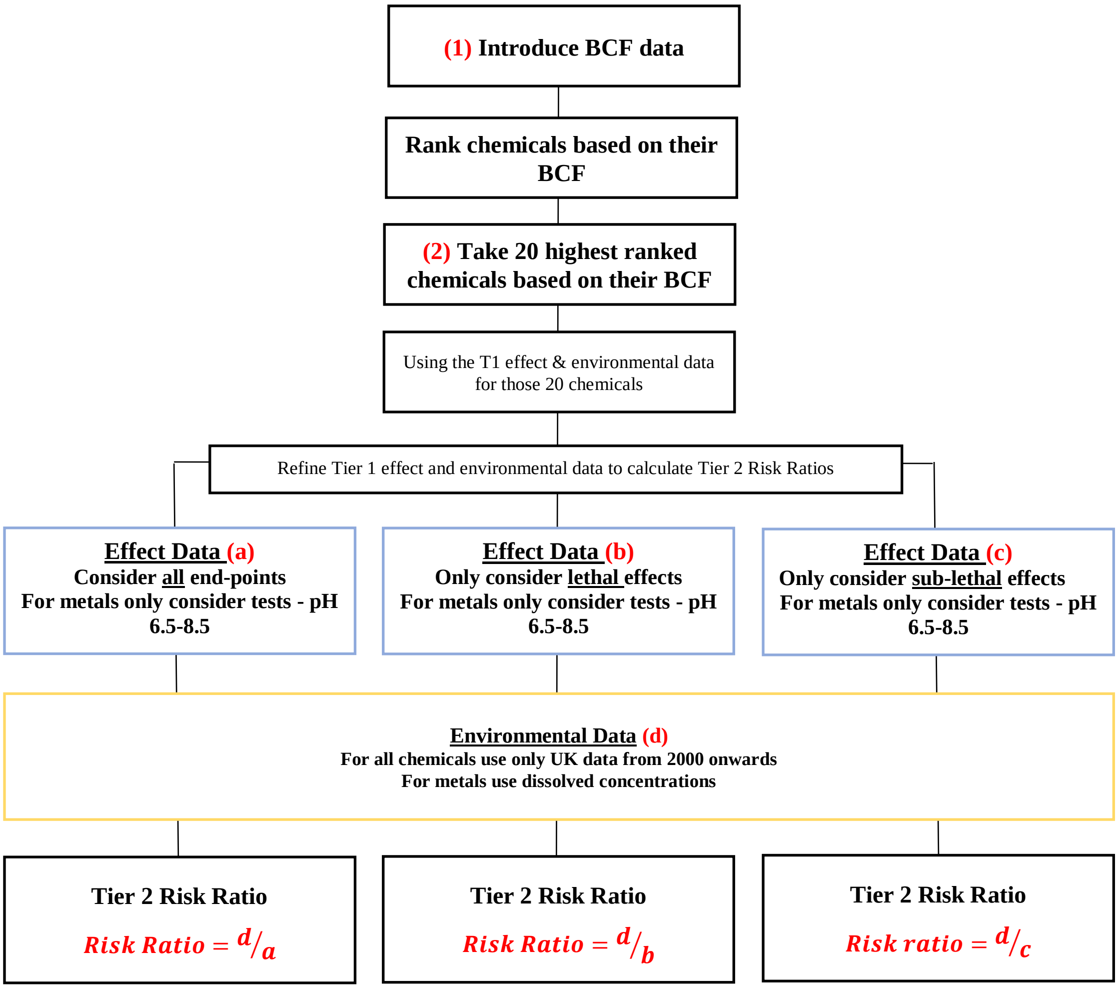


Figure 3-9 - Schematic of part two of tier two: (1) the introduction of BCF data and a ranking based on BCF, (2) a risk-rankings based on BCF and toxicity (the risk-rankings are based on different refinements made to the tier one data)

3.7. QUESTIONING THE APPROACH TO RISK RANKING (CHAPTER 7)

Throughout the project it has been important to critique and question the approach being developed. Not only as a means of understanding the feedback and response from others with regards the approach, but as a key training aspect to the PhD and to understand the potential breadth or development of the approach after the project. Chapter 7 includes three main sections which discuss the different ways the approach has been compared to others and tested. A key aim was to understand if there is a different, better means of ranking the chemicals. The risk-ranking is a) compared with experimental tests obtained as part of this study, b) it is put in context by comparing the ranking to sewage effluent concentrations, and c) summarise feedback following a workshop. The work in this chapter was developed via consultation with experts from the scientific, government and industry community.

3.8. CONCLUSION

The overall objectives and principles of this project are:

- Risk-ranking chemicals was performed on the basis of widespread risk to UK rivers across the country. Thus, this approach did not considered problems with isolated pollution hot spots.
- Aimed to reflect risks to the widest range of relevant species and end-points (not confined to standard test species and end-points).
- Relied as far as possible on measured river concentrations.
- Ranking on the basis of comparisons of the median concentrations and toxicity values was selected as a robust risk ranking tool, it was considered less likely to be deflected by unusual ecotoxicity or measured findings.
- The method is biased towards chemicals that exert their effects through the water column. Some considerations of the bioaccumulation/effect route was included by considering BCF values.

4. TIER ONE APPROACH TO RISK-RANKING CHEMICALS – INDIVIDUAL CHEMICAL CLASSES

4.1. INTRODUCTION

The aim of this chapter is to detail the results of the tier one approach, considering the chemicals on a class-by-class basis. This chapter looks at the metals, nanoparticles, persistent organic pollutants, pesticides and surfactants/others as individual classes of chemicals. It is important to understand which chemical within each class is of greatest concern in relation to the other chemicals in the class. For each class this chapter presents the data underpinning the approach, an initial risk-ranking, a precautionary risk-ranking and risk-rankings at species level.

The aim of this approach was to take an unbiased approach to data collection and risk-ranking. The results described here are based on the tier one methodology as described in Chapter 3. No further refinement has been applied to the data presented in this chapter.

4.2. BRIEF METHODS

Within each chemical class there are a number of chemicals (Table 4-1). These chemicals have been risk-ranked based on the methods detailed in section 1.5 of Chapter 3. The risk-ranking methods are briefly detailed here, but refer back to Chapter 3 for full details.

Firstly, the data underpinning the risk-ranking of each class are presented in a graph to highlight the raw data used for the analysis, to visualise the two datasets (effects and environmental) for each chemical as well as the data for a specific chemical in comparison to other chemicals within the same class.

The risk-ranking approaches used were:

- Median environmental vs median toxicity concentration.

This approach aims to rank chemicals on the basis of typical environmental and ecotoxicity data. The median has been used to remove bias from outliers.

- Median environmental vs 5th percentile toxicity concentration.

This approach places more emphasis on the lower ecotoxicity concentrations, which represent the more sensitive species and end-points, as well as the potentially less reliable data points.

- Species specific risk-ranking of chemicals

This approach focuses only on algae & aquatic plants, or invertebrates or fish (the species are split). This approach tries to determine which species group is the most sensitive to which chemical class.

The chemicals were first ranked within their specific classes and the ranking of chemicals of the same class is discussed. The second part of tier one analysis is the comparison of all chemicals, regardless of class, the results of which will be discussed in Chapter 5.

4.3. RESULTS AND DISCUSSION

The chemicals were ranked and the results discussed based on the risk they present to aquatic wildlife in the UK. Table 10-6 and Table 10-7 summarise the ranking order and risk ratios obtained from all chemical classes. The concentrations presented here are based solely on water column data and chemicals which are water soluble.

For each chemical class, the tier one data and results are discussed based on ranking via the median values, the 5th percentile and the three defined species categories. Further discussion of the chemicals that rank highly based on the different ranking methods for each class are then detailed.

Table 4-1 List of chemicals (divided into chemical classes) investigated in this study

Metals	Pharmaceuticals	Persistent Organic Pollutant’s
Aluminium	Aspirin	Benzo [a] pyrene (B[a]P)
Arsenic	Atenolol	Decabromodiphenyl ether (BDE 209)
Cadmium	Carbamazepine	Dichlorobenzene (DCB)
Chromium	Diclofenac	Dichlorodiphenyldichloroethylene (DDE)
Copper	Ethinyl estradiol (EE2)	Dibutlytin
Iron	Fluoxetine	Fluoranthene
Lead	Ibuprofen	Hexachlorobutadiene (HCBD)
Manganese	Metoprolol	Lindane
Mercury	Naproxen	Polychlorinated biphenyl 153 (PCB 153)
Nickel	Ofloaxcin	Polychlorinated biphenyl 180 (PCB 180)
Silver	Paracetamol	Polychlorinated biphenyl 194 (PCB 194)
Zinc	Propranolol	Polychlorinated biphenyl 52 (PCB 52)
	Sulfamethoxazole	Perfluorooctanesulfonate (PFOS)
		Trichlorobenzene (TCB)
		Trichloromethane (TCM)

Pesticides (1)	Pesticides (2)	Surfactants & others
Bentazone	Pendimethalin	Alcohol ethoxysulphate (AES)
Beta –hexachlorocyclohexane (Beta- HCH)	Permethrin	Alkyl sulfonate (AS)
Carbofuran	Pirimicarb	Benzotriazole
Chlorpyrifos	Simazine	Bisphenol A
Diazinon	Terbuthylazine	Bis(2-ethylhexyl) phthalate (DEHP)
Glyphosate	Tributyltin (TBT)	Linear alkylbenzene sulfonate (LAS)
Imidacloprid		Nonylphenol
Lenacil		Octylphenol
Linuron		Sucralose
Malathion		Triclosan
MCPA		
Mecoprop (MCPP)	**Nanoparticles**	
Metaldehyde		
Metolachlor	Nano Silver	
Methomyl	Nano Zinc oxide	

4.3.1. Metals and Nanoparticles

Metals were the starting point to develop and test the initial methodology. They were chosen because they are well studied and understood in comparison to some other chemicals (e.g. there are 1 million published papers on Lead in the literature). As only two nano-metals were considered they are discussed alongside the metals in this chapter.

There is a great deal of information available in the literature on metal toxicity [97]; studies reporting impacts of metals on the environment have been published since the 1960's. Although there is less information available on nano-metals, these chemicals are of recent concern and have attracted a lot of funding and attention in recent years [98].

When the data for all the metals were analysed, it can be seen that some degree of overlap between environmental concentrations (measured environmental values for metals and predicted environmental values for nano metals) and concentrations that cause effects on aquatic organisms occurs for all metals, except for silver. In comparison, there is no overlap between the two datasets for either nano Ag or nano ZnO (Figure 4-1).

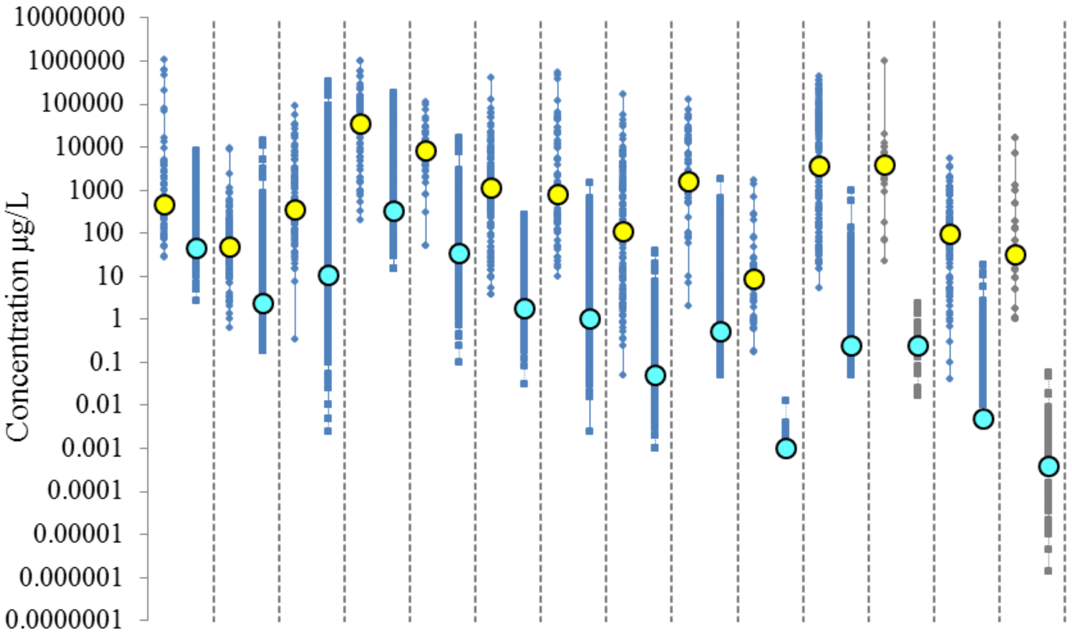


Figure 4-1 Underpinning data of metals (light blue), nanoZnO and nanoAg (nano – dark blue), ranked based on the difference between the median effect (left hand vertical line of each pair: diamonds) and river water concentrations (squares). The median values are plotted as yellow (effect) and blue (environmental) circles. (From left to right – Al, Cu, Zn, Fe, Mn, Ni, Pb, Cd, As, Ag, Cr, nano ZnO, Hg, nano Ag).

4.3.1.1. *Comparison of median environmental concentrations and effect concentrations*

When ranking the metals by comparing the differences in the median river water and median effect concentrations, Al, Cu, and Zn emerge as posing the greatest risk (Figure 4-2). The difference between the median river water and effect values was relativity small (10-fold) for the metals of most concern, such as Al, Cu, Zn, but was larger (10,000-fold) for metals of less concern, such as Cr, Ag and Hg. The greater the risk ratio the greater the concern. The risk ratios for metals and nano-metals range from $1.21x10^{-5}$ to 0.24, with the highest risk ratios of 0.24 (Al) and 0.05 (Cu) and lowest risk ratios of $5.43x10^{-5}$ (Hg) and $1.21x10^{-5}$ (Nano Ag). Thus, there is a 10,000-fold difference between the highest and lowest ranked chemicals within the class. Note this ranking does not yet take into account the impact of natural pH on Al toxicity or metal bioavailability in natural waters.

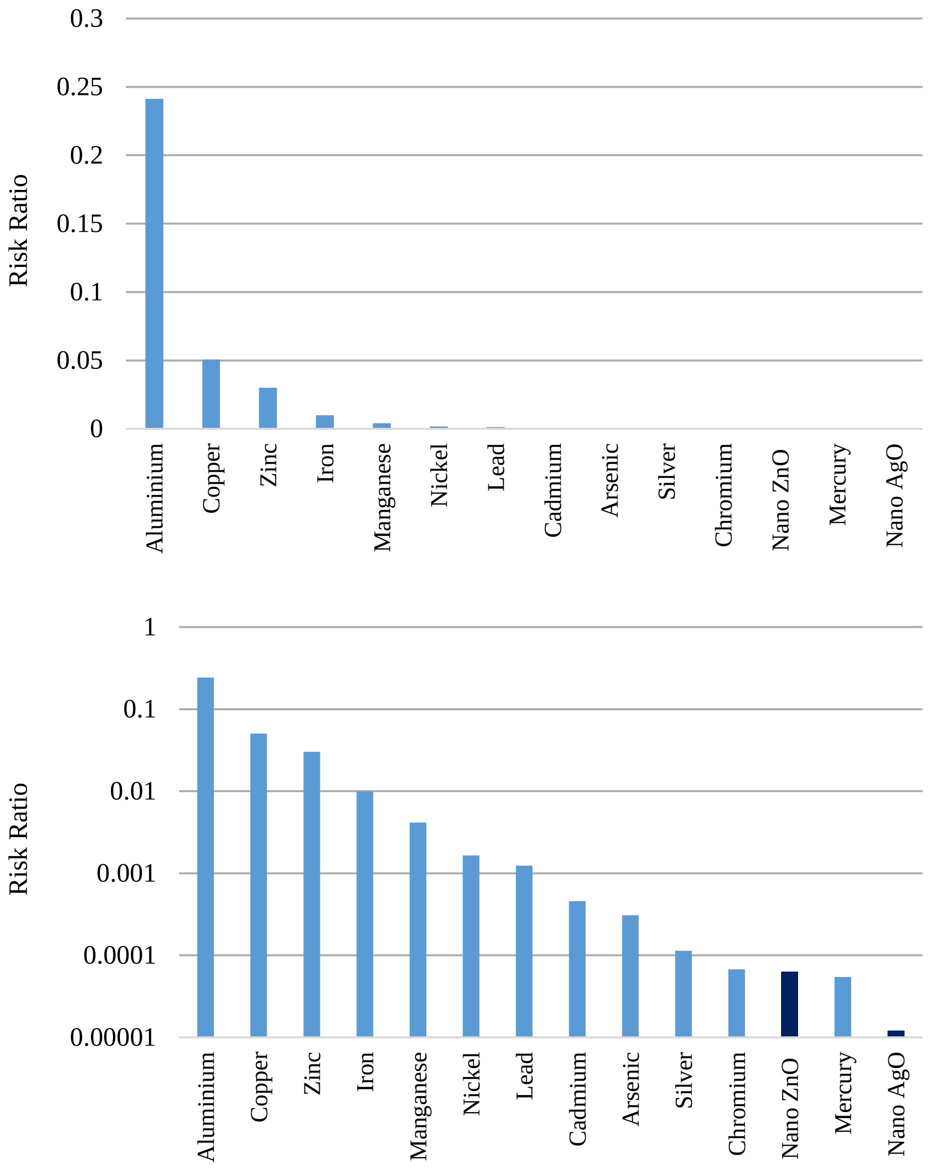


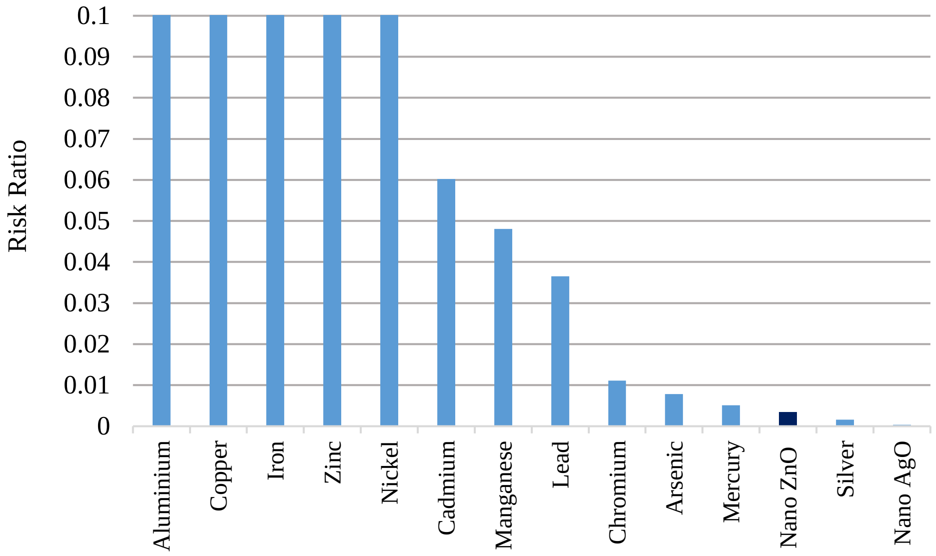
Figure 4-2 Risk ranking of 12 metals (light blue), nanoZnO and nanoAg (dark blue), based on the difference between the median effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

4.3.1.2. *Comparing the median environmental concentration and 5th percentile effect concentration of each chemical*

When using the 5th percentile effect concentration as a comparison to median river water concentrations, Al, Cu and Fe are the metals of greatest concern (Figure 4-3).

This approach gives greater weight to the more sensitive end-points and species.

NanoAg and nano ZnO remain at the lower end of the risk ranking.



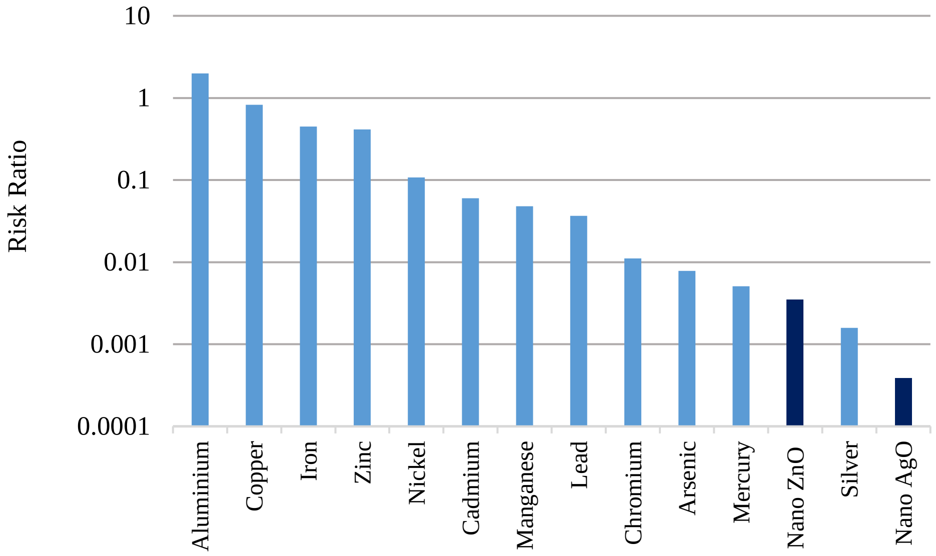
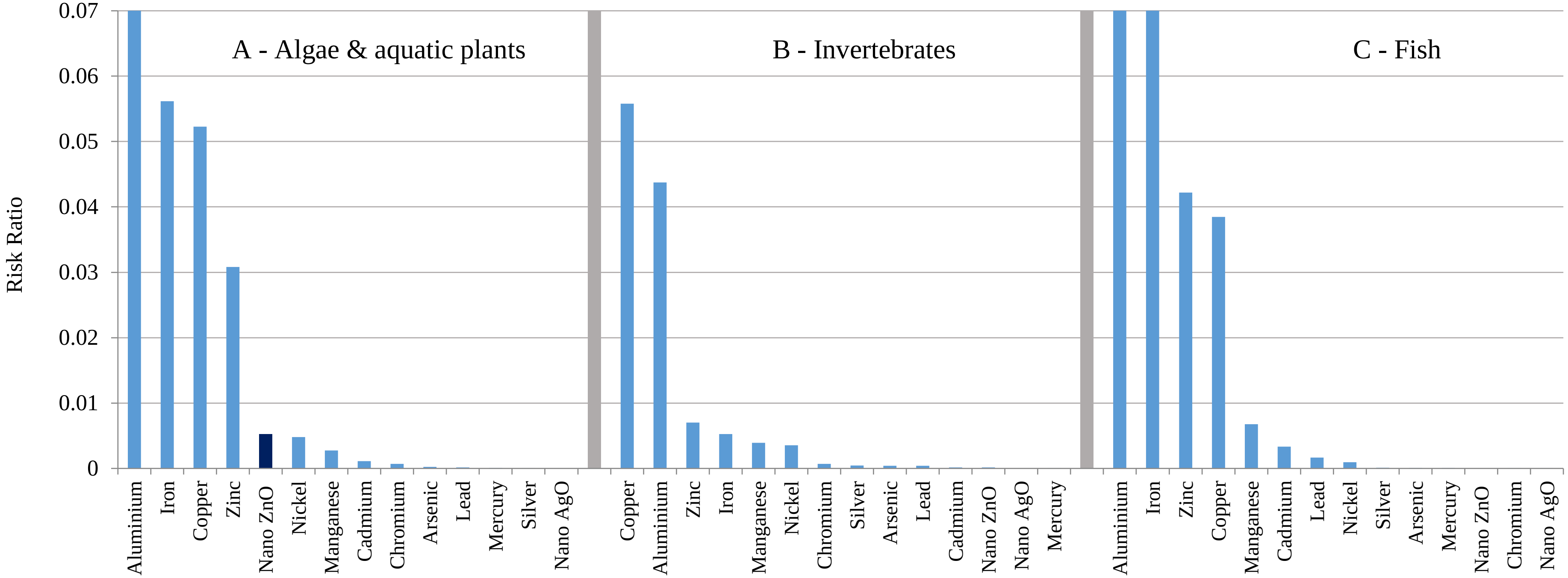


Figure 4-3 Risk ranking of 12 metals (light blue), nanoZnO and nano Ag (dark blue), based on the difference between the 5th %ile effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

4.3.1.3. *Risk-ranking of metals and nanoparticles based on different species*

To understand which chemical is of greatest concern to fish, invertebrates and algae & aquatic plants as individual species categories, the effect data for each chemical were split into fish, invertebrate and algae & aquatic plants. A risk ratio based on the median data was then calculated for each species category. It can be see that aluminium is the metal of greatest concern to fish and algae & aquatic plants, with a risk ratio of 0.44 for fish and 0.46 for algae & aquatic plants, while copper is the metal of greatest concern to invertebrates, with a risk ratio of 0.06. It should be noted at this stage the pH at which the ecotoxicity tests were conducted was not taken into consideration. The nano-metals remain at the lower end of the risk ranking for fish and invertebrates. However, for algae & aquatic plants nano ZnO moves to rank 5th out of the 14 metals and nano-metals considered.


Risk Ratio
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
A - Algae & aquatic plants
B - Invertebrates
C - Fish
Aluminium
Iron
Copper
Zinc
Nano ZnO
Nickel
Manganese
Cadmium
Chromium
Arsenic
Lead
Mercury
Silver
Nano AgO
Copper
Aluminium
Zinc
Iron
Manganese
Nickel
Chromium
Silver
Arsenic
Lead
Cadmium
Nano ZnO
Nano AgO
Mercury
Aluminium
Iron
Zinc
Copper
Manganese
Cadmium
Lead
Nickel
Silver
Arsenic
Mercury
Nano ZnO
Chromium
Nano AgO

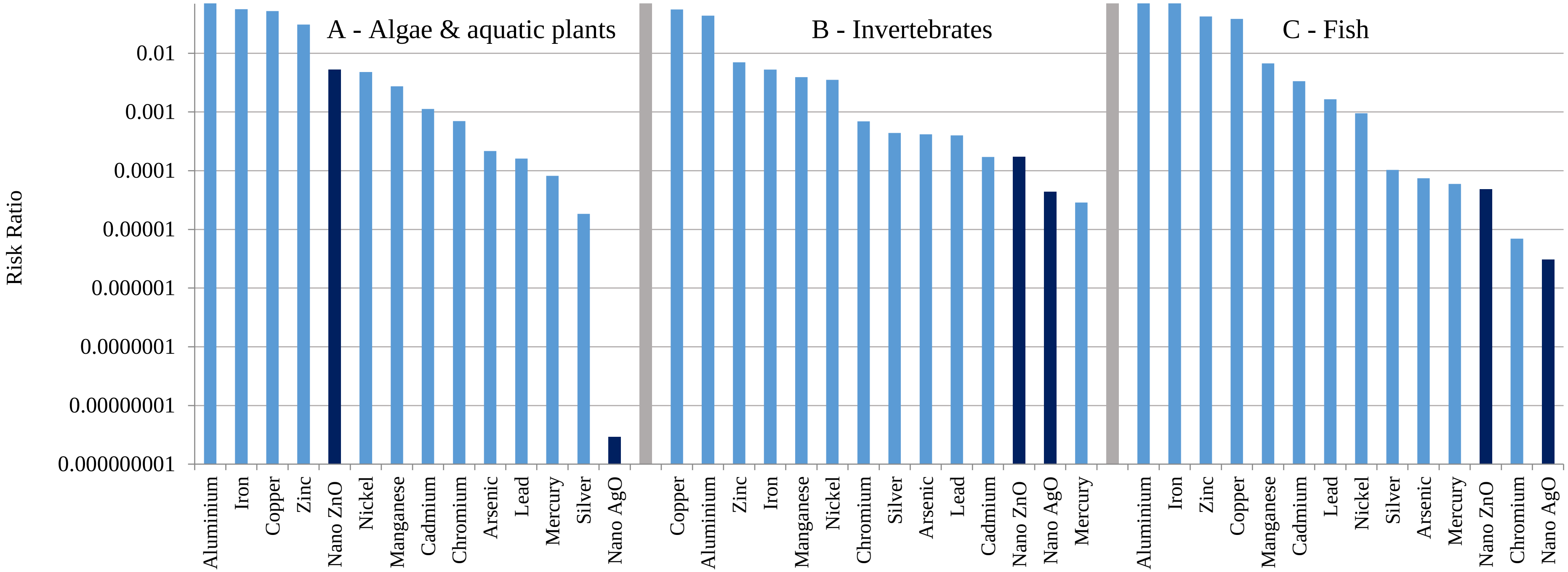


Figure 4-4 Ranking of metals, Nano ZnO and Nano Ag based on their reported effects on algae & aquatic plants (A), invertebrates (B), and fish (C). Rankings are based on a risk ratio derived from comparing the median effect concentration and median river water concentration (presented both as non log-scale and log-scale).

4.3.1.4. *Aluminium*

Aluminium appeared first in order of risk to the environment when water chemistry is not considered (Figure 4-2). Al has mainly been studied in relation to its toxicity in acidic waters [99]. To aquatic organisms, monomeric Al species are the most toxic species [100]. These species of Al are found at pH levels <pH6 and >pH8.5. Below pH6, cations (Al^{3+}, $AlOH^{2+}$, $Al(OH)^{2+}$) are present in the dissolved phase and their solubility increases with decreasing pH. In alkaline conditions the anion $Al(OH)^{4-}$ dominates [41, 43] (Figure 4-5). In the UK, freshwaters are on average found to be of a neutral pH (Figure 3-7) [101]. However, any increase in sources of anthropogenic acidification gives cause for concern with regards Al toxicity to freshwater organisms [102]. Natural causes of fluxes in pH, such as during periods of snowmelt and anthropogenic acidification, can alter the speciation of Al and have been a major concern to freshwater biota [103]. The FOREGS project states a pH range of 6.1-8.5, with an average pH of 7.9 in UK waters (Figure 10-3), although lower pH levels (pH 3.9-6) have been reported in the literature [104-106]. The effect and exposure data included in this study encompass studies conducted at any pH level. The effect of the pH on aluminium toxicity will be considered in Chapter 6.

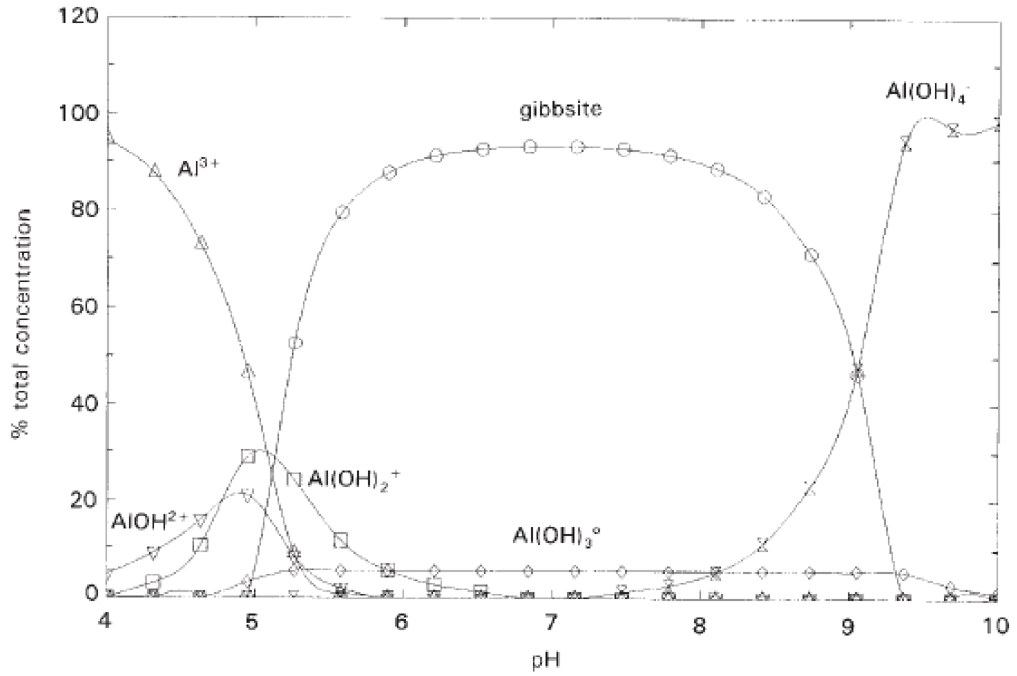


FIGURE 1. Aluminum speciation from a MINEQL+ simulation (Schecher and McAvory, 1992) using five aquatic species of Al plus gibbiste, and varying water pH from pH 4 to 10. The simulation was run using 4 µ*M* total Al (=100%), and 15°C. In the simulation we used 1 m*M* concentrations for Ca, Cl, Na, and NO_3, with the system open to the atmosphere.

Figure 4-5 Aluminium speciation diagram, taken from [41]

4.3.1.5. *Copper*

Copper ranks second based on the risk ratio using the median values. Concentrations of Cu measured in the UK rivers range between 0.18 µg/L and 142,00 µg/L, with a median concentration of 2.4 µg/L. The lowest reported concentration which has harmful effects on freshwater organism was 0.63 µg/L (LOEC), effecting the hatching of zebrafish embryos, at 2.5 µg/L periphyton (algae) experience a 57-81% reduction in productivity at this concentration [107, 108], with *Oncorhynchus mykiss* (Rainbow trout) being affected at 2.8 µg/L [109].

4.3.1.6. *Zinc*

Based on the median data, zinc has been ranked here as the third metal of potential concern to freshwater ecosystems in the UK. The median river water concentration of Zn is 10.70 µg/L. Reported toxic effect concentrations range from 0.34 µg/L to 882 mg/L. *Oncorhynchus mykiss* (Rainbow trout) were reported to be affected at low concentrations, with effects including a decrease in survival at concentrations between 20 and 289 µg/l [110]. Gardner et al. (2012) reported in their study on chemicals present in sewage effluent that the median concentrations of total Cu and Zn are found in the UK were 8.3 µg/L and 30.9 µg/L respectively [82], which as expected are higher than median river concentrations. Sewage effluent is not the sole source of zinc into the aquatic environment, as mentioned previously in section 2.3.1, zinc can enter the aquatic environment via road run off and mine drainage (Table 2-2).

4.3.2. Pharmaceuticals

Of the 1000's of pharmaceuticals currently used for both human and veterinary use, 13 have been included in this study. Pharmaceuticals are a class of chemicals which has received a lot of attention over the last decade amidst fears of the impact they are having on aquatic organisms. In terms of our use of pharmaceuticals it is unlikely that humans' consumption of pharmaceuticals is going to decrease. Pharmaceuticals are now more accessible as they become cheaper alongside an ageing population. In comparison to some chemicals (i.e. the metals), there is limited data available via the literature with regards the ecotoxicity for all pharmaceuticals. For the majority of pharmaceuticals ecotoxicity data are not publicly available and also they have not been measured in the environment. The 13 pharmaceuticals included in this study have varying amounts of ecotoxicity data available. In terms of environmental data, there are limited environmental data, and because of this absence of measured environmental data, predicted concentrations were used based on UK scenarios (as detailed in Chapter 3).

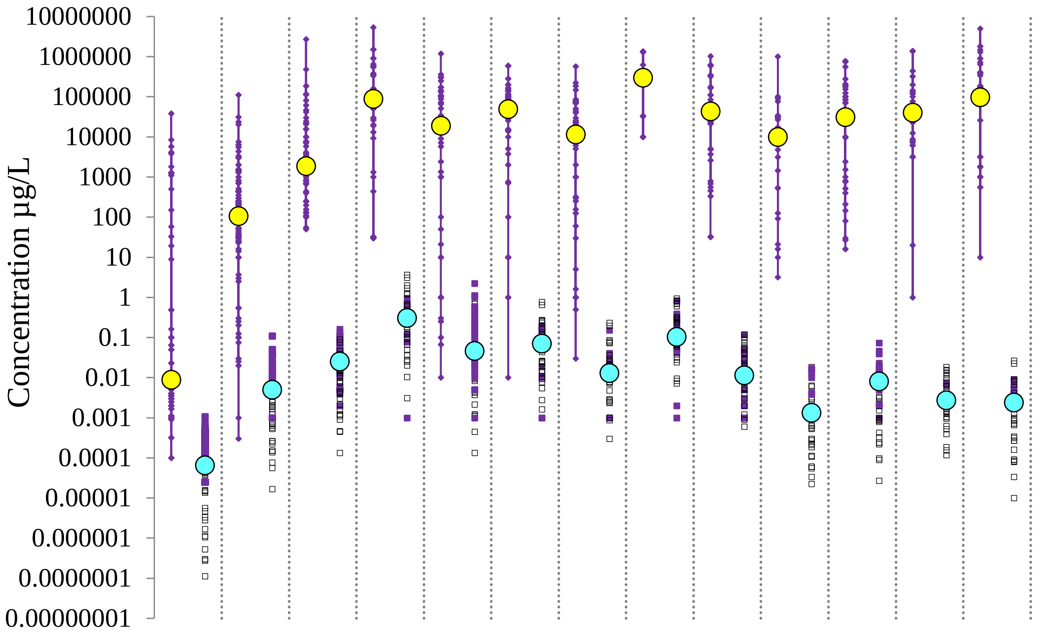
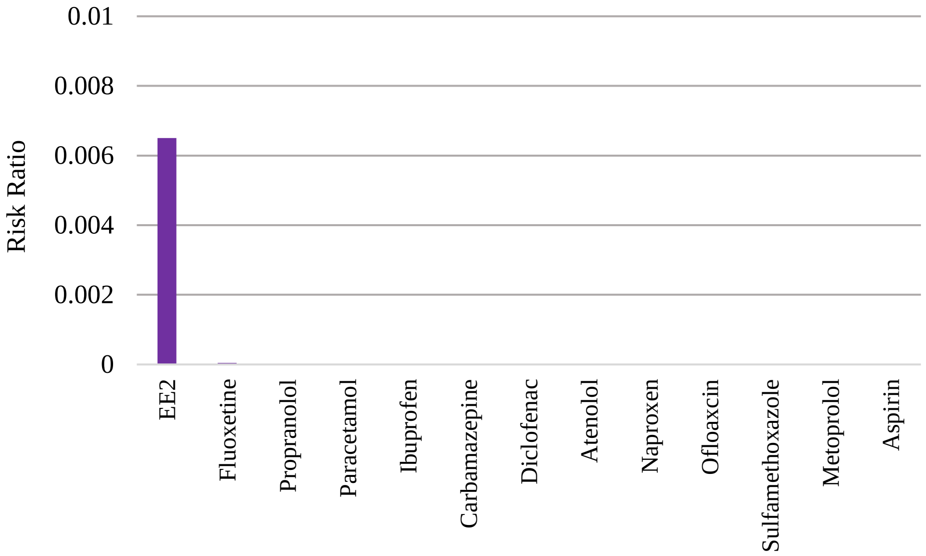


Figure 4-6 Underpinning data of 13 pharmaceuticals, ranked based on the difference between the median effect (left hand vertical line of each pair: diamonds) and river water concentrations (squares). The median values are plotted as yellow (effect) and blue (environmental) circles. (From left to right – EE2, fluoxetine, propranolol, paracetamol, ibuprofen, carbamazepine, diclofenac, atenolol, naproxen, ofloxacin, sulfamethoxazole, metoprolol, aspirin).

When the data for all the pharmaceuticals were compared, it can be seen that some degree of overlap between the measured or predicted environmental concentrations and concentrations that cause effects on aquatic organisms occurs for 5 of the pharmaceuticals, namely EE2, fluoxetine, ibuprofen, diclofenac and carbamazepine (Figure 4-6), with the other 8 of the pharmaceuticals having no overlap between the environmental and ecotoxicity concentration data.

4.3.2.1. *Comparison of median environmental concentrations and effect concentrations*



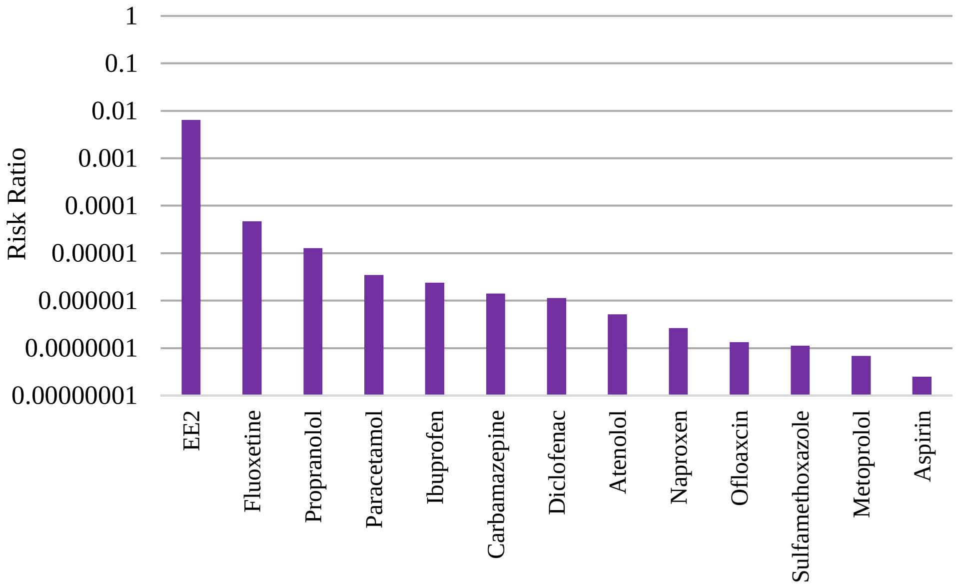


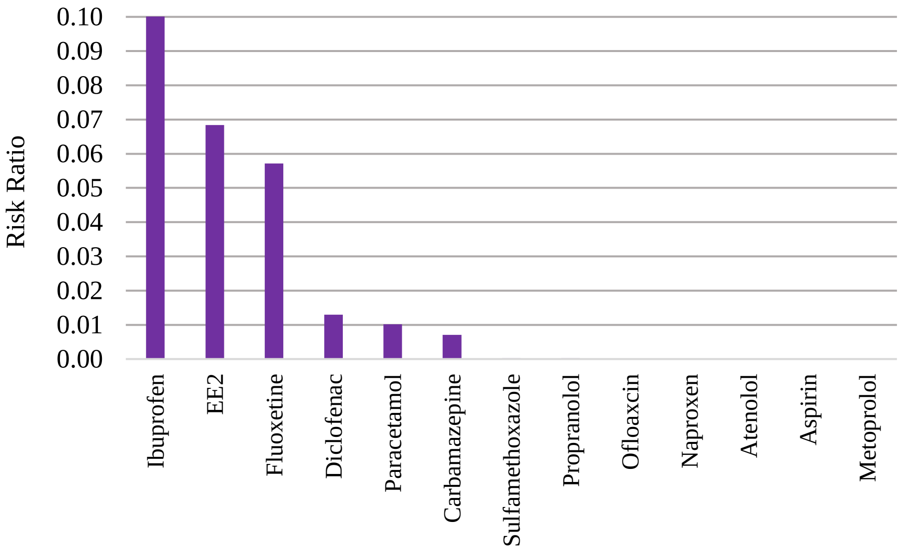
Figure 4-7 Risk ranking of 13 pharmaceuticals based on the difference between the median effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

The pharmaceuticals have been ranked using a risk ratio calculated from the median data (Figure 4-7). The higher the risk ratio, the greater the concern; thus a risk ratio of >1 would be classed as a major concern for aquatic wildlife. For all pharmaceuticals studied apart from EE2, the difference between the two median values was greater than 100,000-fold, giving a risk ratio of 0.00001 or less. EE2 had a risk ratio of 0.007, which was significantly higher than that of the other pharmaceuticals reported here (Figure 4-7).

4.3.2.2. *Comparing the median environmental concentration and 5th percentile effect concentration of each chemical*

When the ranking was based on the difference between the 5th percentile effect concentration and the median river water concentrations, ibuprofen came 1st with a risk ratio of 0.26, followed by EE2 (0.07) and fluoxetine (0.06).

The ranking of pharmaceuticals based on the 5th percentile effect data is a more precautionary approach to ranking, as the focus is on the most vulnerable species and the most sensitive end-points. Even when using the precautionary approach, the risk ratio of all the pharmaceuticals was less than one (Figure4-8).



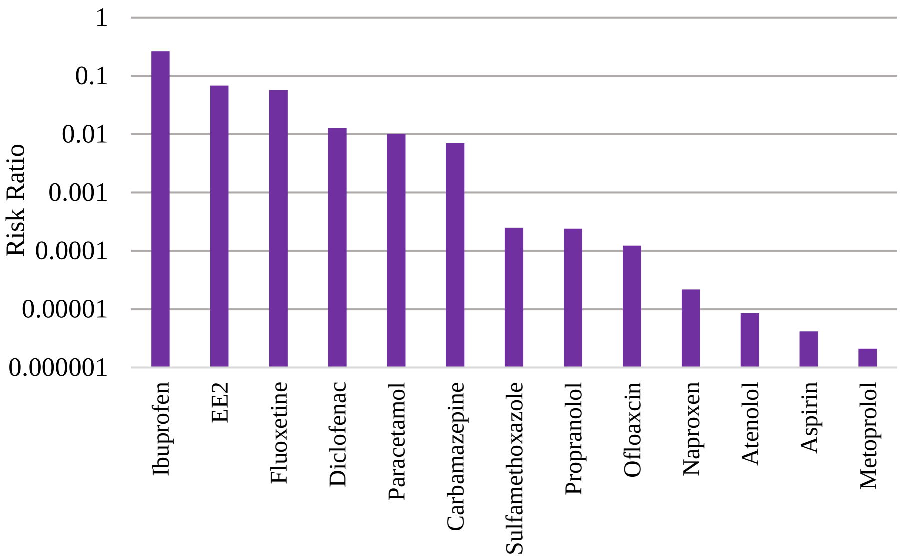


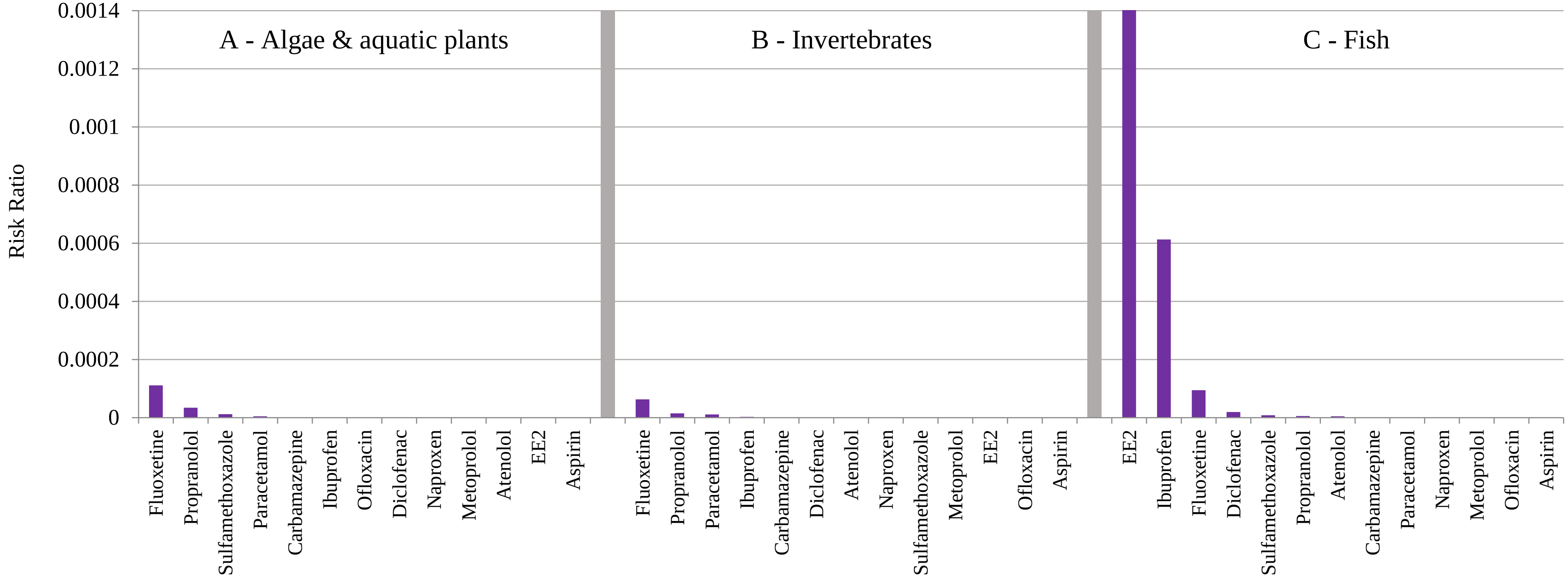
Figure 4-8 Risk ranking of 13 pharmaceuticals based on the difference between the 5th %ile effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

4.3.2.3. *Risk-ranking of pharmaceuticals based on different species*

Pharmaceuticals have often been designed to target specific metabolic and molecular pathways which are often common to all vertebrates. Species with similar targets may be more likely to experience an effect due to the presence of pharmaceuticals in the environment as they have a comparable pathway [111].

The risk to each category of species was assessed based on the risk ratio using the median effect value and the median river water concentration (Figure 4-9). It would appear from the 13 pharmaceuticals selected that fish are the most sensitive species to pharmaceuticals. Given that fish appear to contain many of the same drug targets as humans, this is not surprising [71]. Based on the median data (Figure 4-9), EE2 is the pharmaceutical of most concern to fish, with a risk ratio of 0.016. In contrast to fish, EE2 has a ratio of 5.27×10^{-7} and 7.74×10^{-8} for algae & aquatic plants and invertebrates, respectively, based on the median data. Within each species group, it is likely that there will be intraspecies sensitivity variation as well [112]. Paracetamol, fluoxetine and propranolol rank highly for invertebrates and algae & aquatic plants, based on the median comparison. But these species are 100 times less sensitive to these pharmaceuticals than fish are to EE2.

Fluoxetine is one of the more studied pharmaceuticals. It has been reported to be one of the most potentially disruptive human drugs to aquatic species [113]. Fluoxetine appears to be of concern to all species groups, with algae & aquatic plants being the more sensitive species group. The median fluoxetine effect concentration for algae & aquatic plants reported in this study is 45 µg/L, with the effects reported on the most sensitive algae occurring at 24 µg/L, where fluoxetine affected the growth of *Pseudokirchneriella subcapitata* [114]. The median fluoxetine effect concentration for invertebrates identified in this study was 80 µg/L, whilst the most sensitive species appears to be mussels, on which biochemical effects have been reported at 0.0003 µg/L [115]. Sumpter and Margiotta-Casaluci (2014) have openly questioned some of the reports which claim that invertebrates are exquisitely sensitive to fluoxetine [116].


Risk Ratio
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
A - Algae & aquatic plants
B - Invertebrates
C - Fish
Fluoxetine
Propranolol
Sulfamethoxazole
Paracetamol
Carbamazepine
Ibuprofen
Ofloxacin
Diclofenac
Naproxen
Metoprolol
Atenolol
EE2
Aspirin
Fluoxetine
Propranolol
Paracetamol
Ibuprofen
Carbamazepine
Diclofenac
Atenolol
Naproxen
Sulfamethoxazole
Metoprolol
EE2
Ofloxacin
Aspirin
EE2
Ibuprofen
Fluoxetine
Diclofenac
Sulfamethoxazole
Propranolol
Atenolol
Carbamazepine
Paracetamol
Naproxen
Metoprolol
Ofloxacin
Aspirin

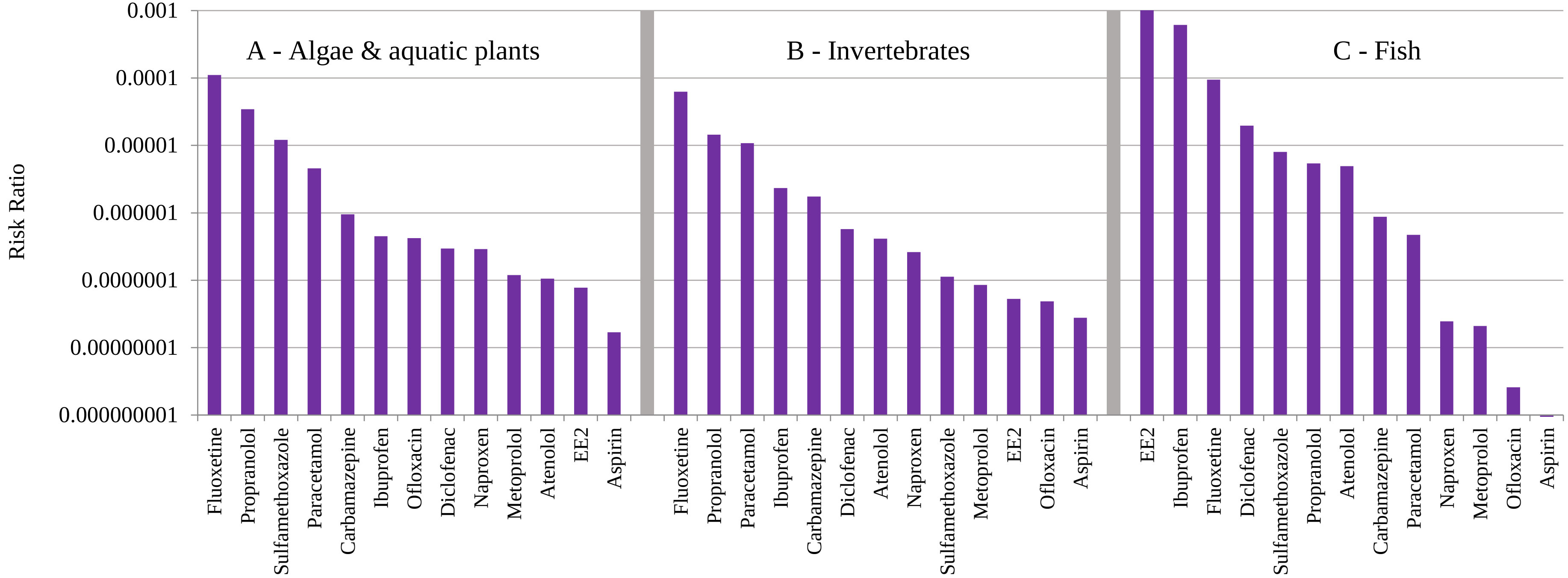


Figure 4-9 Ranking of 13 pharmaceuticals based on their reported effects on algae & aquatic plants (A), invertebrates (B), and fish (C). Rankings are based on a risk ratio obtained by comparing the median effect concentration and median river water concentration (presented both as non log-scale and log-scale).

4.3.2.4. *EE2*

EE2 is the highest ranked pharmaceutical based on the median data (Figure 4-7). EE2 is a synthetic hormone that regulates reproductive functions in vertebrates. It is widely used in contraceptive pills. The reported effect concentration for EE2 ranges from 0.1 ng/L – 37.8 x 10^6 ng/L, with a median of 8.9 ng/L, whereas the environmental concentrations range between 1.13 x 10^{-4} – 1.07 ng/L, with a median value of 0.065 ng/L. The lowest reported effect concentration was 0.1 ng/L, where a stimulatory effect was seen with an increase in the mean number of eggs spawned per pair in *Pimephales promelas* up to 1 ng/ L but at 3 ng/L a decrease in egg production was observed [117].

4.3.2.5. *Fluoxetine*

Fluoxetine was ranked second based on the comparison of median effect and river concentrations (Figure 4-7). Fluoxetine, also known as Prozac, is an antidepressant of the selective serotonin reuptake inhibitor class. Christensen et al (2009) reported it to be very toxic to aquatic organisms [118]. However, fluoxetine is an example of a pharmaceutical where the effect data are extremely variable and not necessarily repeatable [119]. The range of effect concentrations for fluoxetine is 0.0003 – 111,357 µg/L, with a median effect concentration of 106 µg/L.

4.3.3. Pesticides

Pesticides are used globally. Natural pesticides have been used for 1000’s of years, whereas synthetic pesticides have been developed and used since the 19th century. There are some 1,000 pesticides which are available globally, although the specific pesticides used and restrictions on pesticides is very country specific. In the UK there are > 300 pesticides registered for use [120]. Globally, there is a reliance on pesticides to ensure that crops yields are sustained and that the economic implications within agriculture are not affected by the banning of pesticides [121].

Pesticides are designed to have a toxic effect, but depending on their mode of action and application they affect more biota than just the target organism [122]. For example, the discovery of the impacts of DDT was as a catalyst of concern with regards pesticide use and impacts on non-target species and the environment on a global scale [13, 123].

Pesticides continue to be of concern to aquatic ecosystems, with their use and presence being highlighted as a major contributor to chemical risk [2, 124]. For 40 years there have been strict regulations in the EU for pesticide use [125], which has limited the availability of pesticide active substances used in effective plant protection. This legislation has not only limited the use of pesticides, it has introduced control measures for their use, thus reducing the concentrations used. However, this is not the case globally, because there is still unrestricted use of pesticides as well illegal use, as well as the hard-to-monitor domestic use in many countries. The legislation which has been established is a prevention-led approach [126].

It is important to note that with pesticides, river exposure is not usually continuous. The application method and source of the pesticide can dramatically influence the concentrations found in the environment. For example, peak concentrations are associated briefly with the major application period, which is then followed by a return to very low levels [127].

Within this project 21 pesticides were studied. This included 9 insecticides; chlorpyrifos, methomyl, malathion, permethrin, carbofuran, diazinon, pirimicarb, Beta-HCH, imidacloprid, 10 herbicides; bentazone, MCPA, MCPP, simazine, glyphosate, pendimethaline, metolachlor, linuron, terbuthylazine, lenacil, a biocide, tributyltin and a molluscicide, metaldehyde.

Some of the legacy pesticides which are now classed as persistent organic pollutants have been considered within the POPs class and not within the pesticides class.

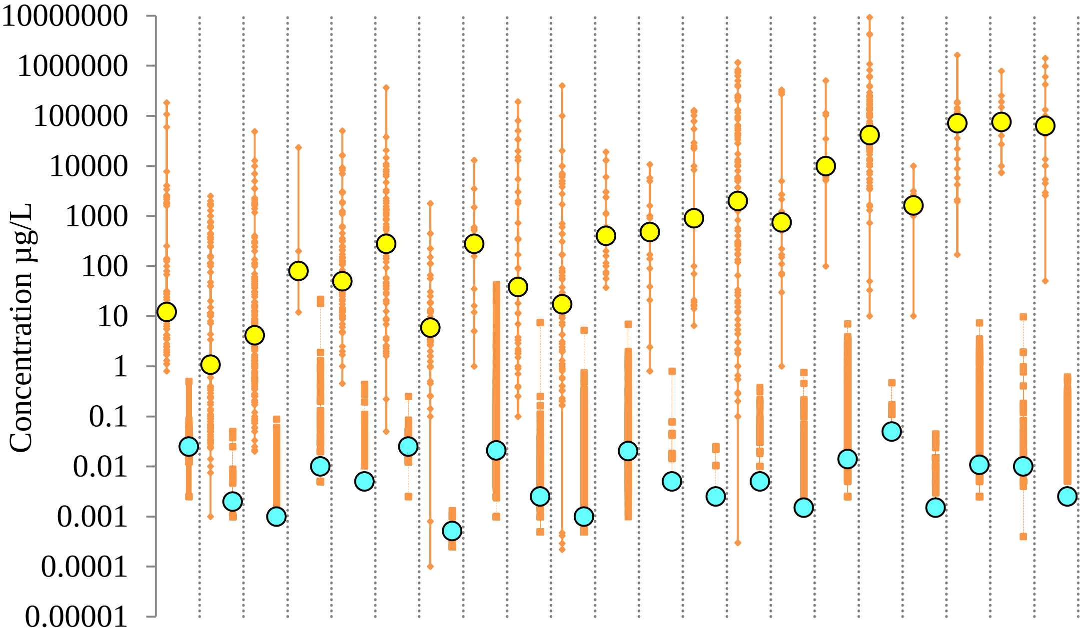


Figure 4-10 Underpinning data of 21 pesticides, ranked based on the difference between the median effect (left hand vertical line of each pair: diamonds) and river water concentrations (squares). The median values are plotted as yellow (effect) and blue (environmental) circles. From left to right – methomyl, chlorpyrifos, permethrin, malathion, lenacil, linuron, carbofuran, TBT, terbuthylazine, diazinon, metolachlor, pendimethalin, pirimicarb, imidacloprid, simazine, MCPP, glyphosate, Beta-HCH, MCPA, metaldehyde, bentazone).

When the data for all the pesticides were compared, it can be seen that some degree of overlap between environmental concentrations and concentrations that cause effects on aquatic organisms occurs for 11 of the pesticides; carbofuran, pendimethaline, diazinon, linuron, lenacil, malathion, chlorpyrifos, imidacloprid, tributyltin, terbuthylazine and permethrin (Figure 4-10), with 10 of the pesticides having no overlap in environmental and ecotoxicity concentration data.

4.3.3.1. *Comparison of median environmental concentrations and effect concentrations*

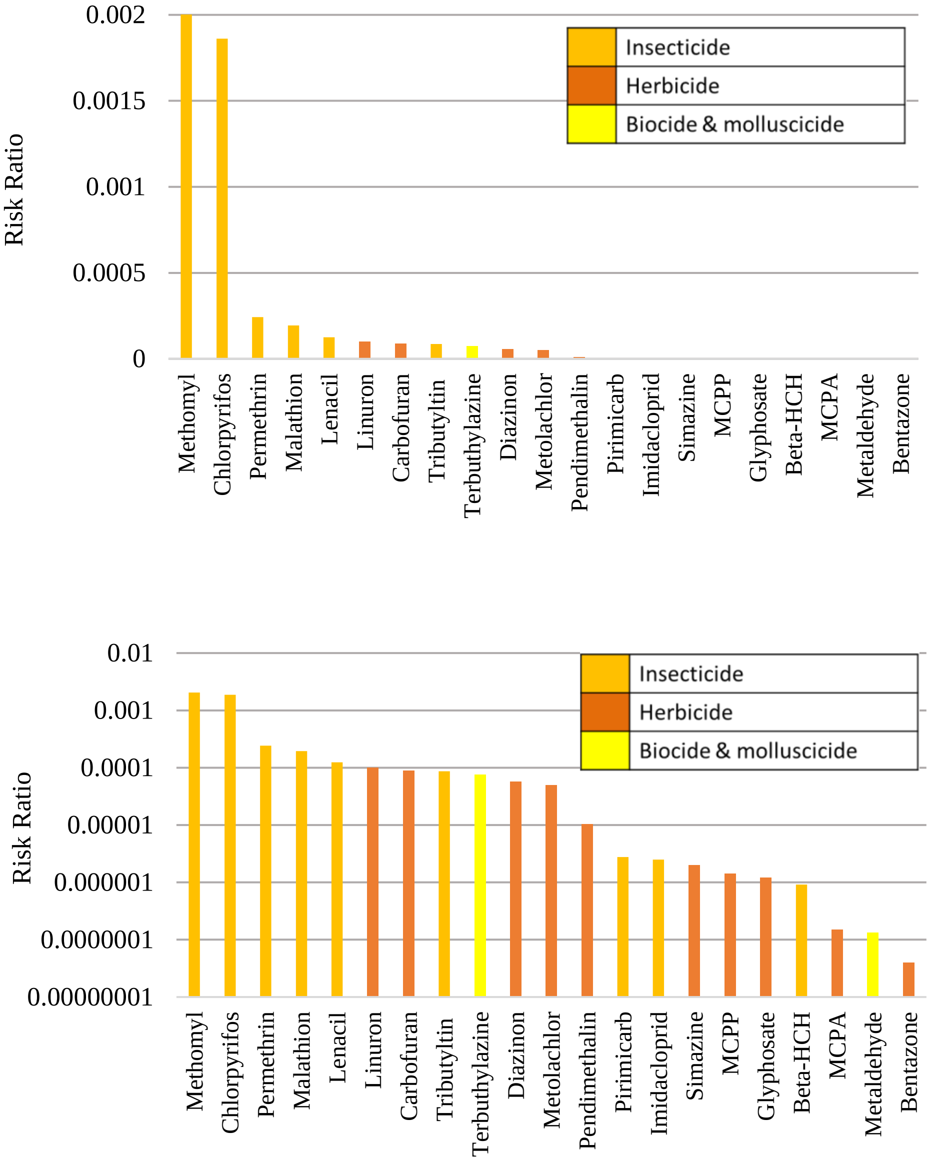
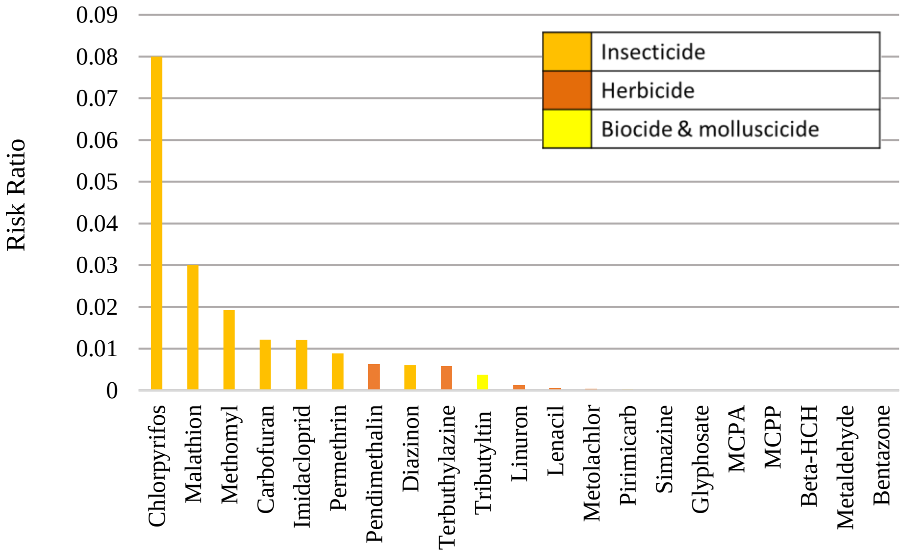


Figure 4-11 Risk ranking of 21 pesticides based on the difference between the median effect concentration and the median river water concentration (presented as log-scale).

When ranking the pesticides by comparing the median river water and median effect concentrations, methomyl, chlorpyrifos and diazinon emerge as posing the greatest risk. The difference between the median river water and effect values was relativity small, (1,000 -fold) for pesticides of most concern, but was larger (>100,000-fold) for pesticides of less concern, such as MCPA, metaldehyde and bentazone (Figure 4-11). The risk ratios for the pesticides ranged from the highest ranked pesticides, methomyl (0.002) and chlorpyrifos (0.002), to the lowest ranked pesticides, metaldehyde ($1.33x10^{-7}$) and bentazone ($3.96x10^{-8}$). There is a fold difference of 10,000 between the highest and lowest ranked pesticides within the class.

4.3.3.2. *Comparing the median environmental concentration and 5th percentile effect concentration of each chemical*

When the precautionary approach is taken chlorpyrifos is the chemical of greatest concern, with a risk ratio of 0.08, followed by malathion and methomyl (Figure 4-12). The neonicotinoid, imidacloprid, moves up the ranking to 5th when this approach is taken. Even when this precautionary approach is considered, none of the pesticides have a risk ratio greater than 0.1 (Figure 4-12).



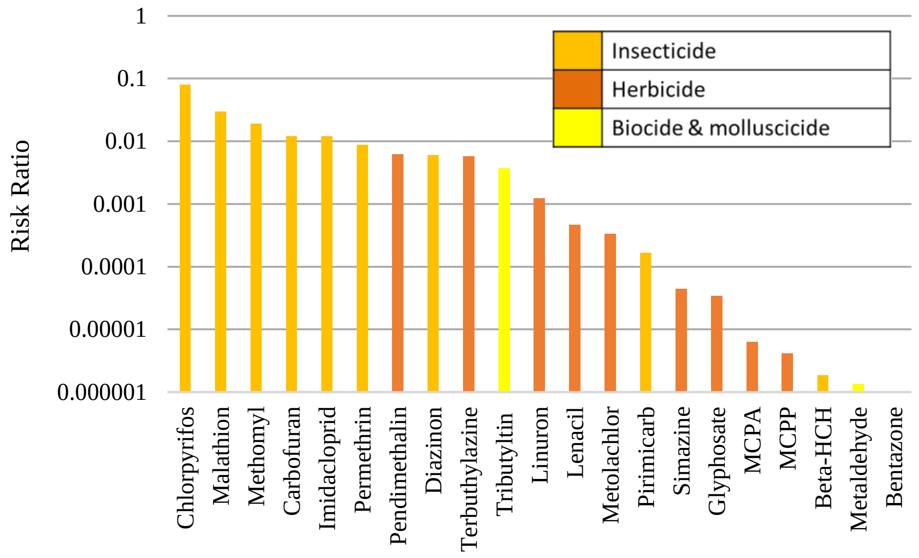
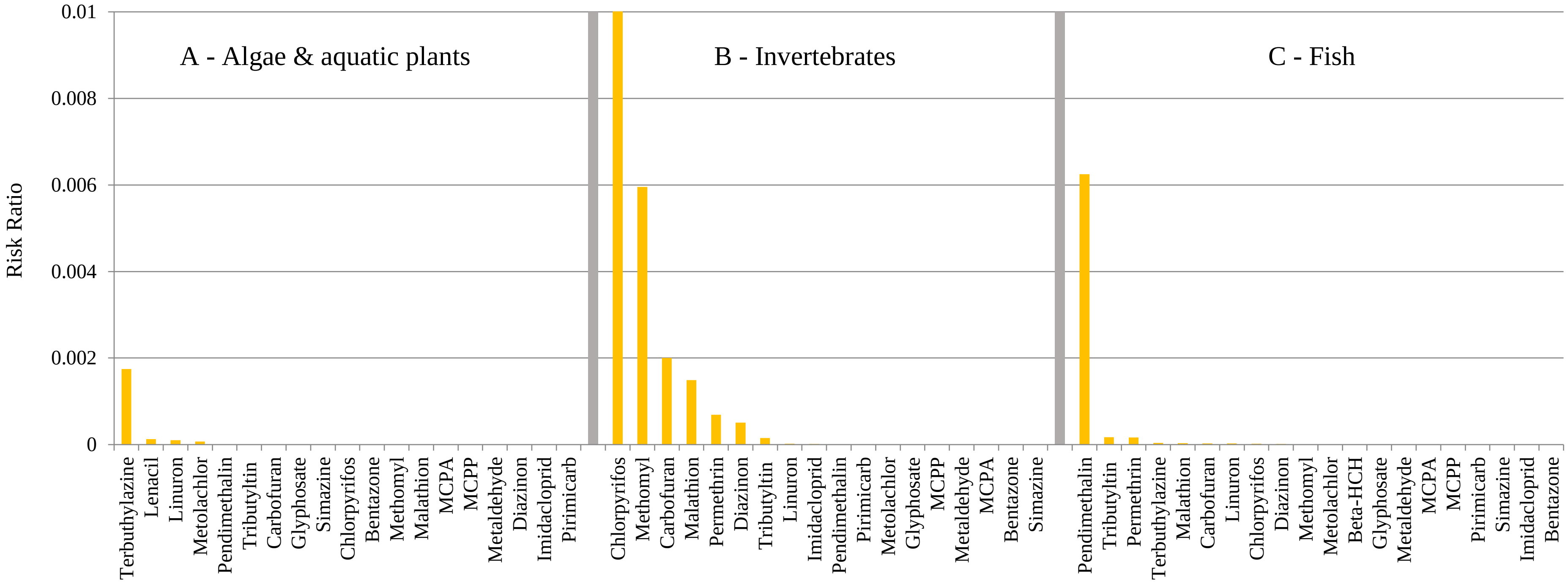


Figure 4-12 Risk ranking of 21 pesticides based on the difference between the median effect concentration and the 5th %ile river water concentration (presented as log-scale).

4.3.3.3. *Risk-ranking of pesticides based on different species*

When the effect data is split into species for fish, invertebrates and algae & aquatic plants. Chlorpyrifos remains the chemical of greatest concern to invertebrates with a risk ratio of 0.014, which is unsurprising as it is an insecticide. Terbuthylazine is the chemical of greatest concern to algae & aquatic plants, this is also not a surprise as it is a herbicide. Pendimethalin is the greatest concern to fish.


Risk Ratio
0.01
0.008
0.006
0.004
0.002
0
A - Algae & aquatic plants
B - Invertebrates
C - Fish
Terbuthylazine
Lenacil
Linuron
Metolachlor
Pendimethalin
Tributyltin
Carbofuran
Glyphosate
Simazine
Chlorpyrifos
Bentazone
Methomyl
Malathion
MCPA
MCPP
Metaldehyde
Diazinon
Imidacloprid
Pirimicarb
Chlorpyrifos
Methomyl
Carbofuran
Malathion
Permethrin
Diazinon
Tributyltin
Linuron
Imidacloprid
Pendimethalin
Pirimicarb
Metolachlor
Glyphosate
MCPP
Metaldehyde
MCPA
Bentazone
Simazine
Pendimethalin
Tributyltin
Permethrin
Terbuthylazine
Malathion
Carbofuran
Linuron
Chlorpyrifos
Diazinon
Methomyl
Metolachlor
Beta-HCH
Glyphosate
Metaldehyde
MCPA
MCPP
Pirimicarb
Simazine
Imidacloprid
Bentazone

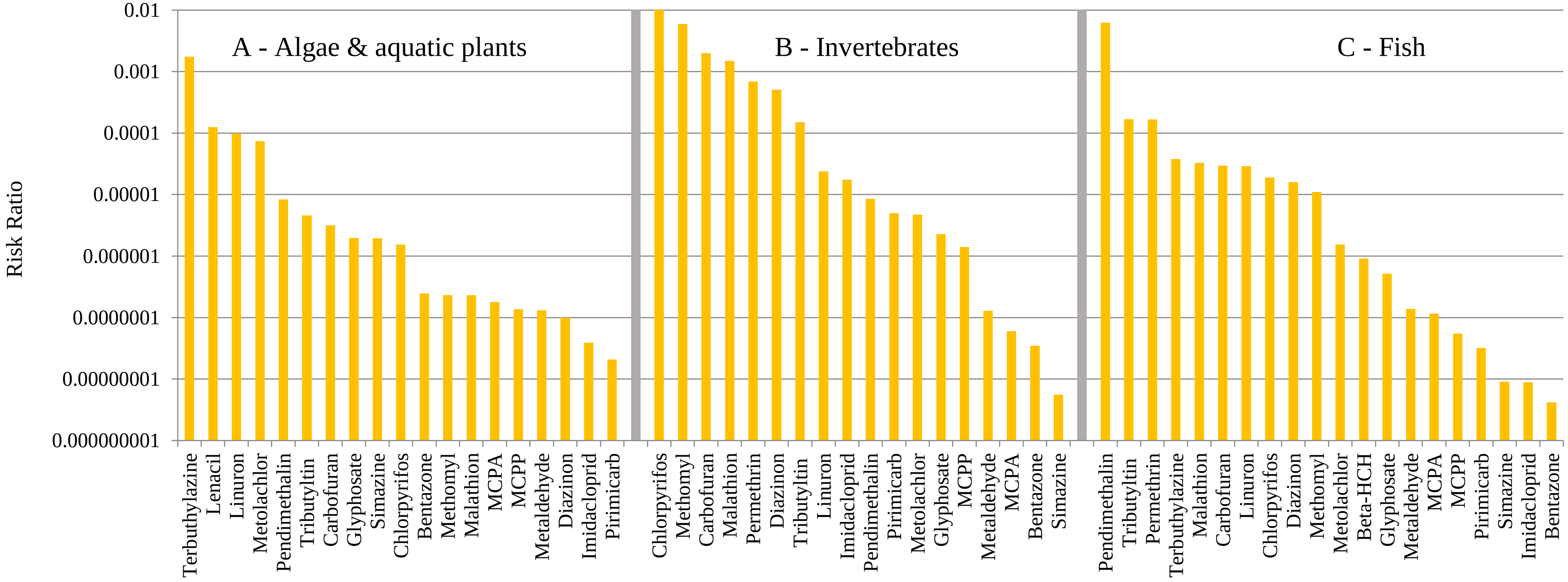


Figure 4-13 Ranking of 21 pesticides based on their reported effects on algae & aquatic plants (A), invertebrates (B), and fish (C). Rankings are based on a risk ratio comparing the median effect concentration and median river water concentration (presented both as non log-scale and log-scale).

4.3.3.4. *Chlorpyrifos*

Chlorpyrifos is the highest ranking pesticide overall. It is a broad-spectrum, chlorinated organophosphate (OP) insecticide. It was first registered as an insecticide in 1965 and was approved for use in the EU [128]. In the UK, chlorpyrifos was used in agriculture. Since this study started in 2012, the EU have banned the sale and use of chlorpyrifos, based on concerns for human health. Its use will be phased out in the following three stages: 1) 12th February 2016: Suspension of sales from manufacturers to distributors, 2) 1st April 2016: illegal to spray any product containing chlorpyrifos on any crop, 3) 1st October 2016: disposal of all stocks of any product containing chlorpyrifos. After the 1st October 2016, it will be illegal to store or use any product containing chlorpyrifos. There is currently one exception to this; the use of chlorpyrifos is permitted on the protected brassica seedling drench treatment applied via automated gantry sprayer [129].

Chlorpyrifos is highly toxic to aquatic organisms, particularly invertebrates, with the lowest concentration to cause an effect reported (in this study) to be 0.001 µg/L affecting gene expression in *Daphnia magna*. Sub-lethal effects on fish are seen at a concentration of 0.36 µg/L (Common carp), with mortality being reported at concentrations of 8 µg/L upwards. Concentrations found in the UK surface waters range from 0.001 - 0.05 µg/L. Based on the data collated for this review, chlorpyrifos does appear to be of concern. The recent ban on its use and imminent stop in its use in the UK should reduce some of these concerns, as its input into the aquatic environment will be reduced.

4.3.3.5. *Lenacil*

Lenacil is the highest ranked herbicide based on the initial median ecotoxicity vs median environmental risk ranking approach (Figure 4-11). It is a uracil pre-emergent herbicide which is used mainly with sugar beet, fodder beet and spinach production in Europe. It is believed to interfere with the photosystem II in plants [130, 131]. The most sensitive aquatic organisms are likely to be plants, but very little ecotoxicity data seems to exists.

The major risk to surface waters from such compounds is associated with herbicide spraying of crops in (typically) spring, when spraying is followed by rainfall runoff events. Detection of lenacil in rivers seems rare, but brief peaks can be found following spring application before concentrations return to a very low baseline [132, 133]. Lenacil can be found in the product Betanal Maxpro, which is used in sugar beet production in the UK. European river water concentrations can reach close to 2 µg/L levels following spring applications in areas where this agriculture is important. But the most sensitive organism based on the literature review was a green alga with an EC50 at 12 µg/L, followed by a macrophyte with an EC50 at 58 µg/L. This suggests we should have some concern for algae & aquatic plants as the lowest effect concentrations are only 6-fold below the highest concentration reported in a European river (France) following spring applications. However, the majority of peaks in rivers measured so far have been below 1 µg/L and the major crops associated with this pesticide are not widespread.

4.3.3.6. *Terbuthylazine*

Terbuthylazine is a chlorotriazine broad spectrum herbicide which appears to be gaining in importance by taking over from the previously popular atrazine and simazine herbicides, which are now restricted in use [134, 135]. It has been described as a vital herbicide for maize production given the demise of many of the other triazines [136]. Terbuthylazine is now one of the top three herbicides used in Italy with an annual consumption of 451 tons in 2009 [135]. It interferes with the photosystem II in plants [131, 137]. Thus, the most sensitive aquatic organisms are likely to be plants. The major risk to surface waters from such compounds is associated with herbicide spraying of crops in (typically) spring when spraying is followed by rainfall runoff events. Concentrations above 1 µg/L have been reported but are very rare occurrences. Terbuthylazine is authorised for use in the EU. It can be found in a number of Syngenta products[136].

4.3.3.7. *Tributyltin*

Tributyltin (TBT) is an organotin, and for approximately 40 years it was used as an extremely effective biocide in anti-fouling paints, to prevent the growth of algae and barnacles on ship hulls etc [138]. TBT is extremely stable, it can have a half-life in water of days to weeks, and a half-life in sediment of weeks, months, or years. Because of its chemical properties and widespread use as an antifouling agent, concerns have been raised over the risks it poses to both freshwater and saltwater organisms. TBT has been found in the water in its dissolved phase, but it readily adsorbs to sediments and suspended solids, where it can persist [139].

In terms of the concentrations found in the environment, the occurrence of TBT has significantly reduced due to bans on its use which have been put in place. Even though TBT has been phased out of the shipping industry, it is still found in the environment. From this literature review, concentrations which was found in the UK freshwater environment ranged from 0.00025-0.001 µg/L (surface water). Concentrations found in sewage effluent have recently been reported by Gardner et al at 0.3 ng/L (50th %ile) 1.3 ng/L (95 %ile) and 1.8 ng/L (97.5 %ile) in the UK [82]. These values exceed the freshwater EQS value of 0.2 ng/L (annual average) and 1.5ng/L (maximum allowed concentration), however dilution will reduce these high effluent concentrations downstream. Both the surface water and the sewage effluent concentrations are in close proximity to the WFD EQS values.

The range of concentrations at which toxic effects occur is 0.041 – 1,782 µg/L. The lowest reported effect from the literature review was 0.041 µg/L, where a change in the sex ratio was seen in zebra fish [140] and 0.14 µg/L, where an effect was seen on Daphnia magna [141].

TBT is of concern because it has been found to be toxic to non-target species. It is can be extremely toxic to aquatic life and is an endocrine-disrupting chemical that causes severe reproductive effects in aquatic organisms [142]. Bivalves have been reported as being the most affected species, possibly because they bioaccumulate more of the chemical. Crustaceans and fish, in relation to TBT, have enzymatic mechanism to degrade it, and thus they bioaccumulate less [143].

4.3.3.8. *Metaldehyde*

Metaldehyde is a molluscicide. It is a synthetic aldehyde pesticide, which has been in use since the 1930's [144]. It is used in both agricultural and non-agriculture capacities to control slugs, snails and other gastropods. In the UK it is estimated that over 8% of the area covered by arable crops are treated with it (Environment Agency Report via [145]). It is a highly specific pesticide; it acts on the mucus cells of the slugs (for example), leading to dehydration and an inability to move, therefore the slugs can become open to predation [146]. This pesticide can result in the depression or excitement of the central nervous system of mammals and thus poisoning is characterized by CNS depression and convulsions [147].

Concerns of toxicity to non-target organisms, such as domestic pets and birds via exposure to metaldehyde have been reported [148]. It is toxic to molluscs via ingestion or absorption through the skin or by secondary poisoning of the other groups of animals via the consumption of contaminated prey [149].

Entrance into the environment, as with other pesticides, is via point source, direct input (i.e. accidental spill) and diffuse source via run off. Kay et al (2014) reported on unpublished work from the Cherwell catchment, and gave a measurement of 1.8% of the active ingredient which being applied was lost to surface waters with peak concentrations as high as 9.8 µg/L [150].

The EU regulatory standard is 0.1 µg/L for a single pesticide (the regulatory standard for the sum of all pesticides is 0.5 µg/L) [151]. Kay et al (2014) reported eight out the nine STPs, and 11 out of the 21 rivers sites, exceeded this limit. The concern lies with the order of magnitude by which the limit is being exceed. From an industry point of view, these exceedances could result in the product being removed from the market. It should be noted that Kay et al (2014) highlight that the 0.1 µg/L limit is an arbitrary figure, and has not been set based on any effect data [150].

There are very limited effect data for metaldehyde, considering how high the concentrations in the environment are. From the data available, all effects are in the mg/L range. The most sensitive end-point, obtained via the EPA database, was an LC50 of 7,300 µg/L for Rainbow trout. For invertebrates, the concentrations at which effects are seen are greater than 77,660 µg/L [152].

From the data available for this study, there is no overlap between measured environment concentrations (both typical and at peak times) and the concentrations at which effects (mortality or immobilisation) have been reported.

Therefore, in terms of concerns to aquatic organisms in the UK, it appears not be of immediate concern, based on very limited ecotoxicity data. Further investigation into its effects on a wider breath of species could be relevant [145]. The surface water concentrations in the UK are exceeding the regulatory limit. A concern with regards metaldehyde is its removal from the water (especially for drinking water). It is not removed via the current method of utilising granular activated carbon, and methods such as chlorination and ozonation do not work, as it can’t be broken down to a simpler molecule. Its half-life in water has been reported to be between 3-223 days, depending on the environment. Thus, this is a potentially persistent chemical. It is also very mobile in the aquatic environment [153].

4.3.3.9. *Imidacloprid a neonicotinoid*

Neonicotinoids are a class of insecticide used to control invertebrate pests. They have been widely used due to their potency. They are synthetic insecticides, which are absorbed via roots or leaves into every cell of the plant, thus ensuring that every part of the plant is protected against pests by being poisonous to them [154]. Sap-sucking insects such as aphids and other insect herbivores die after consuming the treated crop. The insecticide also reaches the pollen and the nectar, which has caused unforeseen environment concern around contamination of non-target organisms, such as bees [155]. The mode of action of the neonicotinoids is to affect the central nervous system of the insect. It binds to the receptors of the enzyme nicotinic acetylcholine (ACh receptor) and excites the nerves, thus causing damage to the nerves and eventual paralysis and death. Invertebrates mobility is affected, thus their behaviours are changed i.e. feeding, therefore they can die of starvation [156]. The concern surrounding the environmental impact of neonicotinoids has been compared to that of DDT in the 1980's, effects of which are still being seen today. The main focus of their environment impact has been the effect that they are having on bees and non-target insects.

Neonicotinoids have been in use since the 1990's. Imidacloprid was used primarily, but others, including clothianidin, thiamethxam, acetamiprid, thiacloprid, dinotefuran and nitenpyram, have now been developed. The use of neonicotinoids has increased in comparison to other pesticides due to the flexibility in their use. They can be applied as seed dressing, foliar spray, granular formula and via soil drenching or water irrigation. This flexibility, coupled with the reduced amount required to have an effect, has made them the dominant pesticides of choice in the UK as well as in the EU and USA.

There has been evidence of environmental concentrations having effects on aquatic and terrestrial organisms. For example, Tennekes et al (2010) reported effects of imidacloprid on invertebrate-dependent bird species [157]. The concern around the effects on non-target organism, especially bees, has resulted in some EU countries introducing a partial ban on the use of neonicotinoids. Counties where bans or partial bans are in place are France, Germany, Italy and Slovenia.

In terms of data in the literature, imidacloprid is the most widely studied neonicotinoids; it has also been the most extensively used [158]. There are environmental data available as well as toxicity data. There are limited data available for the other neonicotinoids.

Following a review by Morrissey et al (2015), it was determined that *Daphnia magna*, the common test species, is actually extremely tolerant to neonicotinoids. Thus, using standard toxicity tests would miss the actual true toxicity of this class of chemicals [159] (this will be discussed further later in the thesis).

Toxic effects have been reported at or below 1 µg/L (acute) and 0.1 µg/L (chronic). This review suggests that ecological thresholds need to be at <0.2 µg/L (acute) and 0.035 µg/L (chronic) to be protective of aquatic organisms. It has been reported that 81% of global surface waters exceed the 0.2 µg/L threshold and 74% of global surface waters exceed the 0.035 µg/L threshold [159].

There is still a lack of environmental monitoring data for neoctinoids in surface waters. This is changing as analytical techniques improve and limits of detection are lowered.

4.3.4. Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. They are characterized by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. They are also semi-volatile, enabling them to move long distances in the atmosphere before deposition occurs. The study of DDT and its properties helped coin the term persistent organic pollutant. POPs are of concern due to their ability to resist degradation, to be transported over long distances, to bioaccumulate and exert toxic effects [160]. The effects of POPs include neurological, immunological, reproductive and genotoxic effects in biota and humans, and based on these many have been banned or restricted in use [161]. The Stockholm Convention on POPs was an international environmental treaty with the aim to eliminate or restrict the production and use of specific POPs. Originally 12 POPs were listed, but this number has since increased to 22. Chemicals included on this list are PCBs, DDT, PFOS, Lindane and TBT. The synthesis of chemicals with persistent and bioaccumulative properties is something that industry is moving away from due to their effects in the environment and their effects on biota and humans.

Even with the bans, restrictions and reduction in synthesis POPs are still found in fish and their persistence has allowed for their global transportation.

Considered in this study under the POPs category are Benzo [a] pyrene (B[a]P), decabromodiphenyl ether (BDE 209), dichlorobenzene (DCB), dichlorodiphenyldichloroethylene (DDE), dibutlytin (DBT), fluoranthene, hexachlorobutadiene (HCBD), lindane, polychlorinated biphenyls 52, 153, 180, 194 (PCB 52, 153, 180, 194), perfluorooctanesulfonate (PFOS), trichlorobenzene (TCB), trichloromethane (TCM).

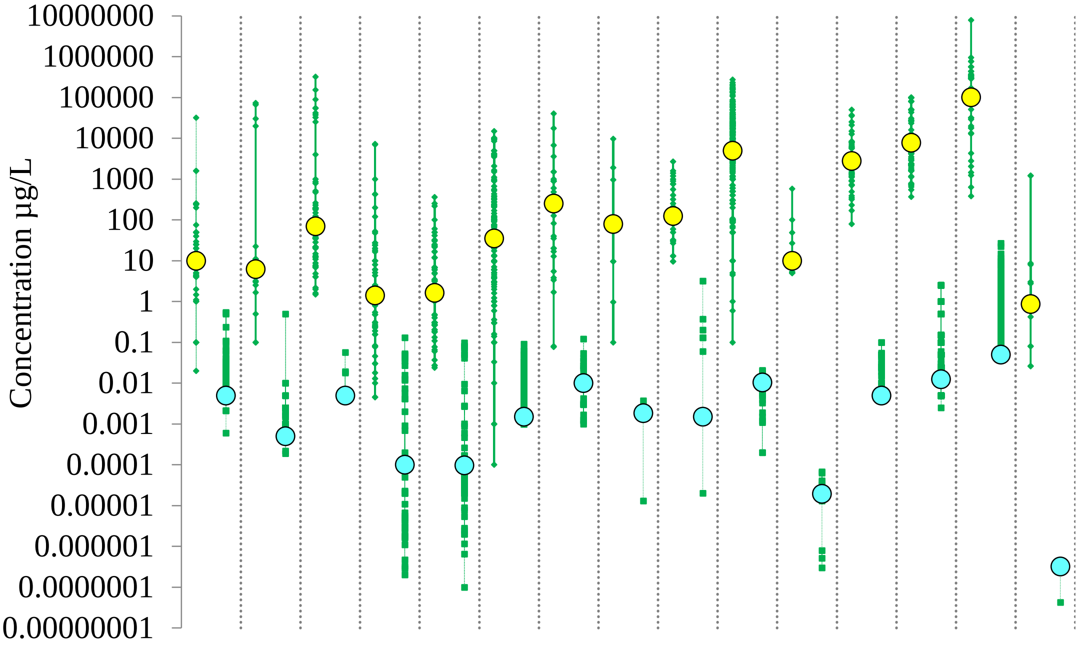
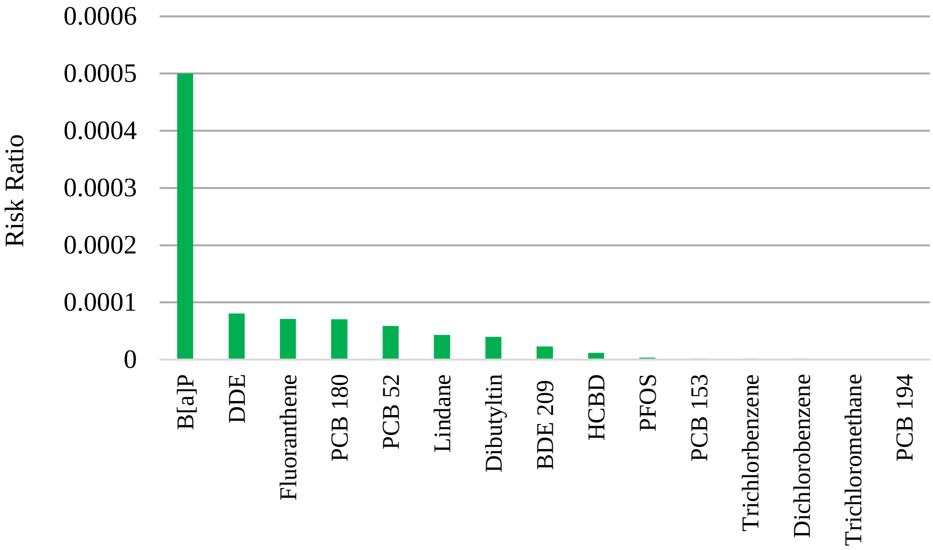


Figure 4-14 Underpinning data of 15 POPs, ranked based on the difference between the median effect (left hand vertical line of each pair: diamonds) and river water concentrations (squares). The median values are plotted as yellow (effect) and blue (environmental) circles. From left to right – B[a]P, DDE, fluoranthene, PCB 180, PCB 52, lindane, DBT, BDE 209, HCBD, PFOS, PCB 153, trichlorobenzene, dichlorobenzene, trichloromethane, PCB 194)

When the data for all the POPs were compared, it can be seen that some degree of overlap between environmental concentrations and concentrations that cause effects on aquatic organisms occurs for 6 of the POPs; PCB 52, PCB 180, DBT, DDE, lindane and B[a]P (Figure 4-14), with 9 of the POPs having no overlap in environmental and ecotoxicity data, based on the data collated for this study. It should be noted that some of the measured effect concentrations reported for POPs are above the solubility limit reported for the individual chemicals, thus in 'water' these chemicals would not be dissolved at these reported concentrations. But via the use of solvents, ecotoxicity tests can be conducted and thus effects are reported above the solubility limit. See Table 10-3 for solubility limits for the 15 POPs considered here.

4.3.4.1. *Comparison of median environmental concentrations and effect concentrations*



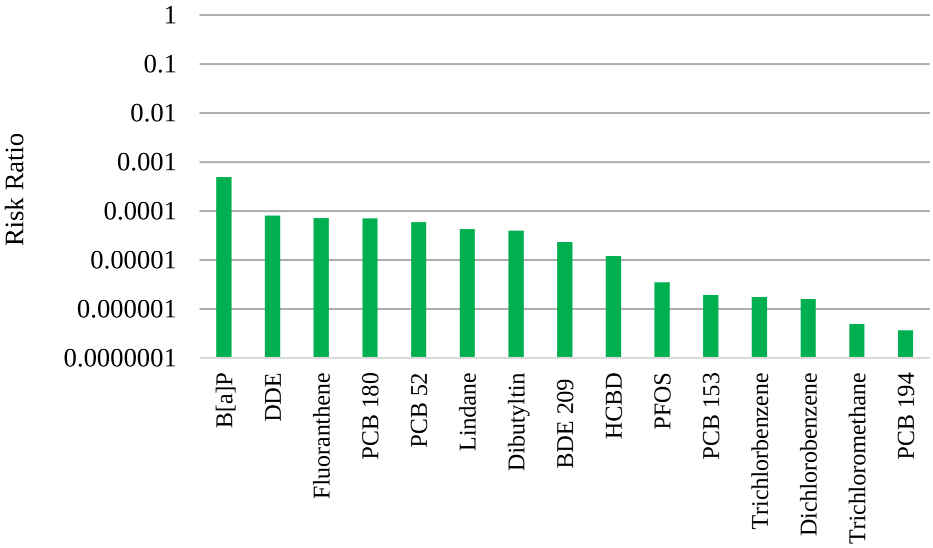


Figure 4-15 Risk ranking of 15 POPs based on the difference between the median effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

When ranking the POPs by comparing the median river water and median effect concentrations, B[a]P, DDE and fluoranthene are the POPs which emerge as the greatest risk (Figure 4-15). The difference between the two median values for the POPs ranges from 1,000-fold for the POPs of greatest concern, to upwards of 10,000-fold for the POPs of less concern such as, trichloromethane and PCB 194.

There is a fold difference > 1,000 between risk ratio of the highest ranking POP, namely B[a]P, and the lowest ranking POP, PCB 194. The risk ratios calculated for the POPs based on the median ecotoxicity and the median environmental data are all below 0.001.

4.3.4.2. *Comparing the median environmental concentration and 5th percentile effect concentration of each chemical*

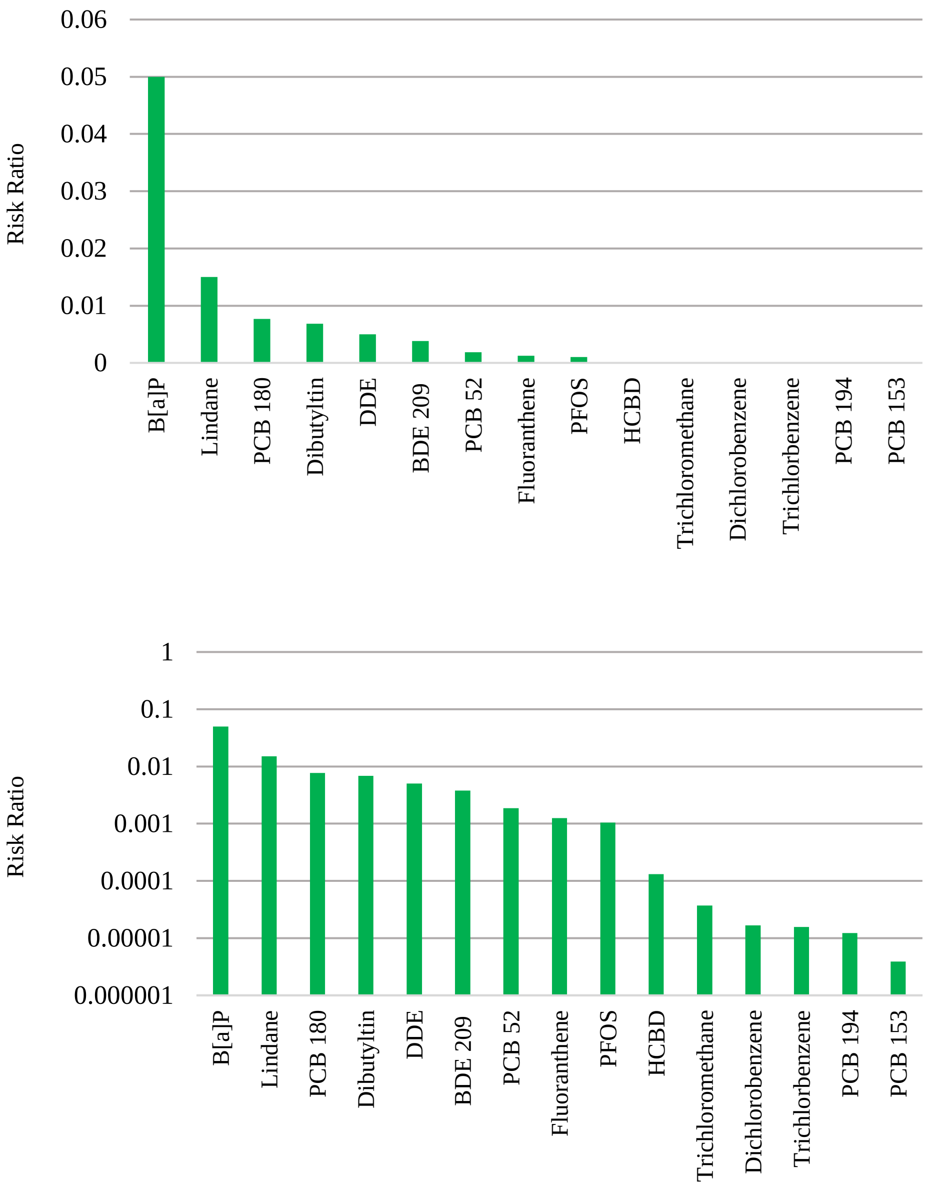
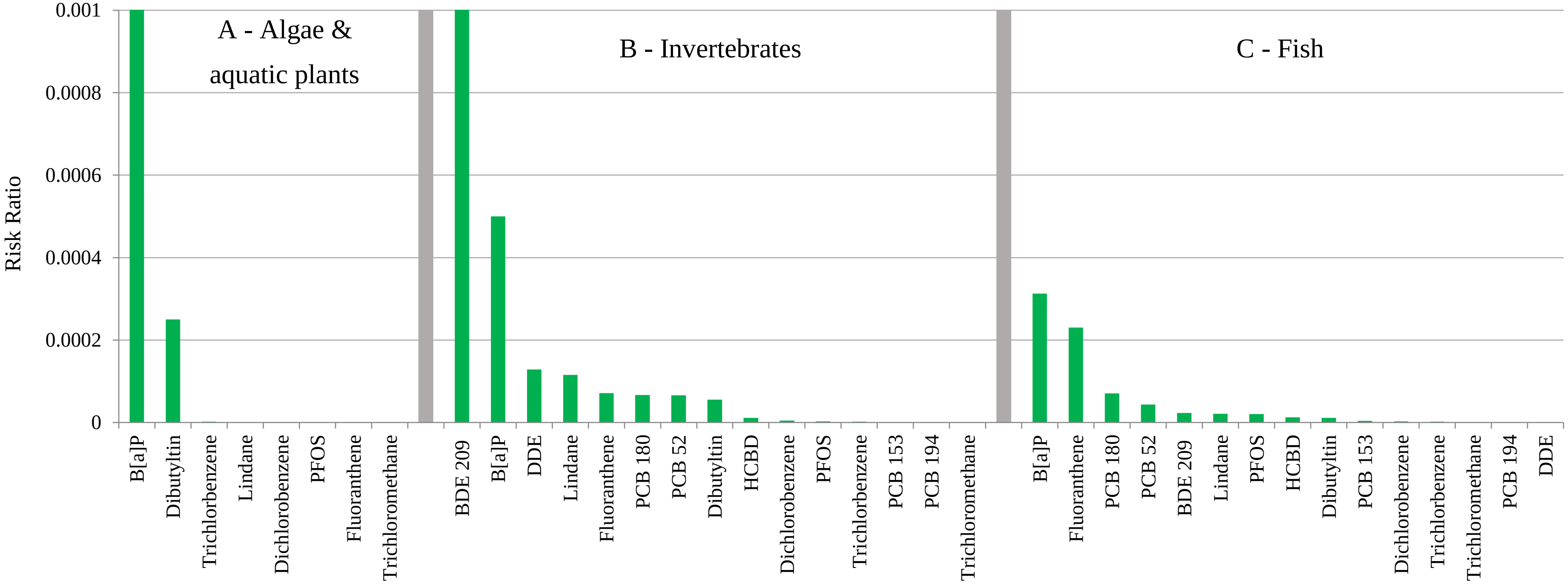


Figure 4-16 Risk ranking of 15 POPs based on the difference between the median effect concentration and the 5th %ile river water concentration (presented both as non log-scale and log-scale).

4.3.4.3. *Risk ranking of POP's based on different species*

When the effect data are split into the data for fish, invertebrates and algae & aquatic plants, the chemical of greatest concern to fish and algae & aquatic plants is B[a]P, while BDE 209 is of greatest concern to invertebrates (Figure 4-17).


Risk Ratio
0.001
0.0008
0.0006
0.0004
0.0002
0
A - Algae & aquatic plants
B - Invertebrates
C - Fish
B[a]P
Dibutyltin
Trichlorbenzene
Lindane
Dichlorobenzene
PFOS
Fluoranthene
Trichloromethane
BDE 209
B[a]P
DDE
Lindane
Fluoranthene
PCB 180
PCB 52
Dibutyltin
HCBD
Dichlorobenzene
PFOS
Trichlorbenzene
PCB 153
PCB 194
Trichloromethane
B[a]P
Fluoranthene
PCB 180
PCB 52
BDE 209
Lindane
PFOS
HCBD
Dibutyltin
PCB 153
Dichlorobenzene
Trichlorbenzene
Trichloromethane
PCB 194
DDE

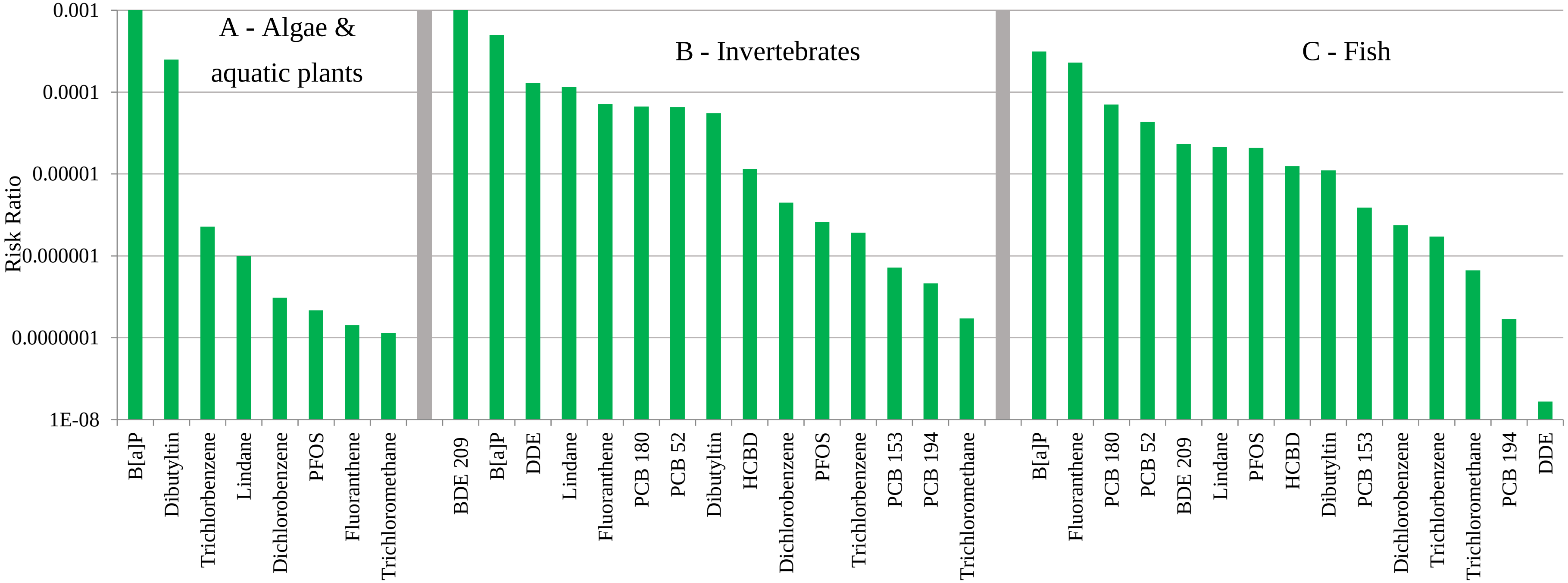


Figure 4-17 Ranking of 15 POPs based on their reported effects on algae & aquatic plants (A), invertebrates (B), and fish (C). Rankings are based on a risk ratio comparing the median effect concentration and median river water concentration (presented both as non log-scale and log-scale).

4.3.4.4. *Benzo [a] pyrene*

Benzo[a] pyrene is the highest ranking POP. It is a 5-ring representative of polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous in the environment and can be found close to their sources in populated and industrialised areas, but also in very remote places such as the Arctic [162]. The release of PAH's (including B[a]P) into the environment is controlled via the UK Pollution, Prevention and Control (PPC) Regulations. In the aquatic environment, they are regulated under the European Water Framework Directive, having been identified as "priority hazardous substances". The regulations in place to address the concern with regards air pollution i.e. from industrial plants include the European Community's fourth Air Quality Daughter Directive (2005/107/EC), which specifies a target value of 1 ng m-3 for the annual mean concentration of B[a]P as a representative PAH, to be achieved by 2012, and the UK Air Quality Objective for PAHs, based on the recommendations of the Expert Panel on Air Quality Standards (EPAQS), is for a maximum annual air concentration of 0.25 ngm-3 B[a]P. At an international level the Convention on Long-Range Transboundary Air Pollution of the United Nations Economic Commission for Europe (UN ECE) and the UN ECE POPs protocol have highlighted them as a concern because of their toxicity and suspected carcinogenicity and mutagenicity. In the marine environment, the Helsinki and OSPAR Conventions have regulations in place which protect the marine environments of the Baltic Sea and north-east Atlantic Ocean respectively. PAHs present in the environment are mostly unintentional by-products formed during incomplete combustion, even at temperatures > 400 °C, that occur during domestic heating, traffic-related fuel combustion, electrical power generation, waste incineration, intentional and accidental biomass burning, etc, but also during the production of tar, asphalt and coke [163]. Apart from this pyrogenic pathway, PAHs were also formed petrogenically, i.e. slowly, over long periods under moderate temperatures (100 – 300 °C) and can be found in fossil fuels [163]. PAHs also form during natural fires and are produced biogenically. Low concentrations of PAHs have always existed in the environment, but levels increased considerably from the middle of the 19th century onwards, when industrialisation and hence the demand for energy increased considerably [164]. The toxicity of PAHs varies and generally increases with the number of aromatic rings and consequently with molecular weight [165]. Therefore, the most volatile and most water-soluble low molecular weight PAHs that are typically the most abundant ones in the vapour phase of the air and in the dissolved phase of surface waters are less toxic than high-molecular PAHs such as B[a]P that tend to bind to particulate matter in both air and water due to their low volatility and water-solubility [166].

Based on the above it can therefore be assumed that B[a]P is fairly widespread in the UK, even if there are only a few studies reporting it in freshwater or freshwater organisms. Moreover, when B[a]P is found, other PAHs, some of them equally toxic, may be present as well, potentially causing a higher risk to sensitive organisms than B[a]P concentrations on their own would suggest.

4.3.4.5. *Lindane*

Lindane (also known as gamma-hexachlorocyclohexane, or γ-HCH) is the second highest ranking POP considered based on the precautionary approach (Figure 4-12). Consumption of lindane declined strongly during the 1980s [167] and since 2009 the agricultural use of lindane is banned internationally under the Stockholm Convention on Persistent Organic Pollutants. However, some other uses are still legal in several European countries, including wood preservation, insect control in public and private areas, and medicinal uses for the control of ectoparasites on humans and animals. Lindane is a relatively volatile persistent organic pollutant and therefore has the potential to transfer from treated fields into the atmosphere. Once transferred to the atmosphere, it can be transported in the air over large distances, making it a ubiquitous chemical that can be found even in very remote environments far from its sources.

Due to its widespread agricultural use until the recent past (an estimated 287,160 tonnes in Europe, and unknown amounts of stored and deposited HCH waste in UK but hundreds of thousands of tons in France, Germany and Spain [168] and relatively high persistence [14] lindane is present ubiquitously in the environment, including freshwater systems. It has been found both in the water body and in sediments [169] and also in freshwater biota. Measurements in the U.K. mainly focus on the River Humber and River Thames and their tributaries. However, given that lindane has also been found in soils and in the air of other regions of the U.K. (e.g.[170]), it can be assumed that it is present in other river systems as well.

Insect larvae were among the organisms most sensitive to lindane. This is not surprising given that lindane is used as an insecticide. Chronic effects have been observed in caddisfly larvae at concentrations as low as 0.1 ng/L [171] and acute effects at 1 ng/L [172]. Some fishes can also be very sensitive to lindane (e.g.[173, 174]). The toxicity data available in the literature does suggest that most of the species tested are not those most sensitive to lindane, and despite the large number of studies, conducted very little data are available on the larvae of generally more sensitive insects such as stoneflies, caddisflies or mayflies. Lindane is one of the European Water Framework Directive priority substances and the EU Directive 2008/105/EC provides environmental quality standards for freshwater. Annual average concentrations (AA) should not exceed 20 ng/L and maximum annual concentrations (MAC) need to be below 40 ng/L.

Although not in use in the UK anymore, lindane still evaporating from past applications both within and outside the UK may pose some risk to the most sensitive freshwater species as the concentrations found to affect them adversely are below some of the concentrations measured in water samples. However, there is not much recent (after-ban) monitoring data available and toxicity data on the species most sensitive to lindane are scarce. Unfortunately, measures to quickly reduce the amount of lindane still present in different environmental media are fairly limited now.

4.3.5. Surfactants and others

This class of chemicals includes surfactants and other individual chemicals which make up the final class of chemical to be considered. Surfactants (surface – active agents) are chemicals which are best known for their use in detergents and cleaning products. They are extremely soluble in water, and are used in vast quantities in both industry and domestic use. There has been concern over the potential risk from surfactants in the aquatic environment due to the sheer quantity (and their tremendous exploitation) in which they are used. Over 1.2 million tons of anionic surfactants were produced in Europe in 2006 [175]. They can and have been measured in high concentrations in the environment [27]. There is current concern over the use of surfactants and their ecofriendliness [176]. The anionic surfactants are the most common surfactants, and hence linear alkylbenzene sulphonic acid (LAS), alcohol ethoxysulphate (AES) and alkyl sulphate (AS) are included in this study [27].

The other chemicals included in this group are bisphenol A (an intermediate in plastic production), benzotriazole (a corrosion inhibitor), nonylphenol (intermediate), octylphenol (intermediate), triclosan (an anti-microbial), DEHP (a plasticizer) and sucralose (an artificial sweetener). In the literature these chemicals are classed as contaminants of emerging concern based on their use, persistence or toxicity [53, 177-179].

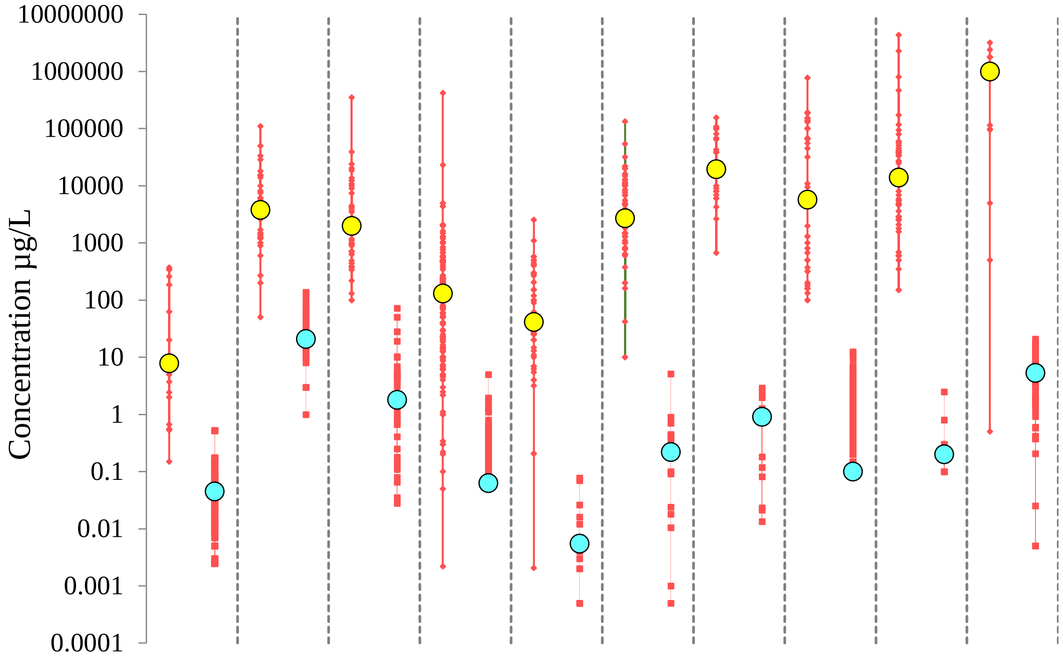


Figure 4-18 Underpinning data of 10 surfactants and other chemicals, ranked based on the difference between the median effect (left hand vertical line of each pair: diamonds) and river water concentrations (squares). The median values are plotted as yellow (effect) and blue (environmental) circles. (From left to right - triclosan, LAS, AES, nonylphenol, octylphenol, bisphenol A benzotriazole, DEHP, alkysulphate and sucralose).

When the data for all the surfactants and other chemicals within this class were compared, it can be seen that some degree of overlap between environmental concentrations and concentrations that cause effects on aquatic organisms occurs for 5 of the chemicals, namely triclosan, LAS, nonylphenol, octylphenol and sucralose (Figure 4-18), with 5 of the chemicals having no overlap between environmental and ecotoxicity concentration data.

4.3.5.1. *Comparison of median environmental concentrations and effect concentrations*

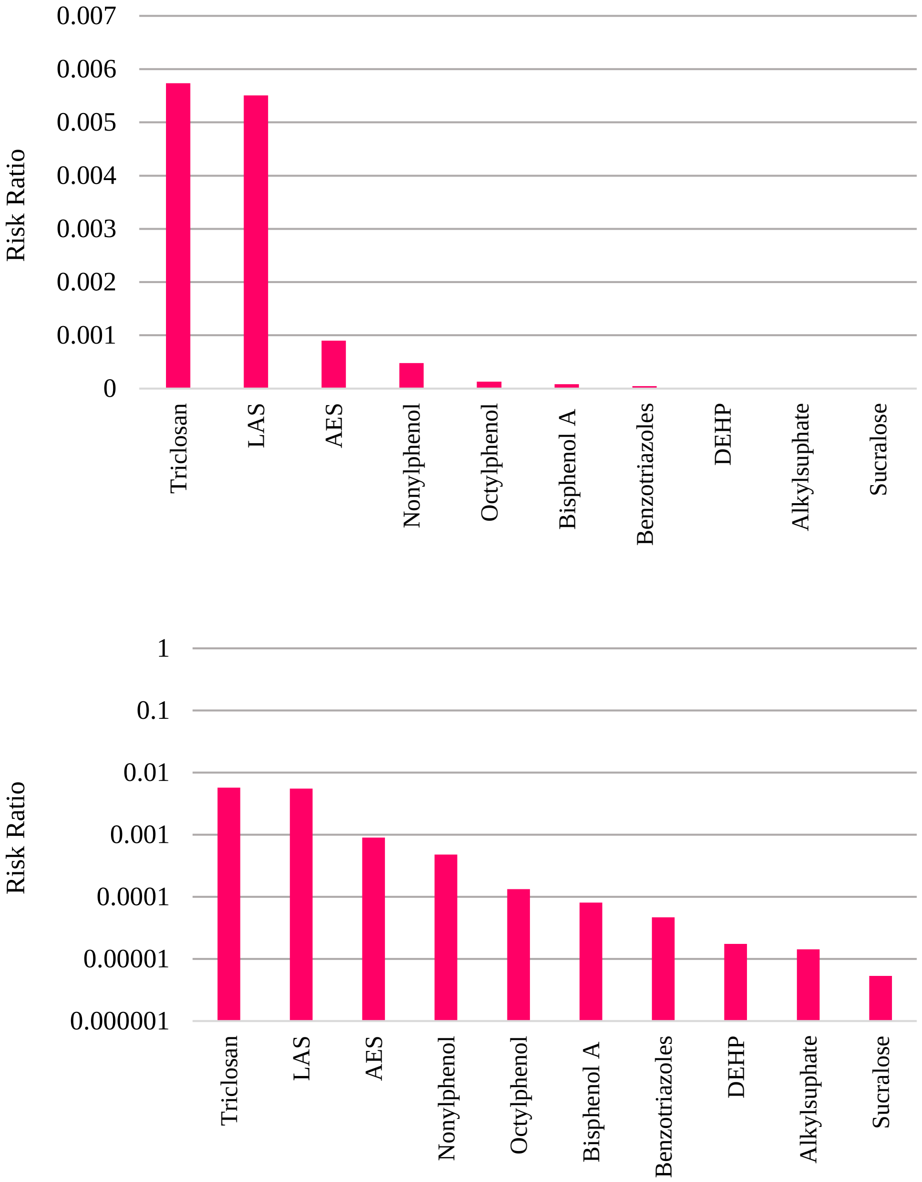
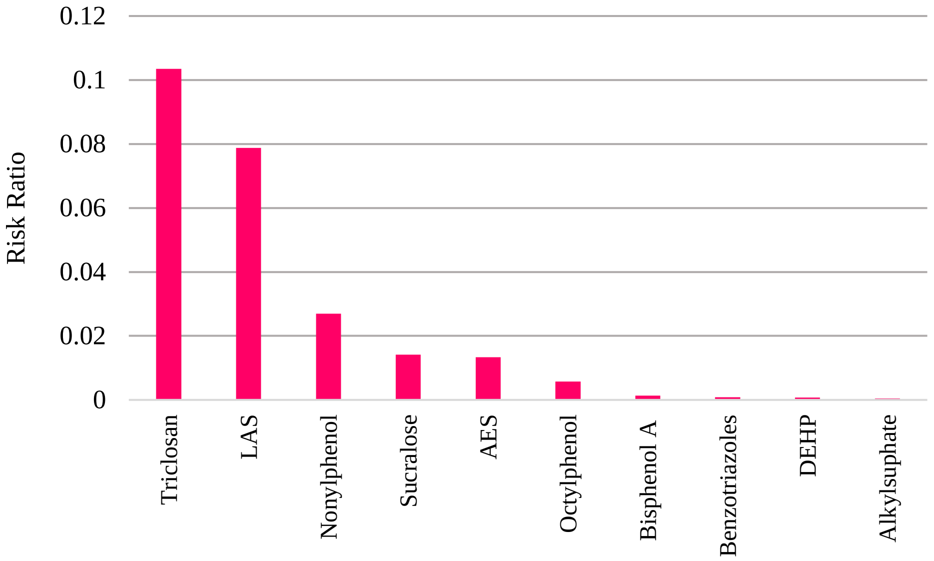


Figure 4-19 Risk ranking of 10 surfactants and other chemicals, based on the difference between the median effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

From the ten chemicals studied within the surfactant and others class, triclosan ranks highest, followed by LAS and AES (Figure 4-19). DEHP, AS, and sucralose rank at the lower end of the class. There is a 1,000-fold difference between the highest ranked chemical, triclosan, and the lowest ranked chemical, sucralose within this class.

4.3.5.2. *Comparing the median environmental concentration and 5th percentile effect concentration of each chemical*

When ranked by 5th percentile triclosan, LAS, nonylphenol and sucralose are the chemicals of greatest concern (Figure 4-20).



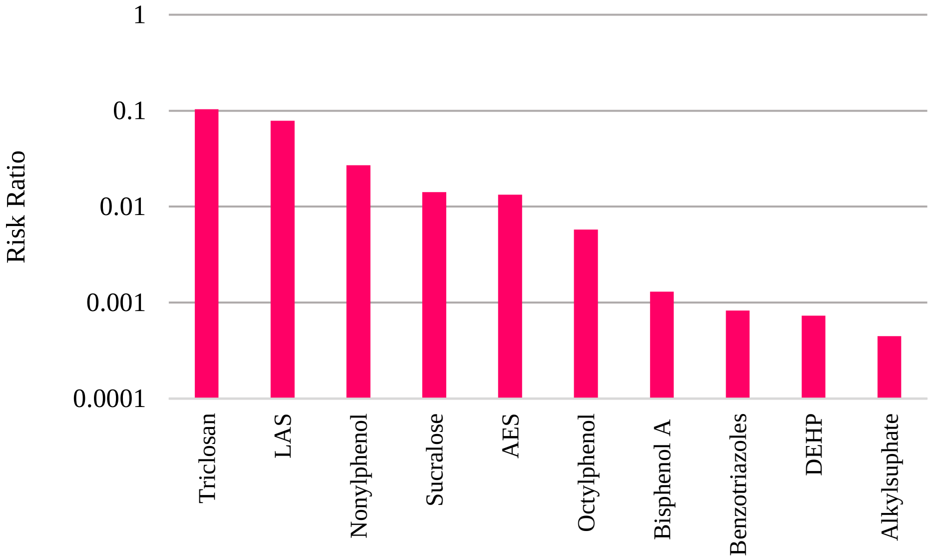
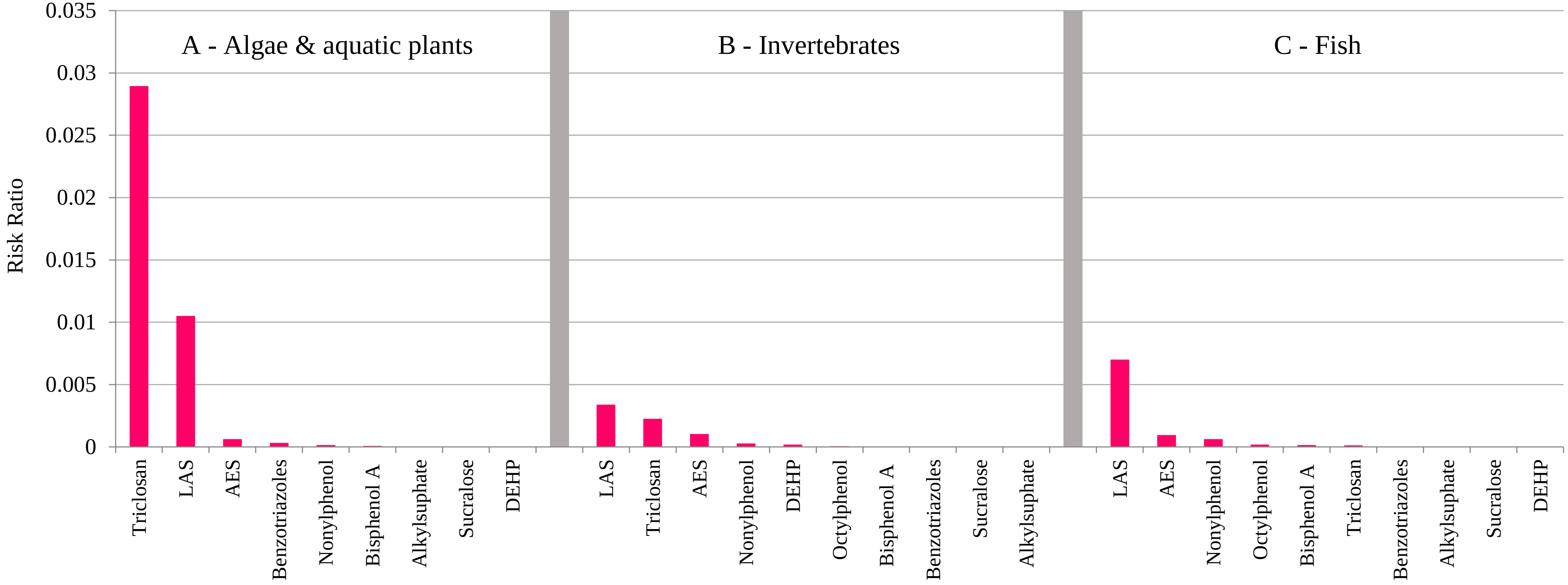


Figure 4-20 Risk ranking of 10 surfactants and other chemicals, based on the difference between the 5th %ile effect concentration and the median river water concentration (presented both as non log-scale and log-scale).

4.3.5.3. *Risk-ranking of surfactants and other chemicals based on different species*



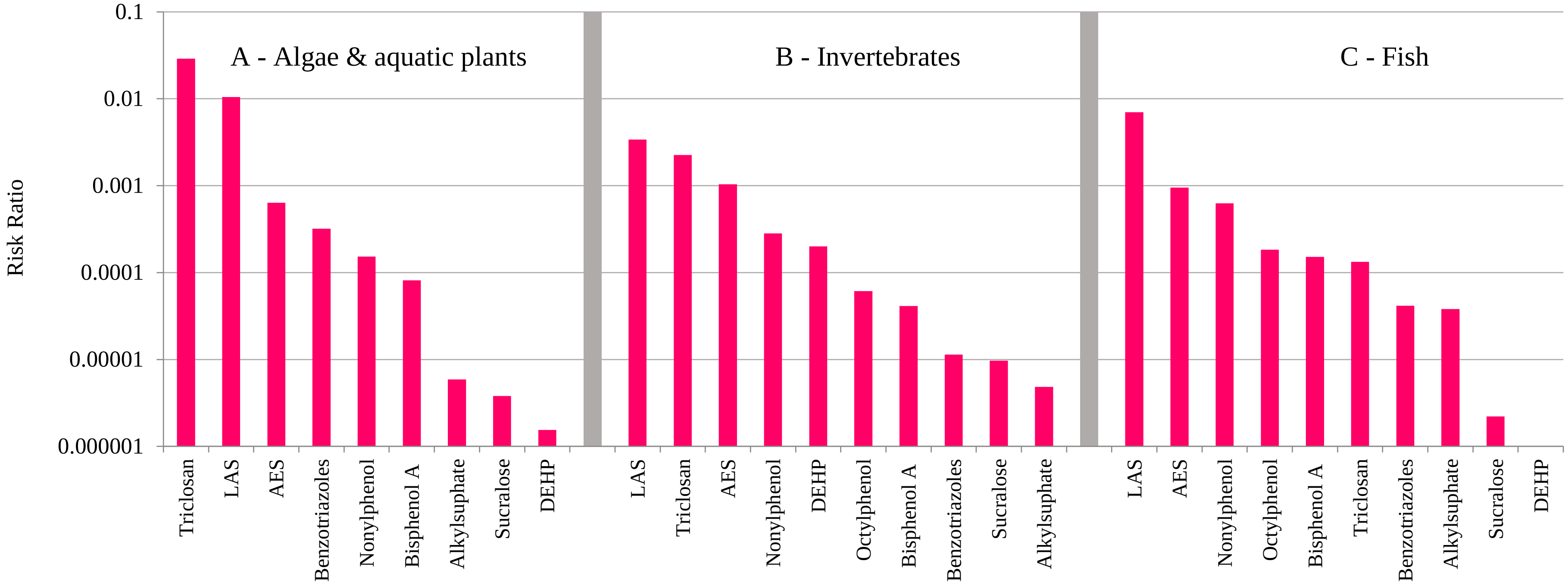


Figure 4-21 Ranking of 10 surfactants and other chemicals based on their reported effects on algae & aquatic plants (A), invertebrates (B), and fish (C). Rankings are based on a risk ratio comparing the median effect concentration and median river water concentration (presented both as non log-scale and log-scale).

4.3.5.4. *Triclosan*

Triclosan is an antimicrobial agent used in personal care products [180]. It is used extensively worldwide on a daily basis in products such as hand soaps, toothpastes and deodorants [178]. Triclosan enters the aquatic environment via waste water treatment plants. With its incomplete removal in sewage treatment [181] it is almost certain to be ubiquitous in rivers receiving sewage effluent. Water concentrations, including at locations immediately downstream of sewage works, are around 10-150 ng/L. Algae have been found to be sensitive to triclosan, with inhibition of growth of reproduction beginning around 500 ng/L, but the effect has been shown to be reversible once the compound is removed from the solution [182]. Similar observations have been made with freshwater biofilms composed of bacteria and diatoms, which by day 16 had largely recovered following a 1-day exposure to 60 µg/L triclosan [183]. The community structure of natural mixed algae populations was reported to change with exposure to 150 ng/L triclosan, with some species declining, others remaining stable, whilst others increased, but overall the biomass remained the same [184]. As algae are a fundamental part of the food web in rivers, their sensitivity warrants particular attention.

Due to concern surrounding its toxicity to aquatic organisms, as well as the concentrations at which it is found in the aquatic environment, triclosan now comes under the WFD as a specific pollutant. An average annual EQS of 0.1 µg/L (100 ng/L) has been established [93].

4.3.5.5. *LAS*

Linear alkylbenzene sulfonate is one member of the anionic surfactant group. LAS is considered to be the world's largest volume synthetic surfactant, with over 4 million tons produced in 2008 [185]. The LAS group were promoted by the detergents industry in the 1960s following the public outcry over foaming sewage effluents and rivers. The previous major surfactant, the alkyl benzene sulphonates (ABS), was not readily biodegradable, whereas the LAS group was [185]. Examination of removal performance in sewage treatment shows generally high removal efficiencies typically 95 to 99% [186], even including the poorer performing trickling filter plants [187].

A wide range of products use LAS and it seems to be one of the more important surfactants in current use in Europe. European river water concentrations are typically in the 10's of µg/L levels. Their discharge into rivers in sewage effluent will be continuous. With a river half-life of 0.5 d, the problem of concentrations accumulating downstream with increasing sewage input is not so important. Having significant economic importance, a creditable amount of research has been carried out by industry on its risk assessment.

Given the ability of surfactants such as LAS to disrupt lipid membranes of wildlife, it is not surprising that a very wide range of organisms have shown sensitivity to this chemical. However, for the vast majority of wildlife, concentrations need to exceed 1 mg/L to have any effect. There are some examples of limited effects at 50-270 µg/L, a review of the toxicity data has suggested a PNEC of 140 µg/L for LAS [188]. Following a risk assessment by HERA, an aquatic PNEC of 270 µg/L was derived and reported [26]. The HERA report provides additional PNECs for other environmental compartments: terrestrial PNEC value (35 mg/kgdw soil), sludge PNEC value (49 g/kgdw sludge), sediment PNEC value (23.8 mg/kgdw sed.) and STP PNEC (5.5 mg/l) [26].

4.3.5.6. *Sucralose*

Sucralose is an artificial sweetener produced from the chlorination of sucrose [189]; its trade name is Splenda. Sucralose is currently used worldwide. It was discovered in 1976, but wasn't approved for use as an artificial sweetener in Europe until 2004. It is used as an ingredient in the food industry (i.e. in fizzy drinks) as well as being an independent product, which can be added to food and drinks as a replacement for sugar (i.e. in tea and coffee). It is used as a sugar alternative due to its non-calorific nature, (i.e. for dieters), as well as being safe for consumption by diabetics. Sucralose is now considered to be ubiquitous in the aquatic environment [190]. Its main source into the aquatic environment is via sewage effluent. When consumed by humans, sucralose passes through the body mainly unchanged. It is a stable compound that is not altered by biological processes in the body [191]. Thus it enters STPs via the influent, where under traditional and advanced sewage treatment processes it remains stable and only a limited amount is removed from the influent. Thus the effluent which enters into the aquatic environment has relatively high concentrations of sucralose. Activated carbon is the only process which is able to eliminate a significant proportion of sucralose from the influent, although it is still not 100% removed [177, 192].

In the UK aquatic environment there is no mainstream monitoring scheme for sucralose, but measured values of sucralose have been recorded in the UK as part of a Europe-wide study looking at polar organic chemicals [190, 193]. The concentrations found in the UK range from 0.005-20.8 µg/L, with a median concentration of 5.3 µg/L. There are limited ecotoxicity data available for sucralose. The lowest reported effects have found that sucralose does effect the feeding and behavioural effects of invertebrates [194]. Concentrations of 0.5 µg/L reportedly affect the body length of Nitocra spinipes, while 500 µg/L affects the ability of Gammarus sp to find food and 5000 µg/L affects Gammarus sp respiration. Wiklund et al (2012) suggested that further studies are needed to get a broader understanding of the effects of sucralose on aquatic organism. No mortality data have been reported based on the effects of sucralose on aquatic organisms [177].

From some of the standard ecotoxicity tests conducted using a range of aquatic organisms, D*aphnia magna* (inhibition), *Lemna minor* (growth) and the green algae

Scenedesmus vacuolatus (reproduction), concentrations > 1000 mg/L of sucralose had no effect on these organisms [195].

In general it has been considered that sucralose is not a concern as an environmental pollutant [194]. Concerns over the lack of ecotoxicity information, especially high quality data has been highlighted [196]. The reason it has been considered a concern by others is due to persistent qualities and its usage, and the ultimate consequences of this potential chronic exposure are not yet understood [197]. From the data collated for this study there is no overlap in the environmental and ecotoxicity concentration data, and hence sucralose unlikely to pose a serious risk to the aquatic environment.

4.3.5.7. *Bisphenol A*

Bisphenol A is a commercially important chemical and one of the highest volume chemicals produced worldwide. In 2003 global production of Bisphenol A was 3.2 million metric tons whereas, in 2011 it was estimated that consumption of Bisphenol A was predicted to exceed 5.5 million metric tons [198, 199]. It has the ability to weakly mimic the effects of natural estrogens, due to the similarity of phenol groups on both BPA and estradiol. Thus synthetic molecules can trigger estrogenic pathways in the body. Bisphenol A is classed as an endocrine disrupting compound [198]. However it was identified as very weakly estrogenic [200], thus the investigation into its pharmaceutical use was not continued as Bisphenol A was 10,000-fold weaker than estradiol [201]. It is used primarily as an intermediate in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastic is used in sheeting, glazing of electrical equipment and electronic goods.

Bisphenol A is usually found in surface waters at low concentrations [202]. The concentrations used in this review range from 0.0005-5.1 µg/L. It enters the aquatic environment via the production process and through wastewater effluent. Sources of bisphenol A into the environment include directly from chemical, plastics coats, from paper and material recycling companies. Indirectly it can enter the environment via leaching from plastics, paper and landfill sites. It is thought that bisphenol A is now ubiquitous in the environment [203]. The proximity to point and non-point sources can drastically alter the concentration of bisphenol A in the water [204]. However, numerous non-detects and also different levels of LOD makes it difficult to combine the results across Europe and get a robust understanding of the concentrations of Bisphenol A in Europe. The reported toxic effects of bisphenol A range between 10 µg/L and 134,000 µg/L. The effects reported included impacts on the growth, reproduction and development of aquatic organisms; fish appear to be the most sensitive organism to BPA [205-207].

4.4. CONCLUSIONS

Many chemicals are present in the aquatic environment. The occurrence of both existing and emerging chemicals globally is an area of research where there has been considerable amount of effort. With the vast diversity of chemicals potentially present in the environment, this is an area of research which is continuing to grow [208]. The prioritisation and investigation into the risk of chemicals to the environment, based on their use, toxicity and occurrence in the environment, is a growing challenge [209, 210].

The aim of this chapter was to investigate different classes of chemicals and establish, based on the data collated for this study, which chemical in each class is of greatest concern. The chemicals chosen for this study are representatives of their individual classes and the focus is on the effects of chemicals via the water column. The data used were obtained from the literature and publicly available databases. Whilst this may not be all the data available for many of the chemicals, it aims to give a good indication of the toxicity ranges for each chemical and the range of concentrations found in the environment (mainly focusing on the UK). From this analysis the following chemicals rank highest in each of the chemical classes based on the median environmental concentration and the median ecotoxicity concentration data (Table 4-2).

Table 4-2 Highest ranked chemicals in each chemical class studied

Chemical Class	**Highest ranked chemicals in each class**
Metals and nanoparticles	aluminium, copper, zinc
Pesticides	methomyl, chlorpyrifos, permethrin
Surfactants and others	triclosan, LAS, AES
POPs	B[a]P, DDE, fluoranthene
Pharmaceuticals	EE2, fluoxetine, propranolol

The chemicals which are highlighted through this analysis within each class are chemicals which have all been reported in the literature previously as being of concern to aquatic organisms [2, 63, 211-214]. This was not the final analysis of chemical risk; it was the first stage of utilising the vast amount of information we have available on chemicals to examine how chemicals could be ranked using a simple method based on current knowledge. Chapter 5 will continue looking at the same 73 chemicals using the same unmoderated datasets per chemical and put these separate classes of chemicals into context together. To understand, based on this approach which chemical out of the 73 is ranked highest? Where do the highest ranking pesticide sit in relation to the highest ranking metals, POPs, pharmaceuticals and surfactants?

5. TIER ONE APPROACH TO RISK-RANKING ALL CHEMICALS

5.1. INTRODUCTION

This chapter compares all 73 chemicals with each other, using the same methods as described previously for chemicals of the same class. Based on the median environmental and median ecotoxicity results, the chemicals of greatest concern for each individual group of chemicals are detailed in Table 5-1. This chapter puts the chemicals into perspective in terms of the relative risk they pose in freshwater ecosystems in relation to each other, regardless of their group. Are all these chemicals of equal concern?

Table 5-1 Highest ranked chemicals in each class

Chemical Class	Highest ranked chemicals in each class in order
Metals and nanoparticles	aluminium, copper, zinc
Pesticides	methomyl, chlorpyrifos, permethrin
Surfactants and others	triclosan, LAS, AES
POPs	B[a]P, DDE, fluoranthene
Pharmaceuticals	EE2, fluoxetine, propranolol

5.2. BRIEF METHODS

The 73 chemicals included in this study were risk-ranked based on methods detailed in section 1.5 of Chapter 3; the risk- ranking methods are briefly detailed here, but refer to Chapter 3 for full details.

Firstly, the data underpinning the risk ranking is presented in a graph to highlight the raw data used for the analysis, to visualise the two datasets for each chemical as well as the data for a specific chemical in comparison to other chemicals.

The risk-ranking approaches used were:

- Median environmental vs median toxicity concentration.

This approach aims to rank chemicals on the typical environmental and ecotoxicity data. The median has been used to remove bias from outliers.

- Median environmental vs 5th percentile toxicity concentration.

This approach focuses on the lower ecotoxicity concentrations, which represents the more sensitive species and end-points, (potentially the less reliable data points).

- Species specific risk-ranking of chemicals

This approach focuses the ranking only on algae & aquatic plants, or invertebrates or fish (species split). This approach tries to determine which species group is considered the most sensitive to which chemical class.

At this stage no moderation to the data has been made; Chapter 6 (tier two) will bring in refinements and moderations to the data in order to understand if the chemicals of concern identified via the tier one method remain the chemicals of concern when a more sophisticated ranking is undertaken.

5.3. RESULTS AND DISCUSSION

The chemicals were ranked and the results discussed based on the risk they present to aquatic wildlife in the UK. Table 10-6, Table 10-7 and Table 10-9 summarise the ranking order and risk ratios obtained from all chemical classes. The tier one data and results are discussed based on ranking via the median values, the 5th percentile and the three defined species categories. Further discussion of the chemicals that rank highly based on the different ranking methods are then detailed.

5.3.1. Underpinning data

As a visual overview of the range of data used in the risk-ranking, Figure 5-1 demonstrates the underpinning data. The chemicals are aligned based on the difference between the median values. When the data for all 73 chemicals were compared, it can be seen that some degree of overlap between environmental concentrations and concentrations that cause effects on aquatic organisms occurs for 39 of the chemicals (Figure 5-1), with the other 34 chemicals having no overlap in environmental and ecotoxicity data (Table 10-5). However, it can be seen that chemicals with no overlap between their two datasets can be present within the higher ranked chemicals, due to the median being the driver behind the ranking – this will be discussed later in the chapter.

There is large variation between the amount of data available in the literature for each chemical (Figure 3-2, Figure 3-3), with some chemicals having an abundance of literature available and others having minimal data, either because they are of new concern or the focus has been elsewhere. The same applies to the environmental data. For some chemicals there is an abundance of environmental data, often obtained using well-established methods in national monitoring programmes, but for other chemicals there is little available environmental data, which could be due to a lack of interest/funding to measure and monitor them regularly, or due to the necessity to develop complex (often expensive) techniques in order to measure them [36].

For some chemicals, even though there is no overlap in the data, for environmental risk purposes a safety factor would be applied, to ensure the regulations in place are fully protective. For example, the effect data may not be based on the most sensitive end-point; or species other than those included in the analysis may be more sensitive [215, 216]. It is difficult to know if this application of a safety factor is taking an over cautious approach. The same argument applies to the environmental data – are the highest concentrations potential common/widespread concentrations, or are they one-off extreme concentrations, reached only in an isolated location? The time of sampling can affect the concentration of a chemical in the water; seasonal variations (i.e. rainfall), can alter the flow of a river and therefore the dilution of the chemical in the water [90]. At the other end of the scale, the concentration of a chemical in the water maybe below the detection limits of the working method and hence that chemical would not be detected even though it was present. At this stage, and using the tier one method, no data were removed based on any concern that they were anomalies or unusual results, as that would bring bias into the analysis. Tier one only reports the data as they were collected from the literature. This isn’t to say there isn’t any bias in this collection method, but the method was developed with the intention to minimise this as much as possible.

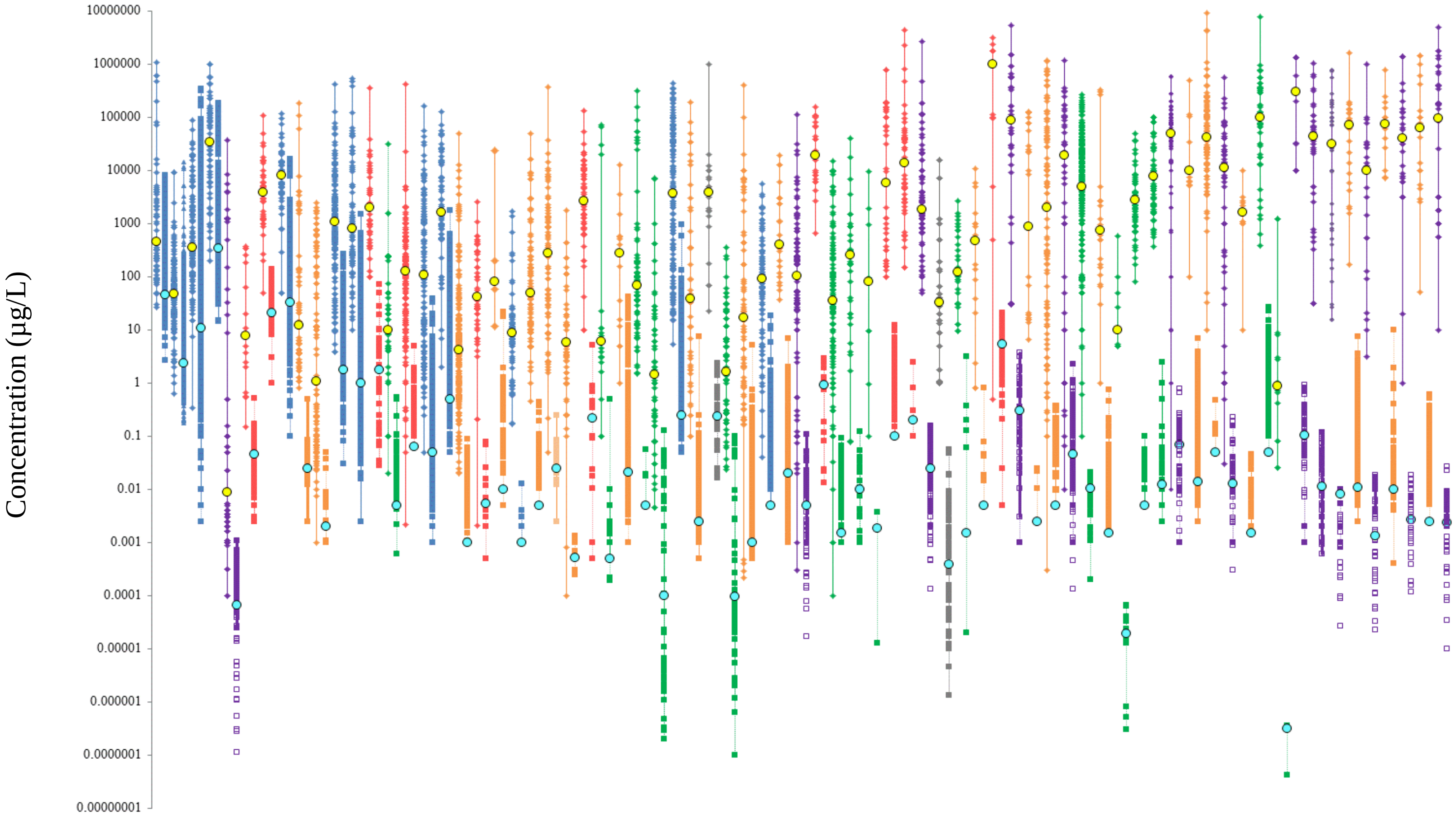


Figure 5-1 Data underpinning the risk-ranking for each of the 73 chemicals ranked based on the difference between the median effect (left hand vertical line of each pair: diamonds) and river water concentrations (squares). The median values are plotted as yellow (effect) and blue (environmental) circles. (See Appendix Section 10.1.2 for risk ranking tables)

5.3.2. Median risk-ranking

The initial risk-ranking approach is based on the proximity of the median environmental concentration and the median effect concentration (Figure 5-2). It takes the typical value for both datasets for each chemical to gain an understanding of the threat each chemical poses to freshwater organisms, and provides a means of comparison between chemicals. When the risk ratio was calculated using the median values, none of the chemicals had a risk ratio estimate that exceeded 1; a risk ratio of ≥1 would highlight a major concern to the aquatic organisms. There is a difference of >100,000-fold between the highest ranked chemical and the lowest ranked chemical based on this approach.

Aluminium, copper and zinc are the only chemicals with a risk ratio greater than 0.01, all other chemicals have risk ratios less than 0.001 (Figure 5-2). These three metals were highlighted as the three highest ranked metals of their class (Table 5-1).

The top ten chemicals of concern that were reviewed, based on this unbiased tier one approach, include metals, an antimicrobial, a surfactant, a pharmaceutical, and pesticides. It can be seen that the metals dominate the higher ranked chemicals: aluminium is the chemical ranked as greatest concern, followed by copper, zinc and iron. EE2, Triclosan, LAS, methomyl and chlorpyrifos were the highest ranked organics. The highest ranked POP is B[a]P, which is ranked 14th out of the 73 chemicals. The highest ranked nanoparticle is nano ZnO, which is ranked 32nd of the 73 chemicals. The surfactants and others are widely distributed across the 73, with triclosan ranking highly, followed by LAS. Sucralose is the lowest ranked surfactant/other (49th). EE2 is the only pharmaceutical in the higher ranked chemicals, followed by fluoxetine which ranked 37th, all other pharmaceuticals are ranked lower. The highest ranked pesticide is methomyl which ranks 9th, followed by chlorpyrifos, which ranks 10th, with bentazone ranking 72nd.

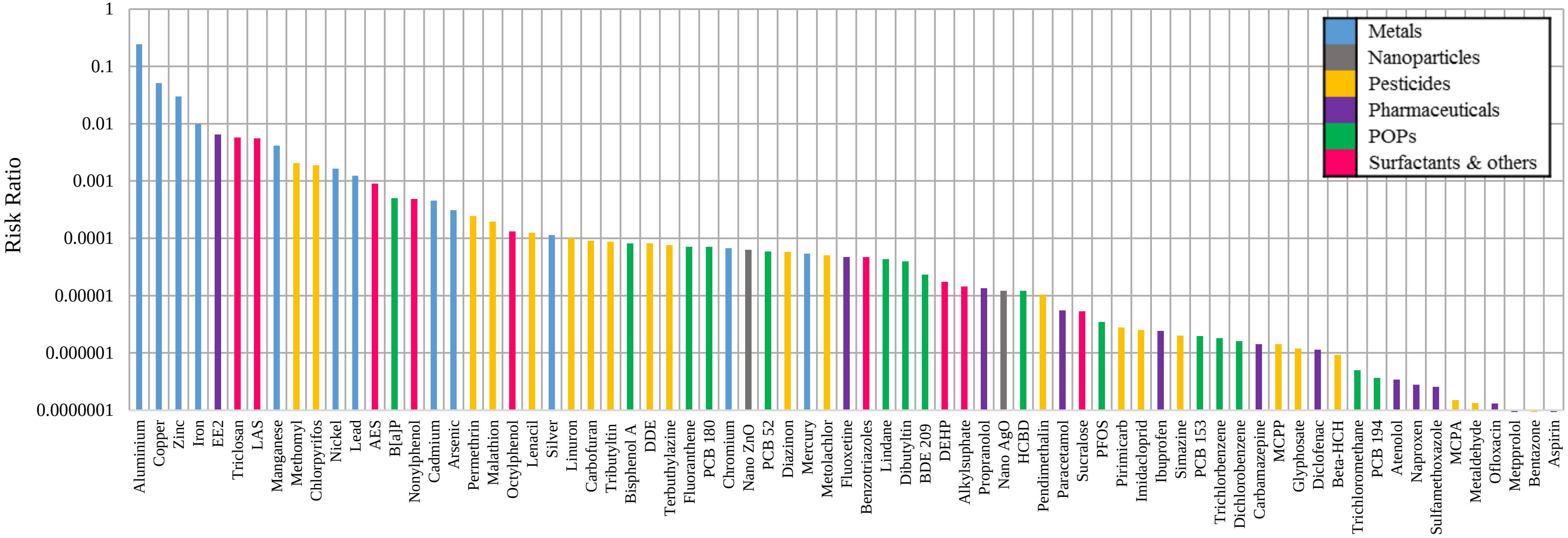


Figure 5-2 Risk-ranking of 73 chemicals based on the difference between the median effect concentration and the median river water concentration (no moderation was made to the data at this stage).

5.3.3. *Precautionary approach*

The more precautionary risk-ranking approach is based on the proximity of the median environmental concentration and the 5th percentile effect concentration (Figure 5-3). In effect, this approach places greater weight on the studies using organisms which have been shown to be particularly sensitive to that particular chemical. Where a great many studies have been carried out, such as with the metals and some pesticides, this approach could well be robust. However, where much less data are available, such as for many pharmaceuticals, there is a concern that this may not be a reliable comparator [119]. At this stage, no data has been removed from the analysis to limit the bias of data selection.

With this precautionary approach there is a difference of >100,000-fold between the highest ranked chemical and the lowest ranked chemical. Aluminium remains the chemical of greatest concern, followed by copper, iron, zinc, ibuprofen, nickel, triclosan, chlorpyrifos, LAS and EE2 (Figure 5-3). Thus, the top ten chemicals of concern are now made up of metals, pharmaceuticals, a surfactant, an antimicrobial and a pesticide. The top seven chemicals all have a risk ratio equal or greater than 0.1, although aluminium is the only chemical with a risk ratio greater than 1. The majority of chemicals have a risk ratio of < 0.01, even when using this precautionary approach.

Using this approach, the order of ranking has changed; for example, some of the pharmaceuticals have moved up the risk-ranking. Ibuprofen has now become the pharmaceutical of greatest concern. Ibuprofen is one of the few pharmaceuticals where the effect data overlaps with the environment data (Figure 5-1). This overlap occurs at concentrations that range between 0.01-0.3 µg/L. A greater understanding of the severity and impacts of these effects is needed to better understand if this is a real risk. EE2, fluoxetine and diclofenac are also within the top 25 of the 73 ranked chemicals.

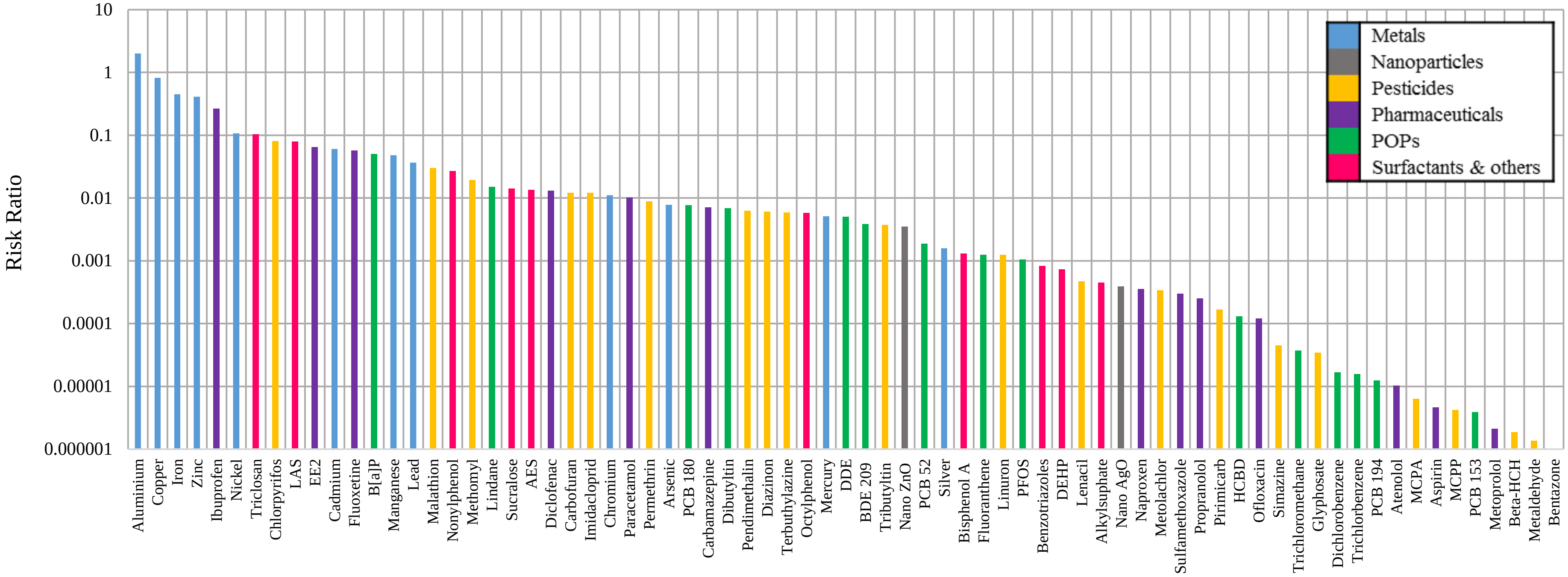


Figure 5-3 Risk-ranking of 73 chemicals based on the difference between the 5th%ile effect concentration and the median river water concentration (no moderation was made to the data at this stage).

5.3.4. Risk-ranking by species

As detailed in Chapter 3 and Chapter 4, the toxicity data were split into three categories: fish, invertebrates and algae & aquatic plants.

The data collated for all chemicals were not based on specific species or groups of species. This was decided in order to try and remove bias from the selection process, as well as take advantage of the array of research conduct on many different species. In the literature there is sometimes an overwhelming amount of data available on the effects of a chemical on aquatic organisms, but the tests employed can be based on a moderate number of species [217]. Common standard test species included are, *Pseudokirchneriella subcapitata* (Algae), *Desmodesmus subspicatus* (Algae), *Lemna gibba* (Duckweed sp), *Lemna minor* (Duckweed sp), *Daphnia magna* (Water flea), *Ceriodaphnia dubia* (Water flea), *Pimephales promelas* (Fathead minnow) and *Oncorhynchus mykis* (Rainbow trout), to name a few [218]. Research shows that these species are sensitive to many chemicals and hence tests done using these species can be used to be protective of other species. However, conflicting studies show that they aren't always the most sensitive species for all chemicals [77, 219].

The sensitivity of a species to individual chemicals can be considered in three ways. Firstly, there can be variation in sensitivity between different groups of species (i.e. fish, algae, invertebrates, etc). Secondly, there is variation in sensitivity between the species within a class (i.e. two different species of fish or two different species of invertebrate). Thirdly, there can be sensitivity variation at the different life stages of an organism. This breadth of variation can cause bias and misinterpretation in chemical risk analysis. What is the most sensitive "combination" of test species for one chemical can be completely different for another chemical. While standard tests have been designed to allow for robust comparison and a form of quality control, the method used here utilised all the data which were available in the literature to take advantage of the array of research which is conducted within the ecotoxicity field.

This approach is precautionary as it permits the inclusion of non-standard species and any end-points.

End-points can be split into various categories (i.e. lethal or sub-lethal and acute or chronic). At this stage of the analysis there was no consideration of what the end-point was. When comparing or categorising end-points there are discrepancies which can bring bias and subjectively into the analysis, this will be discussed in further detail in Chapter 6. The consideration of whether an end-point is reporting harm is also a further consideration. Just because a fish changes its swimming behaviours is this a major concern? Because a pharmaceutical is having an effect on a fish – for example curing a headache - is this a good or a bad thing? Is any effect enough to raise concern? Or is there a need to bring expert judgement into the decision making to determine which are harmful effects and which are ‘just’ an effect? Can an effect ever be a positive outcome?

It is also important to include an array of species and end-points in the analysis, as some of the major historical discoveries within the ecotoxicity field have been unexpected and not predictable (as detailed in Chapter 2). Therefore, it is important to consider a variation of species and end-points to try and include these unexpected results when conducting risk assessment.

The median ecotoxicity data for fish, invertebrates and algae & aquatic plants were compared with the median environmental concentration, to determine what chemical, out of the 73 considered, is of greatest concern to each category of organism. For some chemicals, there are no data for a particular species category, and therefore it has not been included in the ranking. The results for each species category are briefly discussed in sections 5.3.4.1 – 5.3.4.3. The chemicals that rank highly based on this ranking method are then discussed in further detail in sections 5.3.4.4 – 5.3.4.6.

5.3.4.1. *Fish*

When the risks posed by the 73 chemicals are compared based on the median toxicity data (fish only) and the median environmental data, aluminium is the chemical of greatest concern, followed by iron, zinc and copper. EE2 is the highest ranked pharmaceuticals (Figure 5-4). The top five chemicals have risk ratios >0.01 but only aluminium has a risk ratio >0.1. The majority of chemicals, based on the median data for fish, have a risk ratio <0.001. The fold difference between the highest and lowest ranked chemical based on the median effect data for fish and the median environmental data is > 1,000,000.

5.3.4.2. *Invertebrates*

When the risks posed by the 73 chemicals are compared based on the median toxicity data and the median environmental data, copper is the chemical of greatest concern, with a risk ratio of 0.06, followed by aluminium (0.04), BDE 209 (0.02), chlorpyrifos (0.01) and zinc (0.007) (Figure 5-5). The majority of chemicals have a risk ratio <0.001. Pharmaceuticals remain in the lower rankings, unlike the situation for fish. EE2 has now dropped to 66th based on the invertebrate data and fluoxetine has become the highest ranked pharmaceutical. The fold difference between the highest and lowest ranked chemicals based on the median effect data for invertebrates and the median environmental data is >100,000.

5.3.4.3. *Algae & aquatic plants*

When the risks posed by the 73 chemicals are compared based on median ecotoxicity data for algae & aquatic plants and the median environmental data, aluminium is the chemical of greatest concern, with a risk ratio of 0.4, followed by iron (0.06), copper (0.05) and zinc (0.03) (Figure 5-6). Using this analysis, nano ZnO (0.005) ranks within the top ten chemicals of concern, which is the first time in the tier one analysis that one of the two nanoparticles considered in this study have ranked in the top ten chemicals of concern. The majority of chemicals based on the median data for algae & aquatic plants have a risk ratio <0.001. The fold difference between the highest and lowest ranked chemicals based on the median effect data for algae & aquatic plants and the median environmental data is >10,000,000.

5.3.4.4. *Zinc*

It will be noted that zinc has ranked high based on all three species risk ratios, with a risk ratio of 0.04 (3rd) for fish, it also ranked 4th for algae & aquatic plants (0.03) and 5th (0.007) for invertebrates, indicating that zinc appears to be chemical of concern to all three species categories (Figure 5-4, Figure 5-5, Figure 5-6).

Zinc is a natural element and therefore freshwater organisms will be exposed to it naturally. Species exposed to naturally high concentrations of zinc are likely to be acclimatised to the higher levels. They will have evolved to adapt to these levels over a significant period of time. The use of zinc and the mining of zinc will have increased the concentrations of zinc in some locations, but it is also a common constituent in many manufacturing processes, industrial, domestics and agricultural products [17], organisms may not have the ability to evolve at the same rate as the increased concentrations, thus the level cause toxic effects.

A median effluent concentrations for zinc in the UK has been reported as 30.9 µg/L, and surface water concentrations for the UK collated for this study range from 0.7 – 6,900 µg/L. In the EU, zinc has been identified as a concern and an established EQS is available. The EU EQS of 8 µg/L, 50 µg/L, 75 µg/L and 125 µg/L is based on total metal concentration and dependant on hardness. Gardner et al (2012) established a BLM adjusted level of intertest for zinc of 17 µg/L [82].

Hansen et al (2002) looked at the toxicity of zinc to two fish species, to highlight the potential concern that the U.S. national water quality criteria for protection of aquatic life may not be protective of sensitive salmonids with regards exposure to zinc (Cd and Cu were also considered [109, 215]). Mebane et al (2012) reported salmonids to be sensitive to zinc; cutthroat and rainbow trout were reported to be more sensitive to zinc than the invertebrates tested. That study reported that fish were a factor of 10 more sensitive to zinc than the invertebrates. Between the two fish species, rainbow trout were more sensitive than cutthroat trout [110], highlighting the variation in toxicity between closely-related species. Chapman et al (1978), looked at the sensitivity of two different fish species, at different life stages, and demonstrated the toxicity of zinc (Cu & Cd also) to fish and the variation in sensitivity across the life stages of juvenile fish [220].

With regards to effects on algae & aquatic plants, for which zinc is ranked fourth, it has been well documented that zinc at concentrations above those required for optimal growth can cause adverse effects on growth, photosynthesis and chlorophyll concentration [221] [222].

5.3.4.5. *Copper*

Copper is a natural element and therefore background levels are present in freshwater ecosystems. Because copper, as with some other metals, is an essential metal, aquatic organisms have developed strategies for regulating internal copper (metal) concentrations. This has allowed organisms to evolve and adapted to changing concentrations, thus adapting their tolerance level to a metal. For example, when copper concentrations have increased over an extended period of time the organisms have had the opportunity to acclimatise to increased concentrations, and thus evolve to be less sensitive [223]. Copper toxicity to aquatic organisms is primarily due to the ionic Cu^{2+} [17]. The use of copper in industry has increased the concentrations of copper in the aquatic environment. Previously mining was the main anthropogenic source of elevated concentrations of copper in the environment. However, its use for copper pipes and electrical equipment in particular has increased its production globally. Copper ranks as the chemical of greatest concern to aquatic invertebrates based on the data collated for this study. The risk ratio calculated for copper based on median environmental concentration and the median concentration for ecotoxicity data for invertebrates is 0.06. It ranks 4th for fish (0.04) and 3rd for algae & aquatic plants (0.05).

The toxicity of copper to freshwater organisms is well documented in the literature. Studies have looked at the effect of copper on single species, as well as multiple species, under various experimental conditions. Mastin et al (2000) looked at the effect of copper-based herbicides and copper sulphate on freshwater organisms. *Daphnia magna* was reported as the most sensitive organism to copper sulphate followed by *Pimephales promelas* [224]. Maund et al (1992) reported that copper significantly affected the population density and the age composition of *Gammarus pulex* populations, resulting in decreased numbers of juveniles and adults. These sub-lethal effects occurred at levels lower than the reported lethal effect concentrations [225]. Real et al (2003) looked at the effect of copper on a whole food chain; they found copper to affect both the periphyton community and the herbivore *Stagnicola vulnerata*, with the snail being more sensitive to copper than the algae [226]. Species sensitivity distributions (SSD) have been completed and reviewed for copper and demonstrated that invertebrates are the most sensitive species, which our results echo [221] [227]. Based on LC50 or EC50 data a HC5 of 0.009 mg Cu/L (9 µg/L) was reported by Adam et al (2015). This value suggests that at least 95% of the species are protected at this concentration. This value compares to the 5th percentile effect values calculated for this study which range from 2.5 - 6.3 µg/L depending on the effect data used - all species, only algae, only invertebrates or only fish.

5.3.4.6. *Nano ZnO*

Nano ZnO ranks within the top ten chemicals of concern to algae & aquatic plants based on the median effect vs median environmental concentrations, with a risk ratio of 0.005. It ranks 25th and 27th for invertebrates and fish, with risk ratios of 0.0002 and 4.8×10^{-5}, respectively. Nano ZnO is used in paint formulations, sun-screen creams, hair care products, food additives (as an essential nutrient) and toothpastes [228].

Mudd et al (2017) provide a detailed discussion on the world's zinc (and lead) mineral resources. Detailing the increase of the world's annual production of zinc from since 1840 to 2012, with zinc production now estimated to be greater than 13 million t/year [229]. Globally the production of nano ZnO has been estimated at 528t/year, with the global production of all ZnO being in excess of 1.2 million t/year [230]. Therefore, nano ZnO production in context to the total global ZnO production is only 0.04%, and in context to the estimated annual global production of all zinc is only 0.004%.

Via its use in industrial and household products, nano ZnO can enter the aquatic environment in waste water and potentially have harmful effects. Solubility plays a key role in the environmental fate, behaviour, and effects of chemicals, nanoparticles included. Typically, the dissolved fraction of a chemical is considered to be responsible for any biological effects. Nanoparticles vary from insoluble to poorly soluble, partly soluble, or completely soluble. Nano Ag, ZnO and CuO are relatively soluble/partly soluble entities that release ions, which can cause toxicity [231]. The reason the solubility potential is important is because nanoparticles with limited solubility, represent a solid phase with a confined physical shape similar to that of poorly soluble chemical. The dissolution of soluble nanoparticles can describe a large number of the observed effects in fish, crustaceans, and algae [31]. The size of the nanoparticle is reported to affect the toxicity of nano ZnO to algae & aquatic plants [228]. It is considered that there is still a lack of information with regards the effects of nanoparticles in the aquatic environment and their impact of aquatic organisms [221]. Research on the risks of nanoparticles and their unique chemical and physical characteristics is still in the development phase. Therefore, it is difficult for scientists, regulators and industry to know how to manage the potential risks from nanoparticles and the potential threat to the aquatic environment. In most cases nanomaterials are far less toxic than the inherent toxicity of the dissolved metal [231].

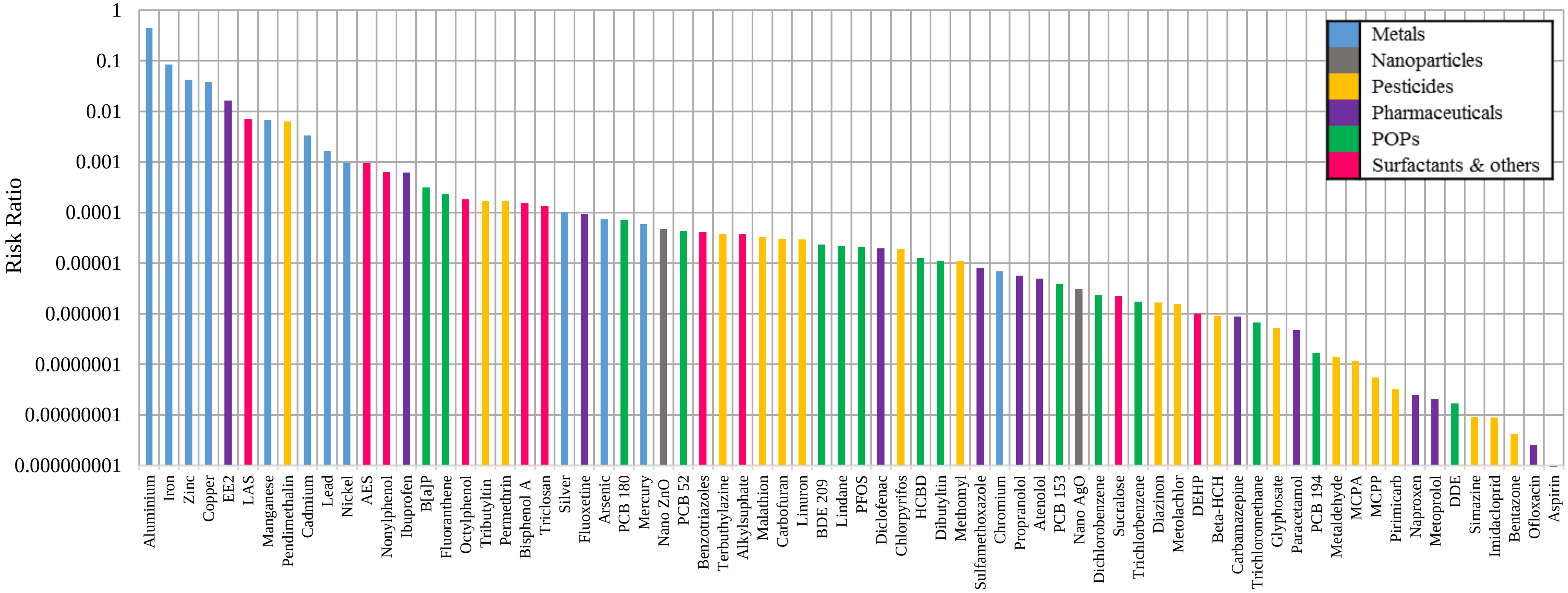


Figure 5-4 Risk-ranking of chemicals based on the difference between the median effect concentration for fish and the median river water concentration.

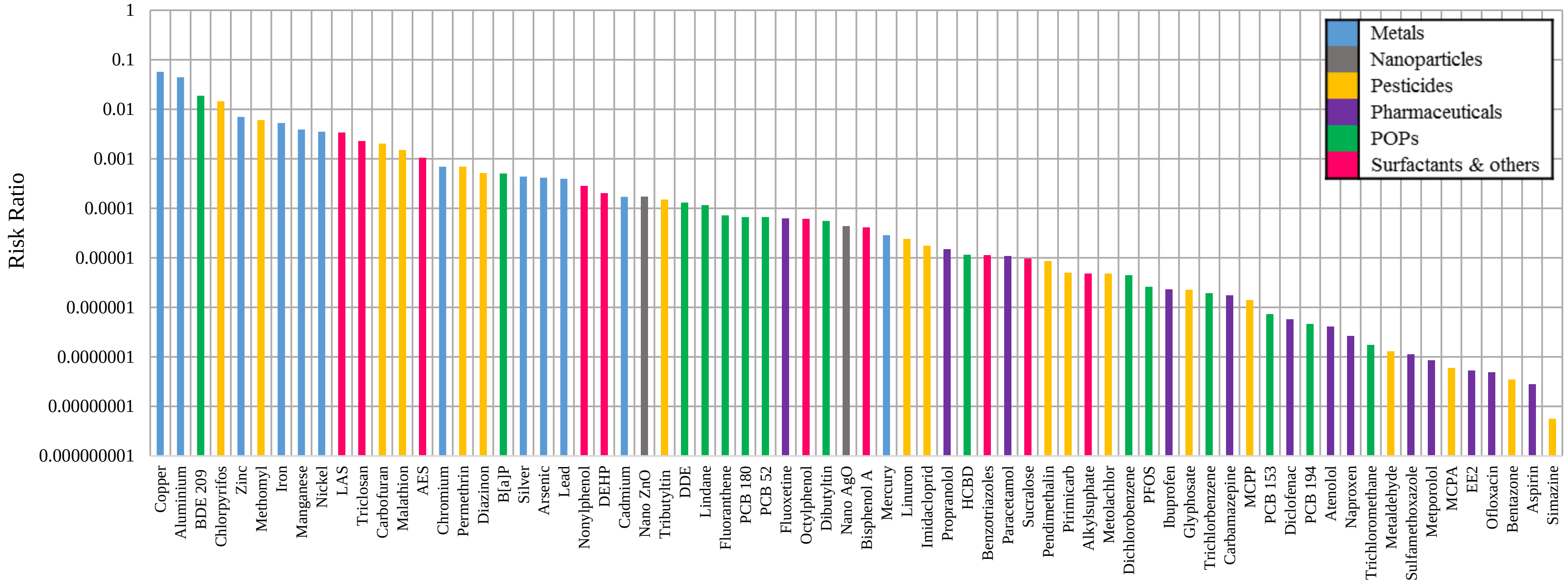


Figure 5-5 Risk-ranking of chemicals based on the difference between the median effect concentration for invertebrates and the median river water concentration.

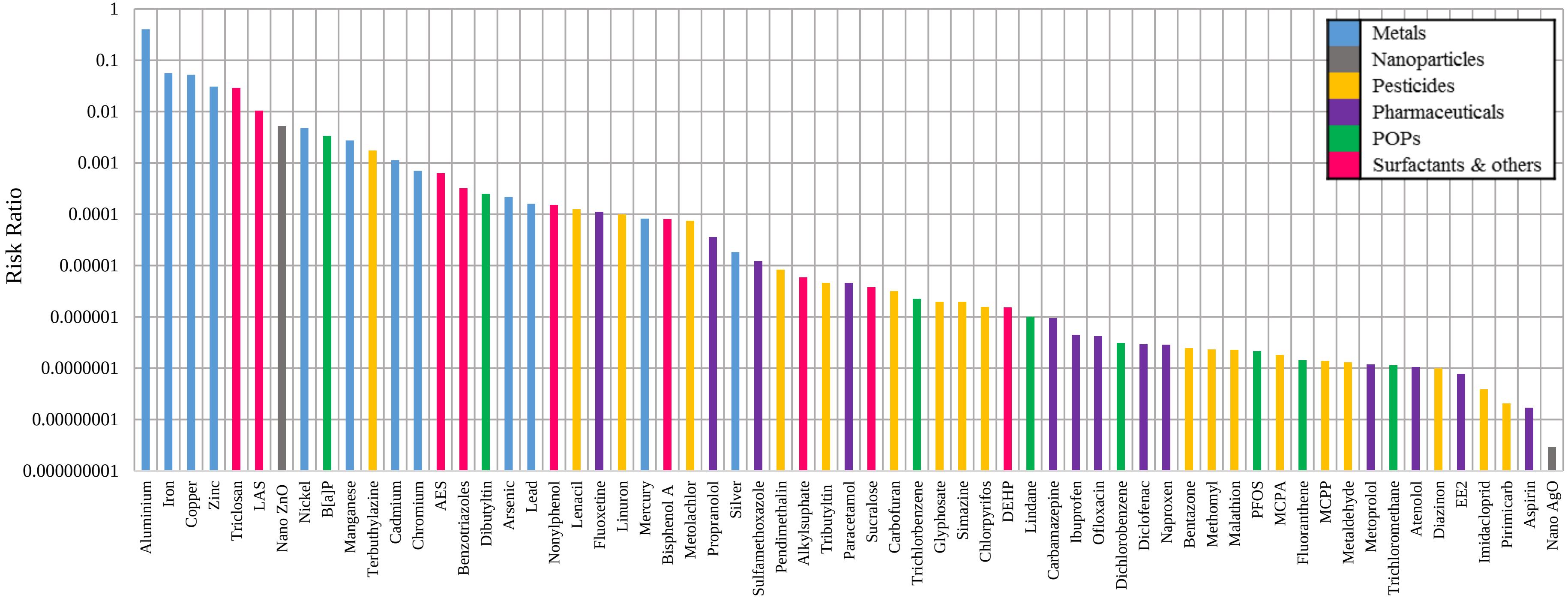


Figure 5-6 Risk-ranking of chemicals based on the difference between the median effect concentration for algae & aquatic plants and the median river water concentration.

5.4. CONCLUSIONS FROM TIER ONE APPROACH

As the number of chemicals used by humans increases it will be important to make sure that monitoring and control of them is appropriate. So that it is possible to understand which chemical is of greatest concern it is important to make use of all the information which is available for ecotoxicity and environmental monitoring.

Chapters 4 and 5 present the results and discussion of the tier one approach. Both provide an uncensored assessment of the environmental and ecotoxicity data for 73 chemicals. The aim was to highlight the chemicals of concern within each class, to understand if, when using this simple method, the results were in line with current opinion. Based on these results, the relative risk between the chemicals studied here using the tier one approach can be radically different (i.e. >100,000 fold). From this initial analysis, it could be stated that the results indicate that metals and pesticides are of concern, with pharmaceuticals being, potentially, of less concern. The POPs remain in the lower ranking, perhaps due to their more hydrophobic properties. The accumulation of chemicals in organisms, which is particularly important when considering the risks of POPs, will be considered in Chapter 6 as a refinement and moderation to the risk assessment.

The approach developed is a very simple and resourceful approach. Although it does not provide a definitive analysis, the aim was to compare chemicals with each other, using an unbiased approach. It did not involve scoring methods and is not as detailed/time consuming as a risk assessment. The method does not attempt to be a full risk assessment but instead tries to identify the relative risk of chemicals.

The final conclusion for the tier one approach will be discussed in Chapter 8, where the risk-ranking results will be compared with the results from the tier two analysis and that of other ranking and prioritisation approaches available in the literature.

6. TIER TWO APPROACH TO RISK-RANKING CHEMICALS

6.1. INTRODUCTION

The question being asked here is, “Would any moderating factors drastically change the results of the tier one based ranking?” What chemical is of main concern after further refinement of the risk-ranking process? If a more sophisticated analysis is completed, is a different result generated?

It is important to understand that there are a range of filters, or moderating factors, which could be applied to both the ecotoxicity data and the environmental data, some of which were mentioned in Chapters 4 and 5. For two reasons not all these various options have been considered; firstly, the practicality of completing an analysis of every potential variable in the time frame of the project, and secondly, the more filtering (or adjustments) of the data, the greater the introduction of subjectivity into the analysis. The analysis at the tier two level was not trying to examine all the different ways to refine the data, or trying to provide a definitive analysis of each chemical, but instead by just looking at a few refinements. With the aim to assess the degree of impact these factors were having on the ranking. If they radically affected the ranking, then it would make it difficult to judge which chemical we should focus on, but if they don’t, then the simple approach of tier one provides a reasonably reliable message.

Refinements which could be included in the analysis include: only focus on chemicals where ecotoxicity data and environment measurements overlap and eliminate the rest, where a chemical has been detected in the environment recently in the UK; the ecotoxicity data can be filtered, thus examining only acute or chronic data, only looking at one phylogenetic group, only selecting chemicals which have a potential to bioconcentrate in aquatic organisms. The data itself could be scrutinised, with regards to the quality of the information, details of a dose-response relationship, the value used for comparison (i.e. median, mean or 5th percentile).

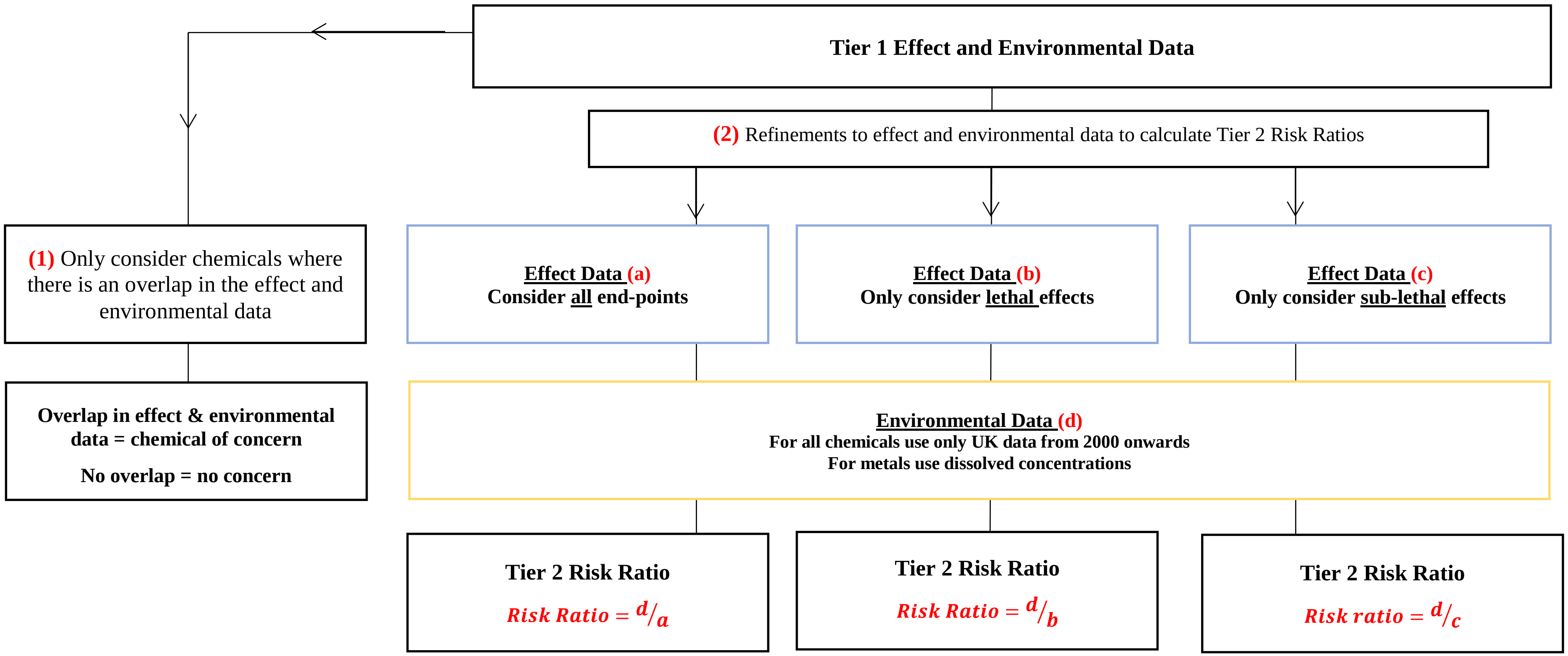


Figure 6-1 Schematic of part one of tier two: (1) is there an overlap in the effect and environment data for each chemical, (2) refinements made to the tier one data to calculate alternative risk ratios based on moderated effect data and environmental data.

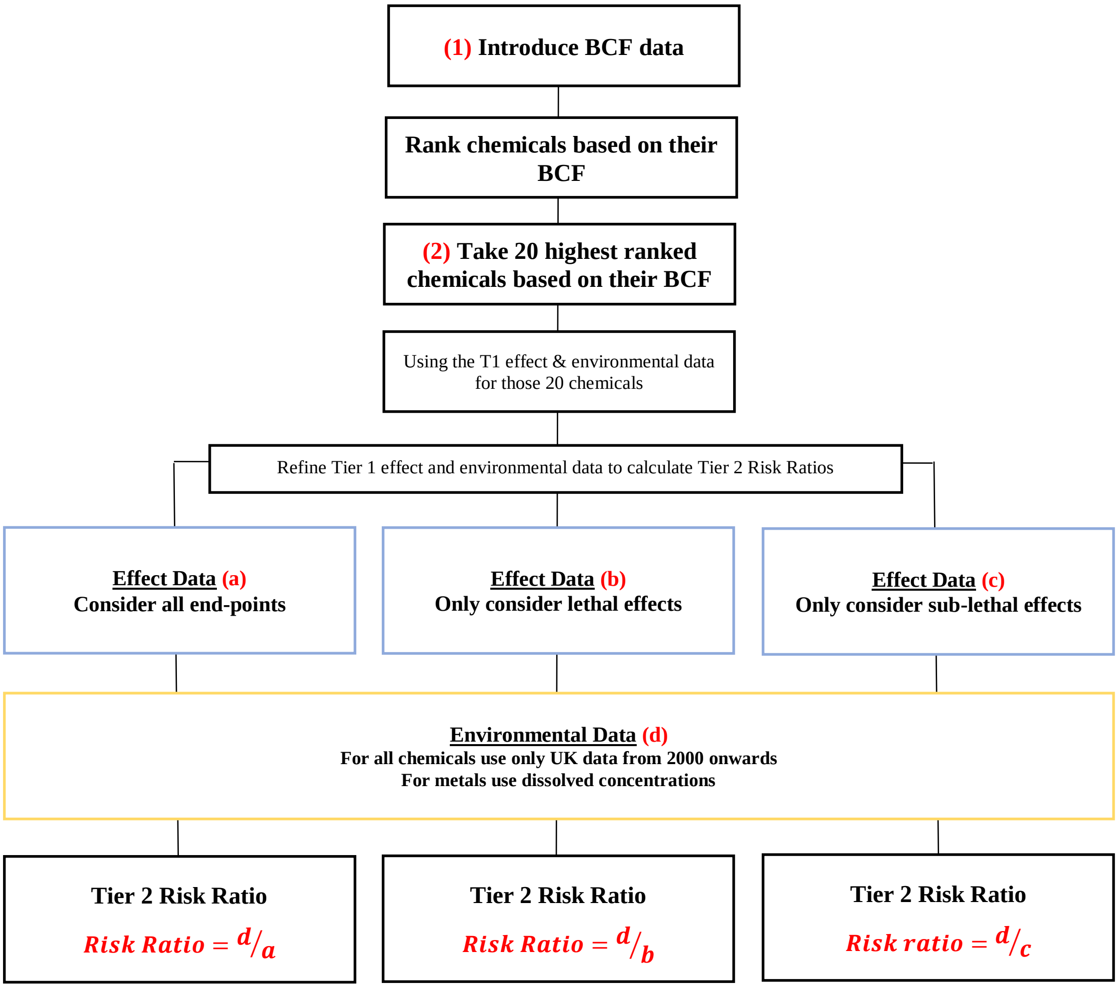


Figure 6-2 Schematic of part two of tier two: (1) the introduction of BCF data and a ranking based on BCF, (2) a risk-rankings based on BCF and toxicity (the risk-rankings are based on different refinements made to the tier one data)

6.2. METHODS

Brief description of methods (See Chapter 3 for details)

6.2.1. Is there an overlap in the toxicity and environmental data?

A first consideration which could be made with regards to the data, is whether or not there is an actual overlap in the ecotoxicity effect concentrations and environmental concentrations? Thus, a filter of the data would be to discard any chemical where there is no overlap in the two datasets, judging it to be of no concern. This is not a moderation of actual data, but instead a simple way of focusing only on those chemicals that appear to be of greatest concern.

6.2.2. Refinement of the data

The tier two analysis used the ecotoxicity data and environmental datasets and applied various moderating factors to generate new risk-rankings based on a sub-set of data (Figure 6-1,

Figure 6-2, Table 6-1). The refinements/moderations made to the data are not all the possible refinements, but a small selection of them (Table 6-1). These refinements were used to filter the two datasets for each chemical to generate new risk-rankings. The details of the refinement methods are discussed in the following section.

Table 6-1 The various approaches used to refine the data

Refinement	Detail
Ecotoxicity Data	For all chemicals consider either Lethal only & Sub-lethal only
Environment Data	For all chemicals consider: -UK only environmental data

	-Measured UK data from 2000 – present -Predicted modelled UK data was included for the pharmaceuticals and nanoparticles as these were considered to be ‘current’
Ecotoxicity & Environment Data	For metals only use dissolved metal concentrations rather than total concentrations For metals include only ecotox data from studies carried out at neutral pH (6.5-8.5)

6.2.2.1. *Refinement of effect data – use of either lethal or sub-lethal data only*

There is an argument that only acutely toxic chemicals need concern us. That in reality, wildlife can cope or adapt to sub-lethal effects. Mortality is an end-point where there is definite evidence of harm occurring to a percentage of the individuals being studied. Therefore, one way to refine the ecotoxicity data is to include only data which have reported mortality.

As ecotoxicity has become more sophisticated and scientific techniques have developed, the ability to measure sub-lethal effects has become possible. Sub-lethal effects include biochemical, physiological, reproductive and behavioural effects on organisms. These sub-lethal effects occur at lower concentrations than are required to kill the organisms, thus basing risk assessment on them is a more precautionary approach. Therefore, another way to risk- ranking chemicals is to consider only sub-lethal effects in the analysis which are presumed by many to be more sensitive and occur at lower levels.

Thus, when calculating a risk ratio, all-inclusive ecotoxicity data can be replaced with either the median effect concentration based only on lethal data or the median effect concentration based only on sub-lethal data.

6.2.2.2. *Refinement of environmental concentration data*

Environmental data were collated for the UK and Europe. As a tier two moderation, only data from the UK were used. The data were also modified by date of collection, with only data from 2000 to the present being included in the analysis. Predicted modelled data was included for the pharmaceuticals and nanoparticles as these were considered to be ‘current’. The aim of these two refinements was to make the environmental data more relevant, to represent, hopefully, the chemicals which are currently present, or have recently been present in UK freshwaters.

Monitoring data are not available for every chemical. Unless the chemical has been of concern and a method exists it is unlikely that there will be monitoring data available for a specific chemical.

6.2.2.3. *Bioavailable concentration of metals*

The definition of the bioavailability of a chemical is ‘*the extent to which a toxic contaminant is available for biologically mediated transformation and/or biological actions in an aquatic environment’* [92]. The bioavailability of a chemical will determine its ability to be toxic to an aquatic organism, as it is the amount of the chemical which is free for uptake by the organism.

The bioavailable fraction of the metals was partly addressed by looking at the dissolved fractions of the metal. Using the Environment Agency WIMS data it is possible to filter out total metal concentrations from dissolved concentrations of the metals. Thus, as a tier two filter, only the dissolved measurements were used, rather than the total metal concentrations. The dissolved measurement is a more accurate measure of the bioavailable concentration of the metal. However, it is acknowledged that the actual toxicity of a metal in water is linked to many complex chemical interactions including competition between metals for the binding sites on ligands or target organs [232]. Thus, a biotic ligand model (Bio-met bioavailability tool, version 1.4.24.11.2011 (Table 10-8) was used, reflecting the differing chemistry of typical UK lowland rivers (Ca 40-120 mg/L, DOC 5.1-8.1 mg/L, pH 7.4-8.1, [233]). When the influence of typical lowland river chemistry (based on the UK rivers Thames, Trent and Calder) was examined via a biotic ligand model, it was found that the Cu toxicity decreased 3-fold, Mn 2-fold and Zn by 14%, values which are all less than an order of magnitude. These relatively modest changes suggested that introducing extra realism would not drastically change the high ranking of the metals.

For all metals, only ecotoxicity tests conducted within a neutral pH range were included in the analysis (most UK rivers have a pH 6.5-8.5). Although there will be environmental conditions where there is a naturally higher pH level, these are not the conditions which freshwater organisms in the UK are typically exposed to.

6.2.3. Ranking of chemicals based on bioconcentration (BCF)

The chemicals were also ranked based on their BCF. By using only the BCF as a ranking tool, without any reference to toxic concentrations, a different ranking order with regard to the concern posed by chemicals to aquatic wildlife can be produced. The BCF is an established ratio, thus values were collected from the literature and the median values used to compare these chemicals. The greater the BCF, the greater the concern based on this ranking methodology. Thus, firstly, chemicals have been ranked based on their BCF alone. The top 20 chemicals based on their BCF were then ranked again based on their using refined water data against the ecotoxicity dataset. This final ranking incorporates both the toxicity and potential bioaccumulation of a chemical.

6.3. RESULTS AND DISCUSSION

Tier two moderations were applied to the data to demonstrate the influence, or not, that the ‘finer detail’ has on the potential risk of a chemical and its ultimate ranking in comparison to the original simple ranking based on all the data. Is there a moderating factor which drastically changes the risk-ranking results from that obtained following tier one analysis?

The chemicals were ranked and the results discussed based on the risk they present to aquatic wildlife in the UK. Table 10-6, Table 10-7 and Table 10-9 summarise the ranking order and risk ratios obtained from all chemical classes. The tier two data and results are discussed, further discussion of the chemicals that rank highly based on the different ranking methods are then detailed.

6.3.1. *Overlap in ecotoxicity data and environmental concentrations*

Following on from the discussions in Chapter 5 (Section 1.2.1), should chemicals with no degree of overlap between the ecotoxicity and environmental datasets be considered of no concern? This is one conclusion that could be drawn from the data. Is there a real concern with regards the threat a chemical presents to freshwater organisms if there is a 10,000-fold difference between the lowest effect concentration and the highest environmental concentration (collated for this study)? Out of the 73 chemicals studied in the project, 39 have an overlap in the two datasets and 34 do not have an overlap in the two datasets (Table 10-5). As discussed in Chapter 5, there is a plausible argument that if there is no overlap in the two datasets, then the literature is telling us that based on these data there aren’t reported concentrations in the UK that are causing toxic effects to aquatic organisms. Some of the chemicals assessed here (based on the data collected for this project), and which do not have an overlap in the reported environmental concentrations and report toxic effect concentrations, include: AS, methomyl, BDE 209, both nano ZnO and nano Ag, propranolol, sulfamethoxazole. Some of the chemicals with and without overlaps in the two datasets are discussed briefly below, to demonstrate the difference between the overlaps in the datasets for a selection of chemicals.

6.3.1.1. *Triclosan*

Triclosan, for example, has a small overlap between the lowest reported effect concentration and highest reported environmental concentration, with a 100-fold difference between the two median values. Triclosan, as discussed in chapter 4, has been reported as a chemical of concern [178]. Under the Water Framework Directive (WFD) a proposed EQS value of 0.28 µg/L has been calculated, however, the report states that this is based on limited information. In Europe, median and average concentrations of 0.07 µg/L and 0.45 µg/L have been reported, with effluent concentrations in the UK being in the region of 0.34-1.1 µg/L, while both up and down stream concentrations ranged from 19-80 ng/L [178, 234]. Triclosan has been reported in the literature and in the media as being a chemical of current concern.

6.3.1.2. *Beta blockers*

Three beta blockers were considered in this study, propranolol, atenolol and metoprolol. Based on the data collated, there is no overlap in the two datasets for any of the beta blockers, the fold difference between the median effect and median environmental data is > 10,000 fold for all three pharmaceuticals.

Beta blockers are frequently prescribed drugs used to treat cardiovascular disease. However, the removal of propranolol via waste water treatments has been reported as being low (17-23%), even using granular activated carbon processes [208]. Therefore, a high percentage of the drug will be released into the aquatic environment via effluent.

Based on the data collected for this study, propranolol has the greatest risk ratio out of the three beta blockers. This is echoed by the results reported by Cleuvers et al (2005) who found propranolol to be the most toxic. The risk ratio for all three beta-blockers based on PEC/PNEC were reported by Cleuvers et al (2005) to be below 1, as with results from this study (based on the median data) [235]. Therefore, the environmental risk, as suggested in the results of this study, are low. However, the concern, as with many scientific investigations, is that not all the potential end-points and scenarios been reported/included. Even though based on the data here there is no overlap between water and effect concentrations, an EQS value has recently calculated for propranolol [236]. Based on that study the EQS value for propranolol is 14 µg/L. The lowest effect data point is 50 µg/L and the median value is 1,870 µg/L according to my data. The highest reported environmental value for this study, based on measured and predicted concentrations is 0.16 µg/L, and therefore the proposed EQS of 14 µg/L is below the lowest effect reported and higher than the environmental data, suggesting little, if any, concern for propranolol.

6.3.1.3. *Lenacil*

Lenacil is a uracil pre-emergent herbicide which is used in Europe mainly on sugar beet, fodder beet and spinach production. As it is a herbicide, the most sensitive aquatic organisms are likely to be plants, but very little relevant ecotoxicity data exists. In this analysis there are only five data points for ecotoxicity, ranging from 12-23,400 µg/L. An overlap in the ecotoxicity data and the environmental data occurs based on the EC50 (growth inhibition) of 12 µg/L reported effect on *Scenedesmus vacuolatus*, following one-day exposure [131] and peak concentrations reported in Belgium and France of 22 µg/L and 1.9 µg/L. Unfortunately, no UK environmental data have been used in this analysis. Therefore, the reason behind the overlap in the two datasets for lenacil is based on a couple of low effect concentrations and a couple of relatively high peak, environmental concentrations. There is not a wide breadth of effect data to include in the analysis. Others have looked at the environmental risk of pesticides, including lenacil, with the conclusion that lenacil is of low risk in European rivers, based on the $MEC_{(max)}$/PNEC ratio

[237]. This example illustrates how risk assessment is sometimes based on relatively little data.

6.3.1.4. *LAS*

LAS has been highlighted as one of the most frequently occurring chemicals in the aquatic environment, due to its extensive use globally [238]. More than 4.2 million tons of detergent products and 1.2 million tons of softener products were used annually in Western Europe 10 years ago [239], of which surfactants were a main component. Influent concentrations are reported in the literature to be as high as 16 mg/L, with concentrations in effluent in tens µg/L [186]. The median environmental concentration reported for this study is 21 µg/L, based on data from the UK and Europe. Current reports in the literature suggest that there is limited or low risk from the presence of surfactants, including LAS, to the environment [240]. Based on the data collated for this study, there is a 100-fold difference between the ecotoxicity median and the environmental median values. However, there is an overlap in the two datasets due to some very low effect data points. Concentrations of LAS in final effluents in the UK range from 0.016-0.029 mg/L [234]. Even though LAS and other surfactants have a high removal rate, due to their continuous and extensive use it is likely that they will always be in the aquatic environment (while that chemical is in use).

6.3.1.5. *Conclusions*

The approach selected for risk-ranking has been the ratio between the median ecotoxicity and environmental concentrations. This in effect selects chemicals which come closest to affecting the widest range of wildlife in the widest range of locations. For some chemicals, the reported river measurements exceed the reported effect concentrations, whereas with others no such overlaps exist. This study has not taken this overlap observation further forward as a metric, although it might be worth doing so in the future. In some cases, where the overlap is due to an individual isolated or extreme value the overlap may be due to unreliable ecotoxicity studies and false or unrepresentative river measurements. Thus, the suggested risk could be based on misleading or inaccurate information, therefore potentially overestimating the risk of certain chemicals. The other side to this argument would be that not all reported effects or environmental concentrations have been included or reported, and therefore the risk of a chemical could be underestimated. Nevertheless, it should be noted that many of the metals, such as Cu and Zn seem to have big overlaps suggesting impacts are occurring in some places in the UK.

6.3.2. Results from applying moderating factors

The refinements to the data are a means of editing the data, potentially making it more realistic, and environmentally relevant, rather than using all the data available. Does this alter the highest ranked chemicals from the tier one analysis?

6.3.3. Filtering the environmental measurement and ecotoxicity dataset

The only change to the effect data was to remove results from the metals toxicity dataset which were conducted at pH level <6.5 and >8.5. The environmental data still included the modelled values but now only information from measurements taken from 2000 onwards. In some cases, this could have a drastic influence on the amount of values available. If there was only one value recorded for this study, for either the ecotoxicity and environmental data, these chemicals were still included; however, I am aware that using only one data point could provide misleading results (this issue will be discussed further in Chapter 7 & 8).

All chemicals studied have a risk ratio <0.1. The four highest ranked chemicals are copper, LAS, zinc and aluminium with risk ratios of 0.031, 0.022, 0.037 and 0.08, respectively (Figure 6-3). These chemicals were highlighted as chemicals of concern in their individual classes and they were in the top 10 in the tier one all-chemical comparisons (as detailed in Chapters 4 and 5). The majority of chemicals have a risk ratio of <0.0001, based on their median values. As with the much simpler tier one analysis, the metals dominate the higher ranking, with pesticides also ranking highly. POPs and pharmaceuticals are generally ranked lower. The two nanoparticles retain their lower rankings. Surfactants and others are widely distributed across the ranking.

Based on the precautionary approach, which used the same environmental data and the 5th percentile of the effect data described above, some of the risk ratios are now much closer to 1.0. In this case copper, LAS, ibuprofen and zinc are the chemicals of greatest concern, with risk ratios of 0.66, 0.32, 0.26 and 0.26, respectively (Figure

6-4). These chemicals were also identified as chemicals of concerns based on the tier one precautionary risk ratio analysis. Based on this approach, the chemical classes become more widely distributed through the ranking, with some POPs and surfactants moving up the ranking. Metals and pesticides are more widely distributed, rather than dominating the higher rankings.

6.3.3.1. *Lethal and sub-lethal risk-ranking*

With regards the effects of chemicals on aquatic organisms, ecotoxicology tests are conducted to gain an understanding of the effects chemicals exert on aquatic organisms, and ascertaining the concentration at which a biological effect occurs due to the presence of a specific chemical. Lethality is/was the most common end-point used in toxicology and used as an end-point for acute toxicity tests. While conducting chronic toxicity tests, sub-lethal effects are generally the end-points of interest. Sub-lethal end-points include effects on behavioural, as well as physiological, biochemical and histological changes.

The effect data collated for this study were split based on lethal and sub-lethal effects. Thus all lethal effects have been considered together (regardless of exposure time or species) and all the sub-lethal effects have been considered together, i.e. growth, reproduction, behavioural, changes to gene expression (regardless of exposure time or species). The aim of this separation was to take reported effects that have caused lethal harm and compare the risk-ranking based on this approach to that of chemicals ranked based on sub-lethal effects. Sub-lethal effects can/do cause harm, but can often be open to interruption with regards to whether or not the effect is harmful or detrimental to the organism. The effects are not immediately lethal to the organisms, although sub-lethal effects can be signals/sign posts to ultimately detrimental effects at the individual, population and community level. The effect data were split between lethal and sub-lethal rather than acute and chronic as it was deemed easier and clearer to use either lethal or sub-lethal effect than it would be to address the potential confusion between separating acute and chronic effects.

The risk ratios have been calculated using the separated lethal and sub-lethal effect data and the modified environmental data. Based on the lethal effect data only, (Figure 6-5), all risk ratios were lower than 0.1. Copper, LAS and zinc are the highest ranking chemicals, with risk ratios of 0.032, 0.015 and 0.014. B[a]P is the highest ranked POP, with a risk ratio of 0.00063 and chlorpyrifos is the highest ranked pesticide, with a risk ratio of 0.008. The chemicals ranked lower include nano ZnO, ranking 23rd with a risk ratio of 0.000069, and propranolol being the highest ranked pharmaceutical (31st with a risk ratio of 0.000013. The majority of the chemicals have a risk ratio <0.005.

Based on the sub-lethal effect data only (Figure 6-6), all risk ratios were lower than 0.1. Copper, LAS, aluminium and triclosan are the highest ranked chemicals, with risk ratios of 0.36, 0.032, 0.026 and 0.020, respectively. EE2 is the highest ranked pharmaceutical with a risk ratio of 0.012.

The risk ratios for the lethal and sub-lethal effects are not substantially different, with the risk ratio for the highest ranked chemical, copper, being between 0.03 and 0.04 in both analyses (Figure 10-18).

6.3.3.2. *Points for consideration*

At first it was thought that dividing the ecotoxicity dataset into either the lethal or sub-lethal categories would be straightforward. However, some issues proved problematic: Firstly, immobilisation of *daphnia magna*. The immobilisation of the organism is often referenced/interchanged with lethal/mortality. Therefore, reported effects that have reported as immobilisation have been included in the lethal effect dataset. The second effect which has caused concern is the reported effect on growth. For invertebrates and fish this was a straightforward decision and the data have been included in the sub-lethal category. For algae and plants, effects on growth have also been included in the sub-lethal category, as they affect the size of the population. However, as this decision means that there is no lethal data for algae and plants, this has warranted further consideration. There is not a reported mortality measure for algae, instead the data reported have included reduction in growth or growth rate. The confusion with regards growth as an end-point for algae is that the test chemical could have killed some of the algae cells, therefore putting a stop to the growth or slowed division of the cells. Or it could have limited the growth rate and therefore slowed down the rate of growth, but not stopped growth altogether. The inclusion of algae growth effects can alter the median value for some chemicals, as a chemical might cause effects on growth only at quite high concentrations, compared to the sub-lethal effects which usually occur at lower concentrations than that of the lethal effect concentration. This has caused the median sub-lethal value for some chemicals to be greater than the median lethal value. This leads into the final point, which is the sub-lethal median value is not always lower than the lethal median value (which is what would be expected) based on sub-lethal effects occurring before an organism experiences a lethal effect. This is due to the datasets containing mixed species and very different sensitivities to some chemicals.

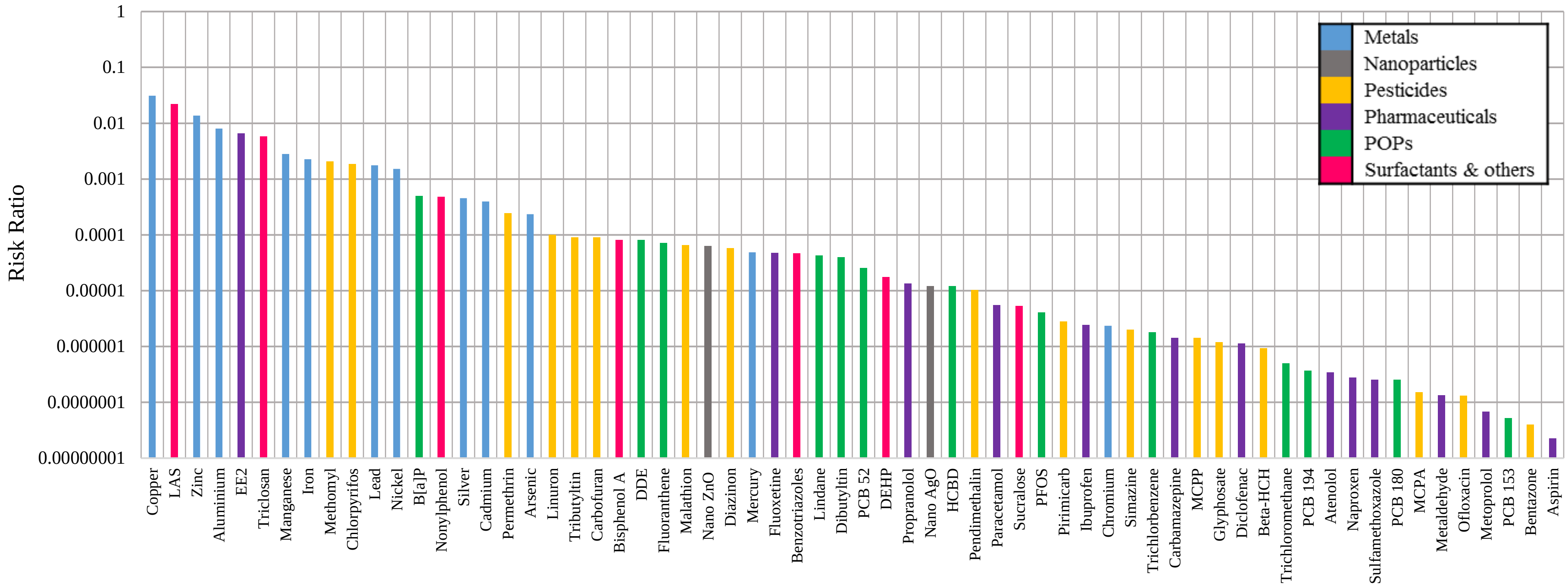


Figure 6-3 Risk-ranking of chemicals based on the difference between the median effect concentration and the median river water concentration. Here only UK river measured or modelled data post-2000 was used and metals ecotoxicity data for neutral pH. Only dissolved metal concentrations were used.

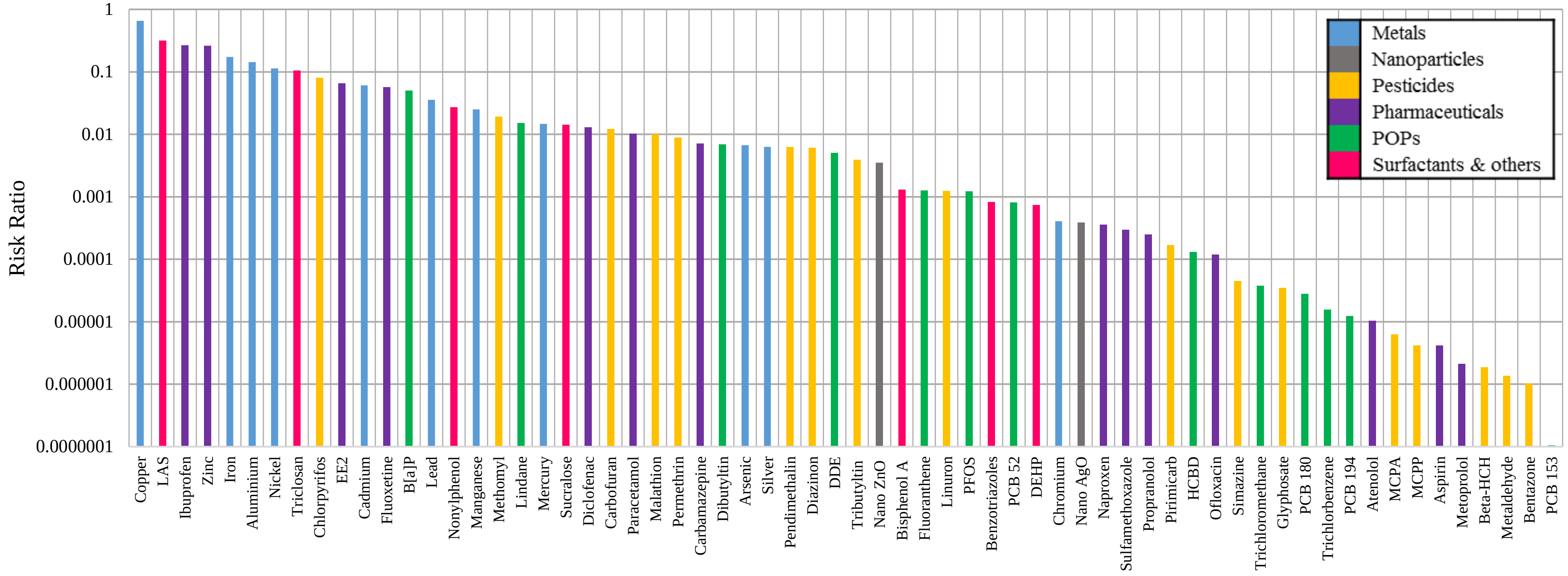


Figure 6-4 Risk-ranking of chemicals based on the difference between the 5th %ile effect concentration and the median river water concentration. Here only UK river measured or modelled data post-2000 was used and metals ecotoxicity data for neutral pH. Only dissolved metal concentrations were used.

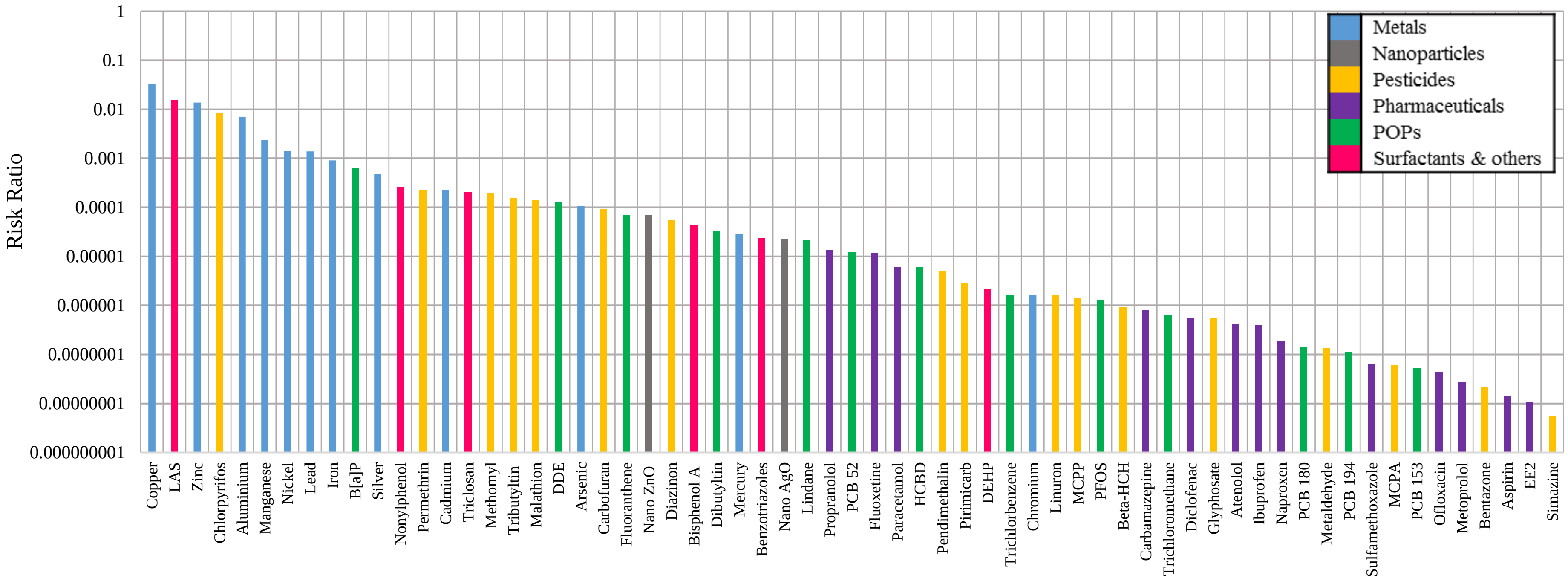


Figure 6-5 Risk-ranking of chemicals based on the difference between the lethal median effect concentration and the median river water concentration. Here only UK river measured or modelled data post-2000 was used and metals ecotoxicity data for neutral pH. Only dissolved metal concentrations were used.

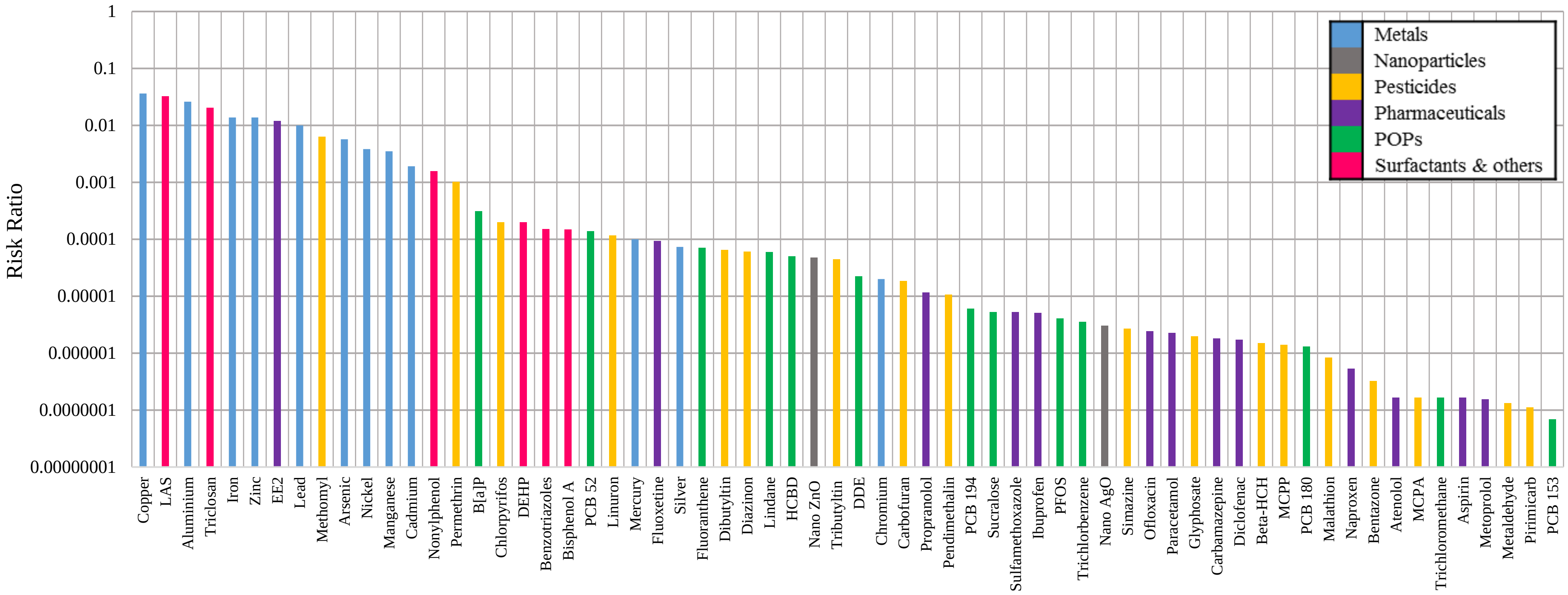


Figure 6-6 Risk-ranking of chemicals based on the difference between the sub-lethal median effect concentration and the median river water concentration. Here only UK river measured or modelled data post-2000 was used and metals ecotoxicity data for neutral pH. Only dissolved metal concentrations were used.

6.3.4. Ranking chemicals based on Bioconcentration Factors

Freshwater organisms are exposed to chemicals via their environment; one result of this is the transfer of the chemical from the external environment to the aquatic organism. The terms used to define the processes that can occur include; bioconcentration – exposure via water; bioaccumulation – exposure via water, air, and diet; and biomagnification – the increasing concentration of a chemical up a food chain. The bioconcentration factor (BCF) is the ratio of the concentration of a chemical in an organism to the concentration of the chemical in the surrounding environment at steady state. BCF is used for regulatory purposes as an assessment of hazard. The BCF of a chemical can be determined experimentally or a predicted BCF can be generated based on computer based models. Obtaining BCF values experimentally is expensive, time consuming and requires a large number of organism (i.e. fish) [241]. The bioconcentration and the bioaccumulation potential of an organic chemical is often inferred from the octanol-water partition coefficient (K_{OW}) [242]. K_{OW} represents the lipophilicity and the hydrophobicity of a chemical and this influences how it thermodynamically distributes. It is inversely related to the aqueous solubility. Other parameters considered in the prediction of BCF values include; water solubility, soil adsorption coefficient, acid dissociation constant (pKa), molecular weight, distribution-coefficient (log D). The parameters used to predict and refine BCF values for chemicals is often explored in the literature. With the inclusion of multiple varies tested to explore the means of achieving an accurate BCF value [243, 244]. The median BCF of each chemical considered in the current study is plotted in Figure 6-7 (Table 10-9). They were obtained from BCF values reported in the literature for a range of organisms. A chemical with a BCF of 2000 is considered to be highly bioaccumulative and a BCF of 5000 is considered very bioaccumulative, according to guidelines in Annex XIII of the REACH Regulation 1907/2006 [245]. Other BCF regulations which are currently in place are detailed in Table 6-2.The higher the BCF value, the greater the concern.

Based on the data collated for this study, PCB 180, PCB 194, and PCB 153 are chemicals which ranked highest when BCF is considered. With eight chemicals having BCF values>5,000 and another five chemicals having BCF values >2,000, 60 out of the 73 chemicals have BCF values lower than the REACH 2000 benchmarks. The chemicals that rank highly based on this ranking method are then discussed in further detail in sections 6.3.4.1 – 6.3.4.

6.3.4.1. *PCBs*

Polychlorinated biphenyls (PCBs) are a man-made group of semivolatile, hydrophobic pollutants. The industrial production of PCBs started in 1929 and peaked in the late 1960s. Their production and use were banned in the US and Europe in the late 1970s due to the serious risks they pose to human health. The PCBs included in this study were PCB 180, PCB 194, PCB 153; and PCB 52, these specific PCBs were chosen based on discussions with colleagues at CEH Lancaster.

All four PCBs rank within the top five chemicals of concern based on the BCF risk-ranking approach, with PCB 180 ranking the highest. All four PCBs exceed the very bioaccumulative benchmark value of 5000. PCB 180 (as with other PCBs) is a highly hydrophobic substance with log K_{OW} between 6.6 and 7.4 (average 7.0) [14]. The high lipophilicity of PCB 180 and its slow metabolism in biota allows it to accumulate in biota, particularly in fatty tissues, as reflected by its high bioconcentration factor.

There is very limited environmental concentration data for PCBs in UK freshwaters. Concentrations observed in other European countries have been reported to be between <LOD and 0.048 μg/L at background levels and up to 0.13 μg/L after sludge from extinguishing a fire had been washed into the stream. These European data suggest that in highly industrial areas of the UK, PCB concentrations may reach levels that are toxic for some organisms. Thus, taking the BCF into account brings

the PCBs into the picture compared with the previously used water-based toxicity data ranking, where they have not been highlighted as a concern.

6.3.4.2. *Mercury*

Hg exceeds the very bioaccumulative value of 5000, with a BCF of 6000. Based on the risk-rankings so far, Hg has not ranked highly compared to other metals. Once BCF is considered, Hg becomes the metal of greatest concern. Mercury is found at very low concentrations in UK waters, with a range of 0.005 – 18.2 µg/L. It is present in freshwater in three main forms: the inorganic forms of metallic Hg^0, inorganic Hg^{2+} and the organic methylmercury [MeHg(I)] [43]. Hg in this study represents all forms of Hg. However, [MeHg(I)] is highly toxic, especially to the developing nervous system, and it accumulates in the food web, whereas the toxicity of the other forms is considerably lower. Thus, comparing total Hg values in the water column with effect concentrations could under-represent risk. The EU WFD has recently set EQS values for concentrations in biota where an EQS based on water concentrations is not considered protective enough. For Hg the EQS value of 0.05 µg/L in water has been supplemented with a biota standard of 20 µg/kg fresh weight. Sources of natural Hg include geothermal and volcanic activity, while anthropogenic sources range from the combustion of fossil fuel in power plants, various types of manufacturing and production processes such as metal and cement facilities, incineration and mining [18]. Although the emissions of Hg have been reduced in Europe, the [MeHg(I)] levels in freshwater fish remain high [246], and hence Hg is still of considerable concern for aquatic wildlife in the UK [247].

Table 6-2 Regulatory bioaccumulation criteria

Regulatory Programme	Regulatory Agency	Categorisation	Criteria	Ref
Reach 2007	European Union	Bioaccumulative	BCF ≥2000	EC 2001
		Very Bioaccumulative	BCF ≥5000	
Canadian Environmental Protection Act	Environment Canada	Bioaccumulative	BAF/BCF ≥5000 Or Log Kow ≥5.0	CEPA 1999
TSCA New Chemicals Programme PBT Policy	United States Environment Protection Agency	Bioaccumulative	BCF/BAF ≥1000 or Log Kow ≥4.2	USEPA 1976
		Very Bioaccumulative	BCF/BAF ≥5000 or Log Kow ≥5.0	
UNEP Stockholm Convention	Countries within the United Nations	Bioaccumulative	BCF/BAF ≥5000 or Log Kow 5.0	UNEP 2001

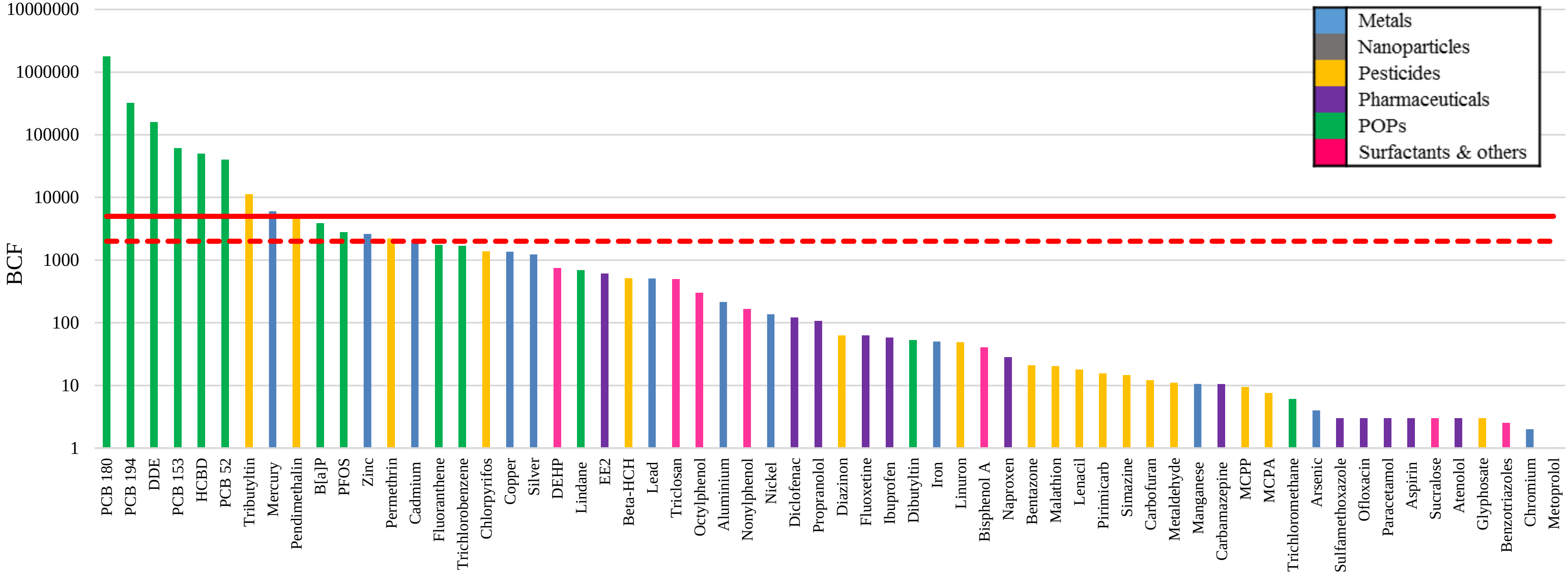


Figure 6-7 Chemicals ranked purely based on BCF data collated from the literature (EU Standards – Bioaccumlative, BCF≥2000 (red dashed line) and Very Bioaccumulative, BCF≥5000 (solid red line))

6.3.5. BCF and toxicity risk-ranking

It should be recalled that a chemical which bioconcentrates need not necessarily be hazardous [248], and hence the final ranking considered here includes an assessment of toxicity. The 25 highest ranked chemicals based on BCF (Table 6-3) have been ranked using the toxicity data and approach used in tier two (Figure 6-2). The aim was to take the 25 highest ranked chemicals based on their BCF (Table 6-3), and then rank those chemicals using the risk ratio calculated following the tier two moderation of the data (as detailed previously), thus bringing together bioconcentration, toxicity and occurrence in the environment.

When this approach was taken, copper ranks as the chemical of greatest concern once again, with a risk ratio of 0.032 based on all the effect data, 0.032 based on lethal effect data and 0.036 based on sub-lethal effect data. Zinc ranks second, followed by EE2, based on all the effect data. Zinc ranks second, followed by chlorpyrifos, based on all the lethal effect data, (Figure 6-9). Triclosan ranks second followed by zinc based on the sub-lethal data (Figure 6-10).

There is a fold difference of >10,000 between the highest ranked (copper) and the lowest ranked (PCB 153) chemicals based on this approach. Whether all the effect data or only lethal or only sub-lethal data are used, the risk ratios for all chemicals are less than 0.1.

Table 6-3 Highest ranked chemicals based on BCF values

BCF	**Rank**	**Chemical**
BCF>5000 (very bioaccumulative)	1	PCB 180
	2	PCB 194
	3	DDE
	4	PCB 153
	5	Hexachlorobutadiene (HCBD)
	6	PCB 52
	7	Tributyltin
	8	Mercury
	9	Pendimethalin
BCF>2000 (bioaccumulative)	10	B[a]P
	11	PFOS
	12	Zinc
	13	Permethrin
BCF>500	14	Cadmium
	15	Fluoranthene
	16	Trichlorobenzene
	17	Chlorpyrifos
	18	Copper
	19	Silver
	20	DEHP
	21	Lindane
	22	EE2
	23	Beta-HCH
	24	Lead
	25	Triclosan

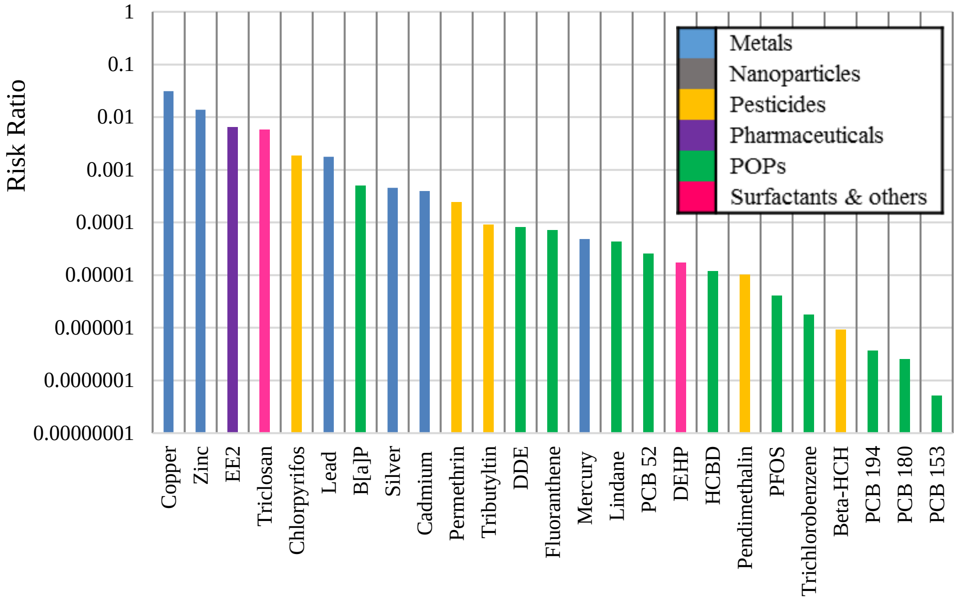


Figure 6-8 Risk-ranking of 25 chemicals based on bioconcentration, all ecotoxicity data & recent UK dissolved water data

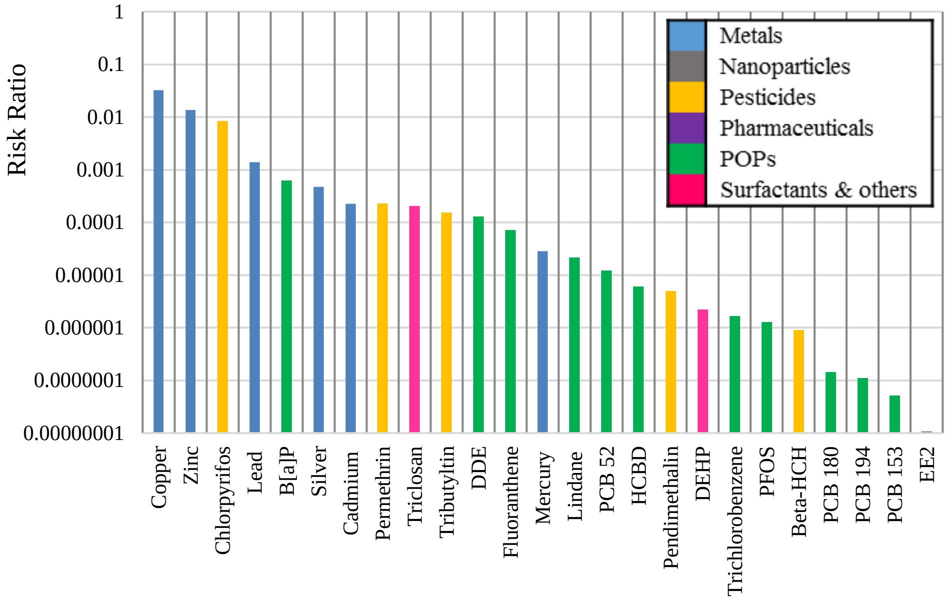


Figure 6-9 Risk-ranking of 25 chemicals based on bioconcentration, the lethal sub-set of the ecotoxicity data & recent UK dissolved water data

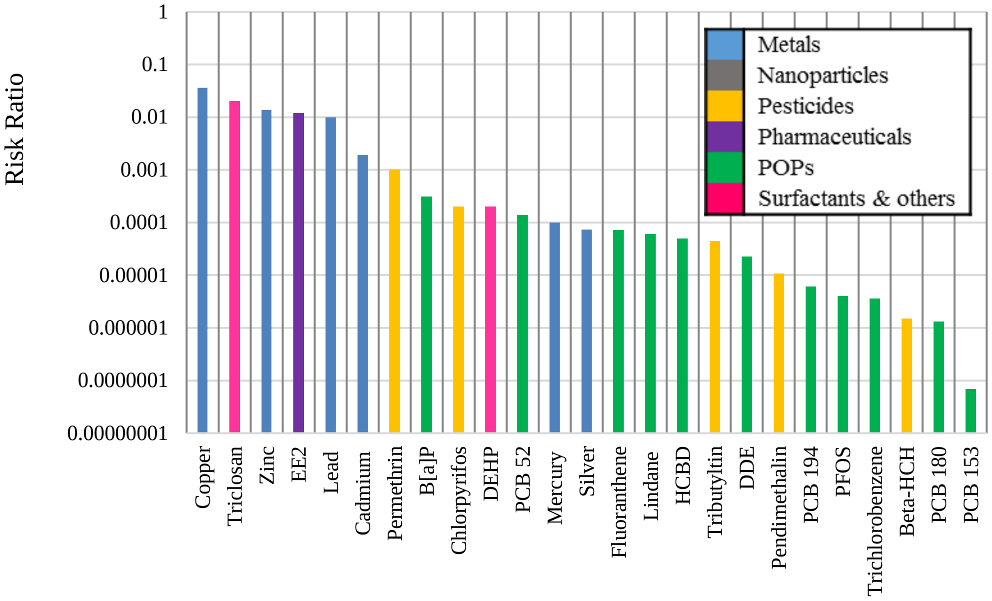


Figure 6-10 Risk ratio of 25 chemicals based on bioconcentration, the sub-lethal ecotoxicity dataset & recent UK dissolved water data

6.4. CONCLUSIONS FROM TIER TWO APPROACH

The effects of chemicals on the environment, and thus pollution, is of concern both in the UK and globally. The overall study aimed to develop a simple, objective approach to investigate chemicals of concern, using the readily available ecotoxicity and environmental data. The aim of this particular chapter was to examine whether the chemicals identified as the highest risk using a simple ranking approach would change when further refinements to the dataset were made. Importantly, this approach does not claim to be a new risk assessment approach, but it was an exploratory project to see if taking a different, perhaps simpler, less subjective approach could be developed and used to investigate chemicals of concern, using the ecotoxicity and environmental data which are available for them.

Following the filtering or addition of moderating factors, no chemical which ranked previously at the lower end of the initial risk-ranking (tier one) climbs/jumps to the higher ranking positions. Metals still remain of most concern, followed by the same pesticides, LAS, EE2 and triclosan.

The results from this chapter and Chapters 4 and 5 (tier one) will be discussed together in the final conclusion (Chapter 8). Prior to the final chapter, Chapter 7 describes the experimental work completed as part of the project and summarises the results and feedback from the workshop held at the end of the project and whose aim was to explore the approach with experts.

7. QUESTIONING THE APPROACH TO RISK-RANKING CHEMICALS

7.1. INTRODUCTION

Throughout the project it has been important to critique and question the approach being developed. Not only as a means of understanding the feedback and response from others with regards the approach, but as a key training aspect to the PhD and to understand the potential breadth or development of the approach after the project. This chapter includes three main sections which discuss the different ways the approach has been compared to others and tested. In this chapter, the risk-ranking obtained from this approach is compared with experimental tests obtained for this study, it is compared to a risk-ranking based on sewage effluent concentrations, and questioned based on feedback following a workshop. The work in this chapter was developed via consultation with experts from the scientific, government and industry community.

7.2. FINAL RISK-RANKING RESULT FOLLOWING MODERATING FACTORS

A final risk-ranking for the 73 chemicals is presented in Figure 7-1. In this result only dissolved metal concentrations were used, the edited metal ecotoxicity concentrations where only studies at pH 6.5-8.5 were included and all the river environmental data for the organics. Thus, if no UK river measurements were available or modelled then European river values were used. This final ranking will be the basis for the discussions in this chapter.

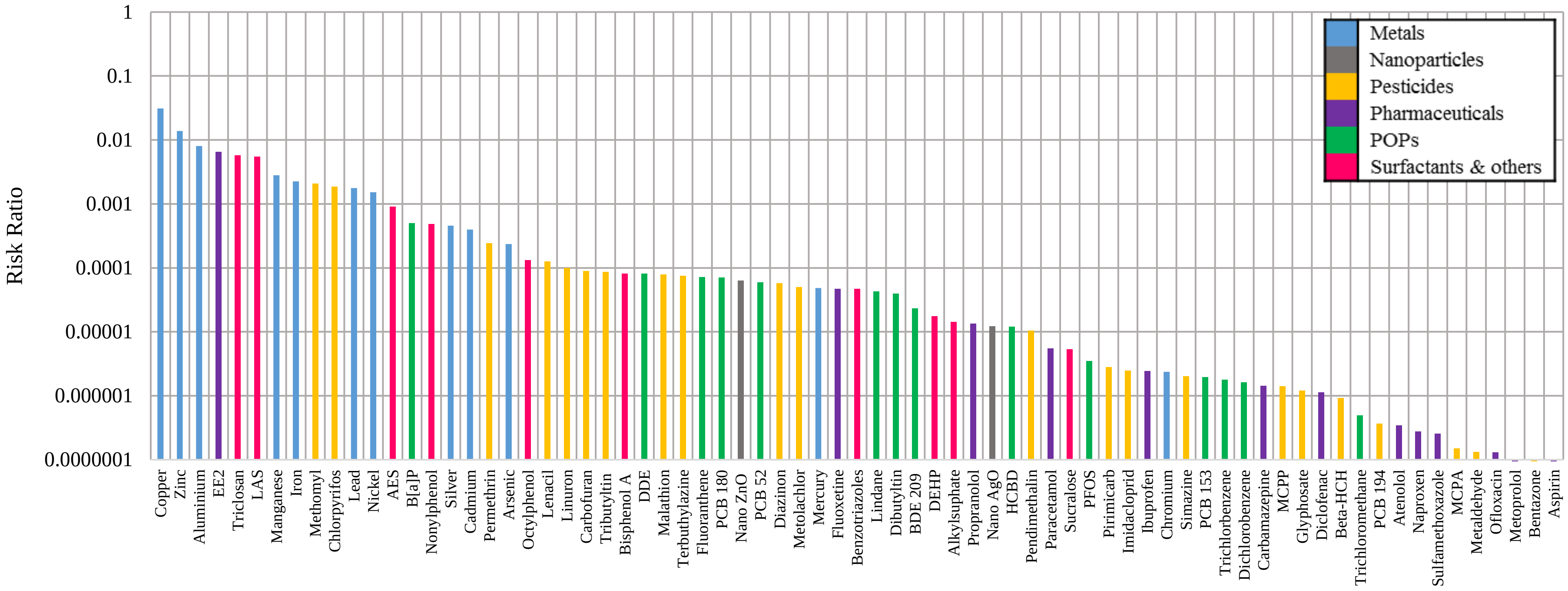


Figure 7-1 Risk-ranking of 73 chemicals based on the difference between the median effect concentration and the median river water concentration. The effect and environmental data have been moderated; the data included is the dissolved metal concentrations, the edited metal ecotoxicity concentrations (limited to neutral pH) and all the environmental data for the organics.

7.3. COMPARING CHEMICAL RISK-RANKING OBTAINED FROM EXPERIMENTAL DATA AGAINST THE LITERATURE BASED METHOD

This study has the long-term aim of prioritising all chemical groups on the basis of risk through analysis of the literature. As a reality check on this literature-based risk-ranking analysis, some of the chemicals from the 73 were selected for toxicity testing in the laboratory. The toxicity tests used representatives of two classes of chemicals and used two test species, *Daphnia magna* and *Pseudokirchneriella subcapitata* (former names: *Selenastrum capricornutum* and *Raphidocelis subcapitata*). The chemical classes were metals (Cu, Zn, Mn and Fe) and pharmaceuticals (diclofenac, fluoxetine, ibuprofen and propranolol). The EC50 results from the experiments were compared with toxicity data from the literature. A risk ratio was calculated using a) this study's experimental results and b) a literature based EC50 median values, and then both values were compared with UK surface water concentrations.

The aim was to see if the ranking of these selected chemicals on the basis of toxicity to an alga and a daphnid carried out in our laboratory would match that from the literature survey.

This experimental work was also a training experience to help understand, at a basic level, the processes involved in conducting simple toxicity tests.

7.3.1. Methods

7.3.1.1. *Effect concentrations - literature-based data*

For each chemical, the effect data for *Daphnia magna* and *Pseudokirchneriella subcapitata* (referred to from now on as algae) were teased out from the main database, to get an understanding from the literature of the reported concentrations (Table 7-1). These data were used to calculate a median effect concentration for each chemical based on effect concentrations reported in the literature.

7.3.1.2. *Environmental concentrations*

Environmental concentrations for each chemical were collated to understand the concentrations which organisms in the UK are typically exposed to (Table 7-1), as described in Chapter 3.

7.3.1.3. *Experimental-based data - MicrobioTests*

The toxicity test kits, Daphtoxkit F Daphnia magna and Algaltoxkit F, were purchased from MicroBioTests Inc. (Belgium). Both tests follow ISO standard methods, ISO 6341 (daphnia) and ISO 8692 (algae). *S*ee http://www.microbiotests.be/ for further details.

For each chemical, a range finder and a definitive test were completed. Using the definitive test results, an EC50 was calculated based on the experimental results. The range finder experimental concentrations were determined based on the range of effects concentrations reported in the literature. The definitive test concentrations were based on the results from the range finder experiments (Table 7-2, Table 7-3).

7.3.1.4. *Preparation of the stock solutions*

The chemicals used in the experiment were $CuSO_4$, $FeSO_4$, $MnCl_2$ and $ZnSO_{4,}$ ibuprofen sodium salt, diclofenac sodium salt, propranolol hydrochloride and fluoxetine hydrochloride. Chemicals were purchased from Sigma; all chemicals were of pure analytical grade.

The chemicals were weighed out using an analytical balance to make either a 10 mg/L, 100 mg/L or 1000 mg/L stock solution, depending on the stock concentration required. Each chemical was transferred to a 100ml flask and the daphnia stock medium or the algae medium was used to prepare the stocks, as appropriate. The flasks were shaken to ensure that the chemicals went into solution and were uniformly distributed. A set volume of the stock solution was pipetted into beakers to make the required concentrations of each chemical. Samples of each stock solution were taken for measurements to confirm actual concentration of the standard solution.

7.3.1.5. *Daphnia toxicity test procedure*

The experiments using the Daphtoxkit F were based on the immobilization of *Daphnia magna* due to the presence of a toxic chemical at a certain concentration. The *Daphnia magna* were hatched from dormant eggs (ephippia) in 3 days under continuous illumination (6000 lx) at 20 °C. Once hatched, the daphnia are referred to as neonates (<24h old). The neonates were fed 2 hours prior to exposure to the chemicals. Twenty neonates were used for each concentration tested in a series of four wells; each well contained 10 mL of the test concentration (Table 7-3) and 5 neonates. The neonates were exposed to the chemicals for 48h at a temperature of 20°C in darkness. The number of immobilised daphnia was recorded at 24h and 48h, by counting the number of active/non-active daphnia per well.

7.3.1.6. *Algae toxicity test procedure*

The 72h algal growth inhibition test was performed in spectrophotometric long cell test vials and was based on the measurement of the optical density (OD) as an estimate of the concentration of algae in the medium. A spectrophotometer was used which measured the absorbance at 670nm.

The algae were initially in algal bead form. After de-immobilisation, an algal suspension was prepared and used to achieve an algal density of $1x10^6$ algae/ml. For each concentration of each test chemical (Table 7-3) in the series there were three 25ml cells with an algal density of $1x10^6$ algae/ml. The cells were randomly arranged using a random number generator and the algae were exposed to the chemical for 72h under continuous illumination (100,000 lux) at 23°C. The density of the algal suspension was measured and recorded at 24h, 48h and 72h using the spectrophotometer.

7.3.1.7. *Measurements of actual exposure concentrations*

The concentrations of the chemicals and deviations from the nominal concentrations were monitored and calculated by taking and analysing water samples. For each compound a sample of the starting stock, pre-experimental dilutions and post-experiment dilutions and controls were taken.

Preparation and storage of the samples varied between species and chemical class:

-Experiments involving exposure of *Daphnia magna* to metals: to all water samples nitric acid was added and they were stored at 5°C.

-Experiments involving exposure of *algae* to metals: to the stock and pre-experiment dilutions nitric acid was added and they were stored at 5°C. Post-experiment water samples were filtered using a 0.45 um filter to remove the algae from the solution, then nitric acid was added and they were preserved at 5°C.

-Experiments involving exposure of *Daphnia magna* to pharmaceuticals: all water samples were diluted with an equal volume of methanol and stored at -20°C.

-Experiments involving exposure of *algae* to pharmaceuticals: stock and pre-experiment samples were diluted with an equal volume of methanol and stored at -20°C.

Post-experimental water samples were filtered using PTFE (Polytetrafluoroethylene) filters, and stored in a 50% methanol solution at -20°C. The PTFE filters were chosen as they had the best recovery (least retention) of pharmaceutical on the filter paper for the pharmaceuticals used in the experiment. The only adjustment need was to the pH of the fluoxetine water samples post-experiment; the pH was reduced to pH 2 to increase the recovery of the fluoxetine (this reduced retention on the filter paper). This action was taken based on advice from the chemists measuring the concentration of pharmaceuticals in the water samples.

Measurements of the metals were conducted during the PhD, however measurements of the pharmaceuticals were not finalised within the time frame of the PhD.

The analysis of the water samples containing metals was carried out using ICP-OES (Inductively coupled plasma optical emission spectroscopy) at CEH Wallingford, by the Water Quality laboratory group. The samples were analysed using a Perkin Elmer Optima 2100 DV inductively coupled plasma-optical emission spectrophotometer (ICP-OES). ICP-OES is a multi-element technique commonly used for trace metal determination. It has a number of advantages for metal analysis including; the ability to rapidly and simultaneously analyse a number of elements, a low detection limit, high precision and a wide linear dynamic range [249]. For quality control purposes, an accredited external reference standard (LGC Aquacheck, Lancashire, UK) and standard solutions of known concentrations were run alongside each set of samples.

7.3.1.8. *EC50 calculations*

EC50 values were calculated based on the guidance provided with the Microbiotest kits, which follow ISO/TS 20281 methods.

EC50 values were determined for each chemical following the algae experiments using a computer programme which uses the Hill model. EC50 values were determined for each chemical following the daphnia experiments by plotting the percentage inhibition and concentration, determining the equation of the line or slope and determining the 50% effect concentration from that dose-response curve.

7.3.1.9. *Risk ratio calculation*

For each chemical there is a literature based EC50 value (Lit) and an experimental EC50 value (Exp) for both daphnia and algae, as well as an environmental concentration (Env Conc).

Using these data, a risk ratio was calculated for each chemical and species:

$$Risk\ Ratio\ = \frac{Env\ Conc}{Algae\ Exp}$$

$$Risk\ Ratio\ = \frac{Env\ Conc}{Algae\ Lit}$$

$$Risk\ Ratio\ = \frac{Env\ Conc}{Daphnia\ Exp}$$

$$Risk\ Ratio\ = \frac{Env\ Conc}{Daphnia\ Lit}$$

7.3.2. Results and Discussion

Table 7-1 Literature-based effect concentrations and environmental data.

Chemical		**Median effect concentration (all species) µg/L**	***Daphnia magna* literature-based median effect concentration µg/L**	***Algae* literature-based median effect concentration µg/L**	**Environmental median concentration µg/L**
Copper	Cu	47.6	20	87.5	2.4
Iron	Fe	34,200	6,690	6,000	337
Manganese	Mn	8,030	9,400	4,980	33.2
Zinc	Zn	356	155	199	10.7
Ibuprofen	Ibu	19,100	32,550	19,240	0.046
Fluoxetine	Flx	106	195	45	0.005
Propranolol	Pro	1,870	2,750	975	0.024
Diclofenac	Dic	11,454	54,000	10,2700	0.013

Table 7-2 Concentrations used in the algae experiments (metals µg/L and pharmaceuticals mg/L)

Test	Chemical	C1	C2	C3	C4	C5
Range finder	Copper	20	40	60	120	210
Definitive test	Copper	15	20	25	30	35
Range finder	Zinc	10	40	120	430	1500
Definitive test	Zinc	10	20	30	40	50
Range finder	Manganese	500	1500	4500	13500	40500
Definitive test	Manganese	5000	7500	11250	16800	25300
Range finder	Iron	1000	2000	4000	8000	16000
Definitive test	Iron	x	x	x	x	x
Range finder	Diclofenac	0.1	1	5	10	50
Definitive test	Diclofenac	50	75	100	125	150
Range finder	Fluoxetine	0.1	1	5	10	50
Definitive test	Fluoxetine	0.01	0.02	0.03	0.05	0.08
Range finder	Propranolol	0.1	1	5	10	50
Definitive test	Propranolol	0.05	0.08	0.11	0.17	0.25
Range finder	Ibuprofen	0.1	1	10	100	500
Definitive test	Ibuprofen	75	100	125	150	175
***Problems with iron participating out of the medium have meant that EC50 values for iron were not reliable and therefore have not been included in the results.**						

Table 7-3 Concentrations used in the *Daphnia magna* experiments (metals µg/L and pharmaceuticals mg/L)

Test	Chemical	C1	C2	C3	C4	C5
Range finder	Copper	20	40	60	120	210
Definitive test	Copper	60	90	140	200	300
Range finder	Zinc	10	40	120	430	1500
Definitive test	Zinc	300	750	2000	4500	12000
Range finder	Manganese	500	1500	4500	13500	40500
Definitive test	Manganese	13500	20000	38000	45000	68000
Range finder	Iron	1000	2000	4000	8000	16000
Definitive test	Iron	x	x	X	x	x
Range finder	Diclofenac	0.1	1	5	10	50
Definitive test	Diclofenac	50	70	90	110	130
Range finder	Fluoxetine	0.1	1	5	10	50
Definitive test	Fluoxetine	-	-	-	-	-
Range finder	Propranolol	0.1	1	5	10	50
Definitive test	Propranolol	2.5	5	10	20	40
Range finder	Ibuprofen	0.1	1	10	100	500
Definitive test	Ibuprofen	10	20	40	80	160
***Fluoxetine was only tested once due to availability of the pharmaceutical at the time of testing**						

Table 7-4 Experimentally obtained EC50 values for each chemical for *Daphnia magna* and *Pseudokirchneriella subcapitata* (µg/L).

Chemical	*Daphnia magna* EC50 µg/L	*Pseudokirchneriella subcapitata* EC50 µg/L
Copper	100	23
Zinc	3,460	65
Manganese	28,560	16,230
Iron *	x	x
Diclofenac	69,650	149,000
Fluoxetine	454	31
Propranolol	142,000	469
Ibuprofen	91,170	483,000

*Problems with iron participating out of the medium have meant that EC50 values for iron were not reliable and therefore have not been included in the results.

*See Appendix Section 10.1.3 for data graphs

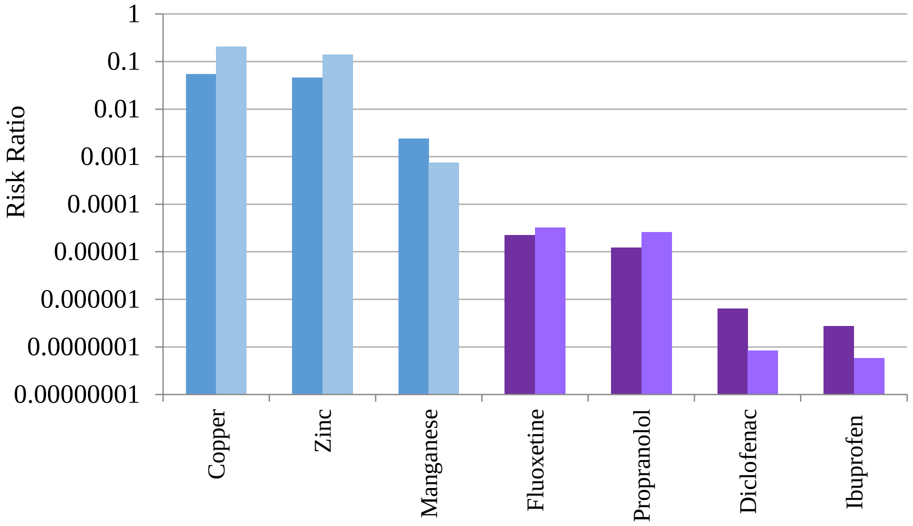


Figure 7-2 Risk-ranking of the chemicals based on their threat to *Pseudokirchneriella subcapitata*. The risk ratios are based on the data from the literature for metals (dark blue) and pharmaceuticals (purple). The risk ratios based on the experimental data I obtained are light blue (metals) and violet (pharmaceuticals).

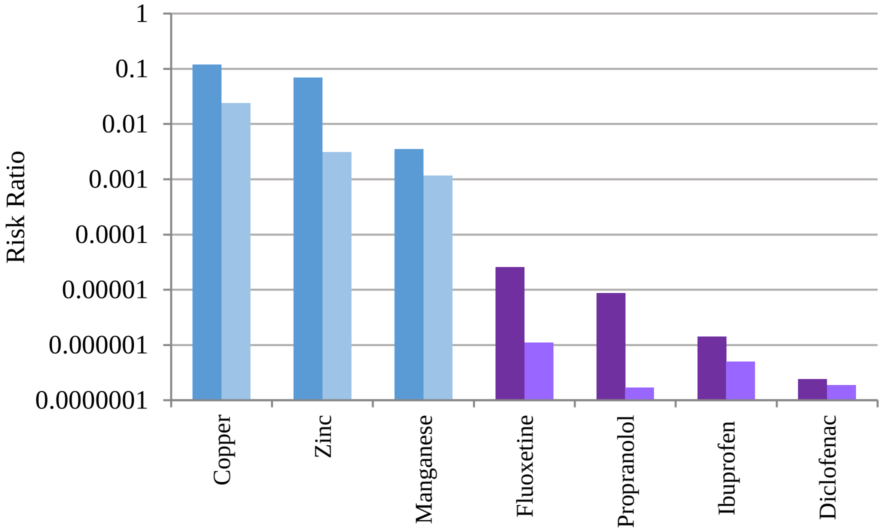


Figure 7-3 Risk-ranking of the chemicals based on their threat to *daphnia magna*. The risk ratios based on the data from the literature for metals (dark blue) and pharmaceuticals (purple). The risk ratios based on the experimental data I obtained are light blue (metals) and violet (pharmaceuticals).

The aim of the experimental work was primarily as a training exercise, as well as a means to understand if the same risk-ranking order was achieved by using an experimental value rather than a median value based on literature data. The EC50 values from the experiments conducted in this study, for all chemicals and both species (Table 7-4), fell within effect concentration ranges reported in the literature. Two risk ratios were calculated for each chemical, both using the median environmental concentrations and either the literature-based effect data or the experimentally-obtained effect data (Figure 7-2, Figure 7-3). The higher the risk ratio, the greater the concern.

7.3.2.1. *Algae*

The risk-rankings calculated using a median effect concentration from the literature and a median environmental concentration give the same ranking as that calculated using an EC50 value calculated from the results of the Microbiotests and a median environmental concentration (Figure 7-2). Copper is the chemical of greatest concern from this group for algae based on either approach, followed by zinc and manganese. The highest ranked pharmaceutical of those used here was fluoxetine followed by propranolol. Based on the data reported in Chapters 4 and 5, where a risk-ranking based on algae and higher plant data (regardless of end-point or exposure), the same risk-ranking is found.

The risk ratio value for each chemical varies slightly, depending on the source of the EC50 values, thus the scale of the potential risk does vary. However, this variation was never greater than 10-fold between the two methods used and the risk ratios for all chemicals are all <1.

7.3.2.2. *Daphnia magna*

Copper was the chemical of greatest concern for *daphnia magna* based on either approach, followed by zinc and manganese (Figure 7-3). The highest ranked pharmaceutical was fluoxetine based on either approach. However, propranolol was the second pharmaceutical of concern based on the literature and fourth based on the experimental ranking. Ibuprofen and diclofenac were ranked 2nd and 3rd (experimental approach) or 3rd and 4th (literature approach). Based on the data reported in Chapters 4 and 5, where a risk-ranking based on invertebrate data (regardless of end-point or exposure), the same risk-ranking of metals and pharmaceuticals is found, apart from the ranking of propranolol.

As with the algae risk ratios, the risk ratio values for *daphnia magna* for each chemical vary slightly per chemical, depending on the source of the EC50 values, thus the scale of the potential risk does vary. However, this variation was never greater than 10-fold between the two methods used and the risk ratios for all chemicals remain <1.

7.3.3. Conclusion to experimental work

The chemical of greatest concern from those tested, to both algae and daphnia, was copper. The pharmaceutical of greatest concern to algae and daphnia was fluoxetine. The proximity between the metals effect concentrations and river concentrations still makes them upwards of 1,000-fold greater risk for both algae and daphnia than the pharmaceuticals. Thus, metals remain the chemical class of greater concern for these organisms, when considered in this context. As discussed previously, the median was used as the best estimate of a typical value, being less influenced by extreme outliers (refer to Chapter 3).

These experiments were conducted to ascertain if the risk-ranking order obtained using only data from the literature could be verified using my own results from simple experimental tests. The results suggest the approach taken in the project to collect as much representative literature as possible to derive a risk-ranking can be supported by the results obtained from fresh laboratory studies carried out *de novo*.

7.4. INDEPENDENT EXAMINATION OF THE METHODOLOGY AT A WORKSHOP

As part of the project, a workshop was organised and delivered to consult established experts on the approach developed. The objective of the workshop was to gain an understanding of whether these experts could support the approach presented to them. Attendees of the workshop included scientific experts (within ecotoxicity) from universities across the UK, colleagues from DEFRA (both familiar and unfamiliar with the project), the Environment Agency, Astrazenaca, and Thames Water, as well as project members from the Centre for Ecology and Hydrology and Brunel University. (The attendance split across the different stakeholder groups was: four attendees from DEFRA, four attendees from industry and eight attendees from UK universities). In preparation for the workshop participants were provided with the two papers published to date from the project. The participants were also asked to complete a pre-workshop task by answering the following question:

If you could only regulate/control one chemical which is routinely discharged into UK freshwaters, which would it be?

This must be on the basis of its direct adverse effects (no, we are not talking about nitrate, phosphate or carbon dioxide). You do not have to be scientific about this, feel free to use your gut instincts! Please do not confer with colleagues, we want your personal opinion.

The pre-workshop question and papers were provided to give participants an indication of the theme of the workshop. It was also emphasised that the workshop was not to be a lecture, but instead the aim was to have group discussions based on the project and topic. The aim of the homework exercise was to get an indication of the thoughts from the attendees. There was no consensus in the answers from participants in response to what they thought was the chemical of greatest concern. (Table 7-5). Even though this homework exercise was a brief consultation, it shows that a room of scientific and industry experts, when asked independently, did not identify a unanimous chemical of greatest concern to UK freshwater ecosystems, thus highlighting the need for this project. The participants were also asked to explain why they had chosen their specific chemical X rather than one selected by other colleagues. However, participants were reluctant to answer this question, even when prompted.

Table 7-5 Chemicals named in response to the pre-workshop task

Chemicals names submitted in response to the homework exercise	Chemical class/use
Aluminium	Metal
Benzo [a] Pyrene	Polycyclic aromatic hydrocarbon
Ciprofloxacin	Antibiotic
Copper	Metal
EE2	Estrogen
Estradiol	Estrogen
Mercury	Metal
Pentabromodiphenyl ether (PBDE#99)	Brominated flame retardant
Tefluthrin	Pyrethroid (pesticide)
Tramadol	Narcotic painkiller
Triclosan	Antimicrobial

The aim of the workshop was to seek advice, feedback and criticism from a room of experts, to ask for input and ideas which might change, disprove or support the ranking. Attendees were encouraged to give feedback on the method developed. The key points from the workshop are summarised are in Table 7-6.

Table 7-6 Key points from the chemical risk-ranking workshop

Key points of discussion from the workshop	Project team response
The data is not comparable as the effect data is not compared based on a like for like i.e. comparison between the same species or the same end-point.	• As broad a range of end-points and species were collected to encompass all effects and species, as some of the common test species may not be the most sensitive, depending on the chemical being tested. • Tests have been conducted on a broader range of species and end-points, thus it seems logical to take advantage of as much information as possible in order to try and best protect aquatic wildlife.
How reliable is the data? Has the reliability of the data been considered?	• This is a possible limitation within our approach, but the reason the quality of that data has not been addressed is to reflect the breadth of evidence. • The ranking is not sensitive to occasional poor studies since we use the median of a large dataset.
73 chemicals have been considered here, have enough chemicals been considered?	• The chemicals have been chosen based on what the community consider their potential threat is to aquatic organisms. The aim was not to consider every chemical but trial the method with a representative sample. • It is difficult to say how many out of the 100,000's of chemicals in use and potentially in the environment this approach could be used for. To some extent the number would be controlled by the availability of data.

What about local issues, they have not been considered.	• The aim of the project was to understand the widespread effect of chemicals to a typical, average UK river. The local situations could be the next stage to be considered.
Water soluble chemicals only can be investigated.	• This approach works for water soluble chemicals, but the concentration of chemicals in sediment has not been considered.
Bioaccumulation is underplayed	• The project considered toxicity more important than bioaccumulation. When the two were considered together by ranking the top BCF chemicals the metals still came near the top. • New EQS values being brought in that are based on the concentration of a chemical in organisms could bring chemicals currently not highly ranked up the agenda to the forefront.
Endocrine disruptors and PBTs are currently the key focus in legislation	• This hasn't been a specific consideration in this project. • The key aspect for wildlife is chemical toxicity in any form. Then this is compared to exposure.
The bioavailability of metals could be taken further by considering BLM data	• Could be considered a further tier two refinement or separate tier three consideration.
Conduct a tier three analyses based on further moderating factors?	• For example, taking a more precautionary approach by ranking chemicals based on the sub-lethal effect data and environmental data (metals considered to BLM level).
Development of tier one or tier two risk-ranking approaches	• An additional consideration at tier one or tier two could be to rank the chemicals based on the ratio between the lowest

	effect concentration and the highest environmental concentration.
Could the sub-lethal data be subdivided further?	• Currently equal weighting has been given to all sub-lethal effect data. Within the sub-lethal data, we could only consider reproductive, growth and developmental effects to see if this changes the ranking. • This may help satisfy the like for like comparisons concerns. • The majority of the sub-lethal data is reproductive, growth and developmental effects. • Should the sub-lethal effect data be split to species level?
Could the chemicals be ranked only by the environmental data?	• Does a ranking based only on the concentration in the environment echo the ranking when effect and environmental data are considered? This was examined further
How does the data compare to available EQS values?	• Use EQS values as the environmental parameter? • How do the environmental medians we have generated compare to EQS values? This was examined further
Taking the investigation further could this approach be used for nutrients?	• This approach could be used to bring i.e. ammonia into the ranking to understand how it ranks in relation to the chemicals currently ranked.
Only interesting effects are reported in the literature, therefore bias could be brought in by using published data.	• The approach cannot go beyond what is in the published literature. Only ‘effects’ are published – uninteresting effects aren’t published thus this data will not be included in our analysis, if this information was included it would be

	assumed that it would increase the median effect value.
How does this approach compare to other approaches?	- Other approaches used in regulation tend to use PEC/PNEC. It is considered that a PNEC is unsuitable as an indicator of relative risk as it is derived from only a small part of the ecotoxicity dataset and involves a variable adjustment factor. This may not be a fair comparator. - This approach is more sophisticated than a crude hazard score but not as sophisticated as a risk assessment. - Its benefits are the approach and the visualisation both of the data and the rankings is very clear.

7.4.1. Further analysis based on the discussion at the workshop

Some of the ideas from the workshop were explored here to assess their impacts. Using linear regression analysis, the relationship between different potential drivers of the ranking where investigated (Table 7-7).

Table 7-7 Methods used to test the ideas arising from the workshop

Point for consideration	**Methods employed**
Would ranking chemicals by environmental data alone pick out the same chemicals as high risk?	Regression analysis
Would ranking chemicals by effect data alone pick out the same chemicals as high risk?	Regression analysis

Does your risk results not merely reflect the number of data points for the effect data and environmental data for each chemical?	Regression analysis
How do EQS values and environmental data compare?	Comparison

7.4.2. Results

The response from the workshop was extremely positive, despite the participants being very vocal about the methods used and their limitations. The new results obtained following the workshop are discussed here.

7.4.2.1. *Environmental data*

Chemicals are found in the environment at varying concentrations. The concentration at which a chemical is found in the environment will vary based on numerous factors such as the usage (both the amount i.e. nanograms, milligrams, tonnes and the frequency) of that chemical, its properties and thus behaviour in the environment. The concentration at which a chemical is found may or may not be a concern depending on the concentration at which the chemical causes an effect on aquatic organisms. This varies from chemical to chemical: 1 mg/L of chemical A could be highly toxic while 1 mg/L of chemical B has no effect. Therefore, the occurrence of chemical A in the ng/L range may cause detrimental effects while the occurrence of chemical B in the mg/l range may have no observed effect on aquatic organisms. Here the question being asked is: does a higher median environmental concentration mean that a chemical is more likely to rank highly (be a greater threat to aquatic organisms), based on the risk-ranking produced from this study.

If the chemicals are ranked based solely on their median environmental concentration, the 5 chemicals that rank highest are iron, LAS, manganese, zinc and aluminium. The majority of chemicals have a median environmental concentration <1µg/L.

A linear regression of log-transformed data was conducted to understand the relationship between a chemical's risk-ranking position (out of the 73 chemicals) and the median environmental concentration calculated for this study (Figure 7-4). There was a significant correlation between the risk-ranking position and the median environmental concentration; (F(1,71)=11.56,$p<0.05$) with an R^2 of 0.14). Although a significant relationship has been found, the low R^2 value indicates a very weak relationship.

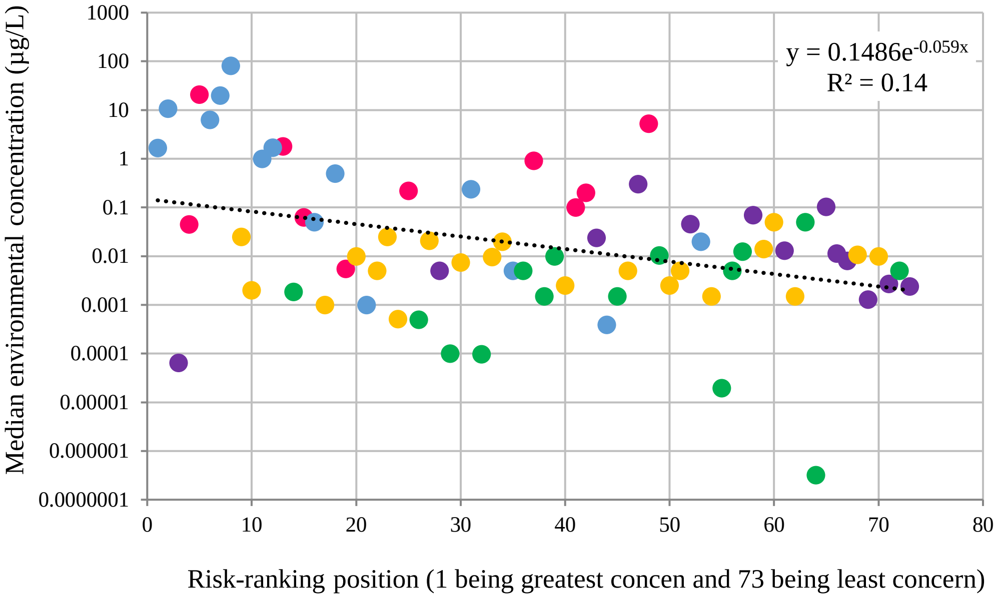


Figure 7-4 Linear regression of the median environmental concentration and a chemicals risk ratio (therefore a chemical's ranking position)

7.4.2.2. *Effect data*

As mentioned above, the concentration at which a chemical has an effect on an organism is not uniform across all chemicals. For each chemical the concentration at which it has an effect on different organisms is also not uniform, nor is the effect it has (as detailed in chapters 4-6). Here the question being asked is: does a lower median effect concentration mean that a chemical is more likely to rank highly, based on the risk-ranking produced from this study.

If the chemicals are ranked based solely on their median effect concentration, the 5 chemicals which cause an effect at the lowest concentration are EE2, PCB 194, chlorpyrifos, PCB 180 and PCB 152. The majority of chemicals have a median effect concentration >10µg/L.

A linear regression of log-transformed data was conducted to understand the relationship between a chemicals risk-ranking position (out of the 73 chemicals) and the median effect concentration calculated for this study (Figure 7-5). There was a significant correlation between risk-ranking position and the effect median (F(1,71)=23.18, $p<0.05$) with an R^2 of 0.25). Although a significant relationship has been found, the reasonably low R^2 value indicates a fairly weak relationship.

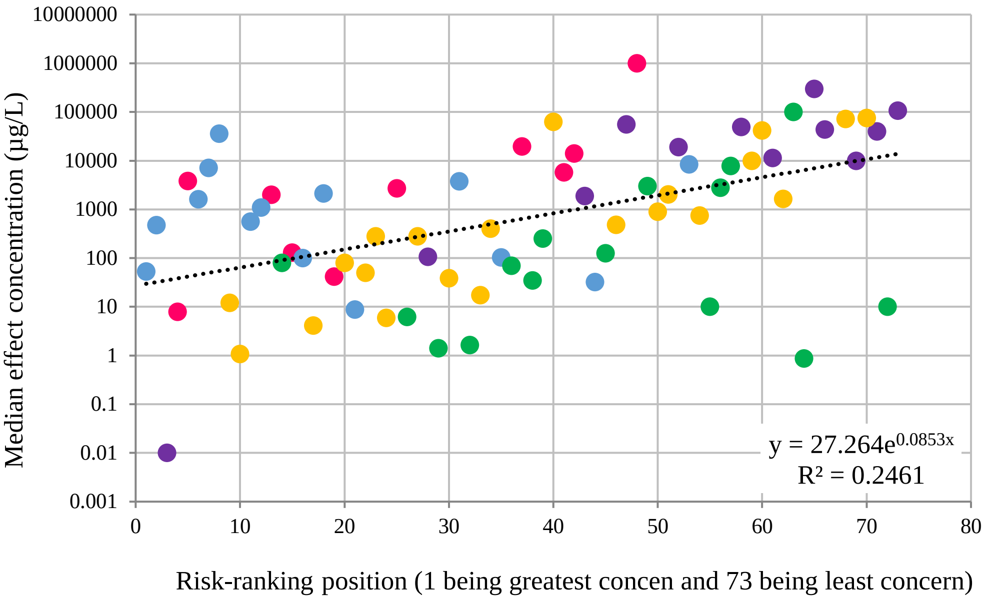


Figure 7-5 Linear regression between the median effect concentration and a chemical's risk ratio (therefore a chemical's ranking position)

7.4.2.3. *Impact of the number of data points*

The data for this study were collated from the scientific literature and various databases. The amount of data available for each chemical, both with regards the effect data and the environmental data, varied from chemical to chemical. The data collated will not be all the data available for each chemical, it will be a sub-set from the literature based on information found using simple search terms, and using available databases such as the WIMS database, FOREGS database, Waterbase and the ECOTOX database. The number of data points used for each individual chemical ranged from 1->1,000. Here the question being asked is: does the number of effect or environmental data points used for each chemical mean that a chemical is more likely to rank highly, based on the risk-ranking produced from this study. Put another way, is there a relationship between ranking based on our approach and number of environmental data points or effect data points used in the analysis?

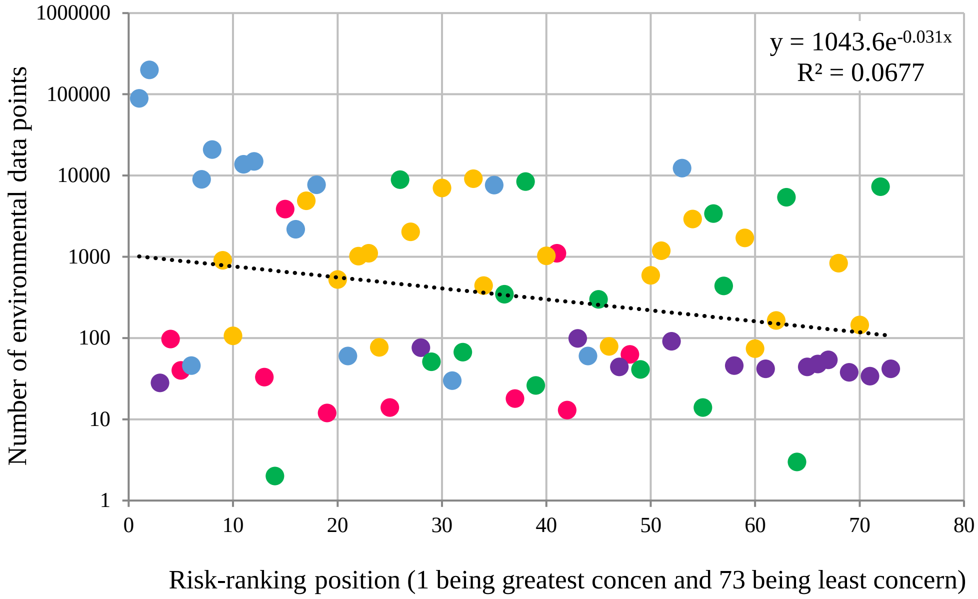


Figure 7-6 Linear regression between, the number of environmental data points for each chemical and a chemical's risk ratio (therefore a chemical's ranking position)

A linear regression of log-transformed data was conducted to understand the relationship between a chemical's risk-ranking position (out of the 73 chemicals) and the number of environmental data points used for each chemical (Figure 7-6) There was a significant correlation between the risk-ranking position and the effect median ($F(1,71)=5.16$, $p<0.05$) with an R^2 of 0.07). Although a significant relationship has been found, the low R^2 value indicates a weak relationship, one could imagine the chemicals of highest concern get measured the most.

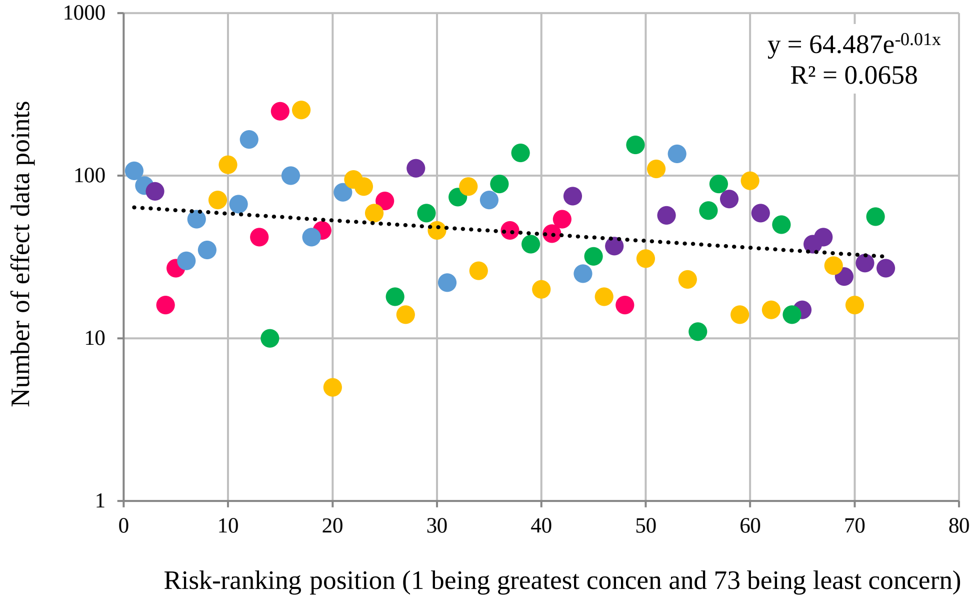


Figure 7-7 Linear regression between, the number of effect data points for each chemical and a chemical's risk ratio (therefore a chemical's ranking position)

A linear regression of log-transformed data was conducted to understand the relationship between a chemical's risk-ranking position out of the 73 chemicals and the number of effect data points used for each chemical (Figure 7-7). There was a significant correlation between risk-ranking and the effect median (F(1,71)=5.00, $p<0.05$) with an R^2 of 0.07). Although a significant relationship has been found, the low R^2 value indicates a weak relationship, one could imagine that more ecotoxicity studies are carried out on chemicals considered of high risk.

7.4.2.4. *EQS values and the median environmental concentration*

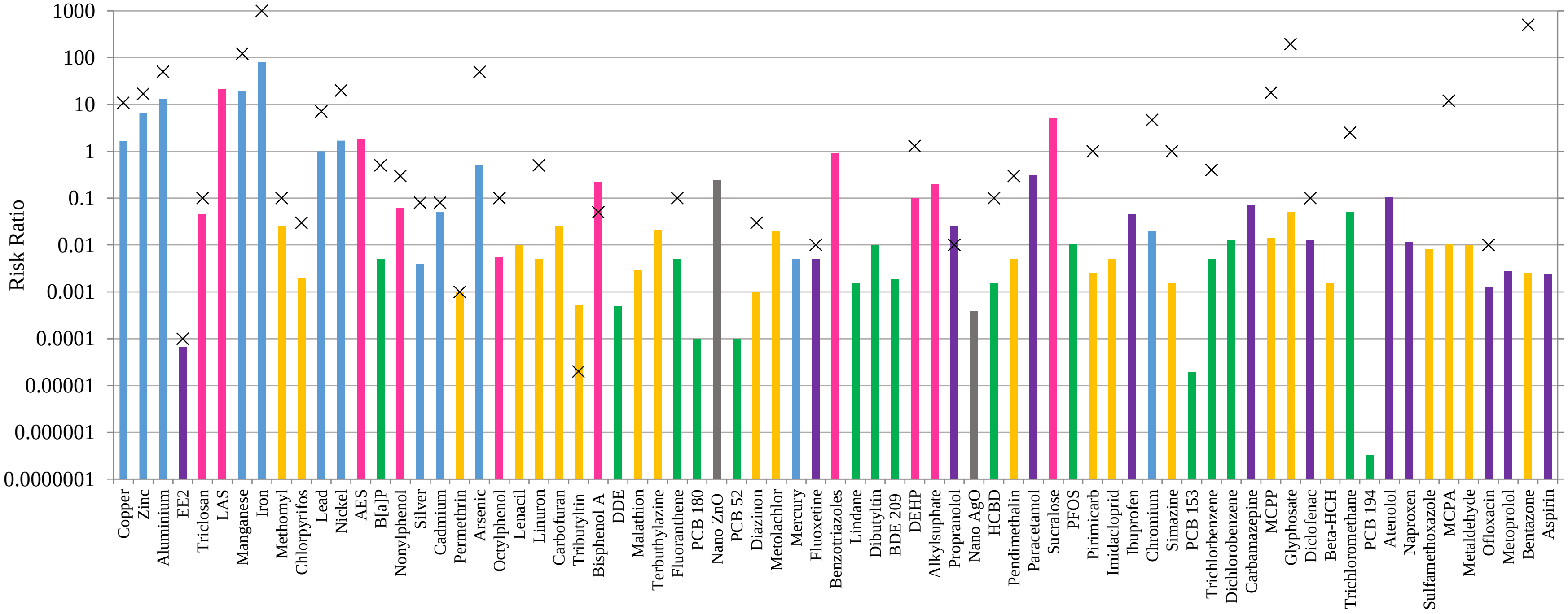


Figure 7-8 Chemicals median environmental data plotted in order of the chemicals risk-ranking position (left to right - higher risk to lower risk, as per Figure 7-1). X **marks the concentration of a specific chemicals EQS, EQS values were sourced from Water Framework Directive & proposed EQS values by Gardner et al (2012)**

For some of the chemicals studied there are established EQS values. Figure 7-8 is the median environmental concentrations collated for this study plotted in the risk - ranking order as detailed in Section 7.2 and Figure 7-1. EQS values are marked with an X. Not all chemicals which are at the higher end of the risk-ranking have established EQS values, i.e. Aluminium ranks 3rd, the EQS value reported here is a proposed EQS, LAS ranks 6th out of 73, it does not have an EQS. The median environmental value for three out the 73 chemicals exceeds the EQS value. Tributyltin (EQS 0.0002 µg/L), bisphenol A (0.05 µg/L) and propranolol (0.01 µg/L). The environmental median reported for these three chemicals are all based on UK data, reported since 2000, sourced from the literature and from the WIMS database.

Using the EQS as an alternative to the median environmental concentration to understand the risk of a chemical would not generate the same risk ranking, and could lead to misplaced concern as some of the median concentrations for the chemicals studied here are significantly lower than the established or proposed EQS value.

7.4.3. Conclusions from the workshop

The workshop proved extremely valuable and allowed the approach and data to be explored further. Following the workshop feedback and analysis of the tests completed as suggested by the workshop participants, the results suggest that although there are significant relationships between the risk-ranking position and the variable considered, there is not a strong relationship between the risk-ranking result and any of the variables.

7.5. WHAT ARE THE HIGHEST RISK CHEMICALS FOUND IN UK SEWAGE EFFLUENT BASED ON THEIR EFFLUENT CONCENTRATIONS?

The potential threat of chemicals to UK freshwater organisms within this project was based on either measured or predicted surface water concentrations, which if possible were data for UK freshwaters.

Sewage treatments plants (STPs) are the main source for many chemicals into the aquatic environment, thus it can be assumed that at the point of entry this is where chemical concentrations will be at their highest, before dilution and degradation in the natural environment. Therefore, sewage effluent could be considered as the more extreme environment in which organisms live with regards to chemicals in the environment (omitting concentrations found at accidental spills, etc). Thus, organisms exposed to sewage effluent are at the most risk.

Gardner et al (2012) sampled the final effluents of 162 STPs across the UK, with each site being sampled 14 or 28 times over a one-year period [82]. The samples were analysed for more than 70 chemicals, including metals, pharmaceuticals, herbicides, and consumer chemicals. Gardner et al (2012) reported 5th, 50th, 95th & 97.5th percentile values for average concentrations of each chemical in those effluents.

Of the 70 plus chemicals included in the Gardner et al (2012) study, 25 of those chemicals were also included in this study. Using the ecotoxicity data collated for this study and the effluent concentrations reported by Gardner et al (2012), a risk ratio for the 25 chemicals was calculated with the aim of comparing the risk ratio for a small selection of chemicals based on a) surface water and b) sewage effluent. The first question this comparison will answer is: what is the potential risk of a selection of chemicals based on effluent concentrations? Secondly, how does this ranking differ from the risk ratio calculated based on surface water concentrations. As areas of high effluent are considered to have higher concentrations of chemicals it could be assumed that if organisms are not at risk in effluent (risk ratio <1) then they are less likely to be at risk in surface water [90]. Finally, this analysis addresses the questions: are the top chemicals of concern the same when effluent concentrations are considered instead of surface water concentrations? Risk ratios have been calculated based on median effect data, environmental data collated for this study and sewage effluent based on the 50th and 95th percentile of the STPs data (Figure 7-9).

7.5.1. Results and Discussion

Of the 25 chemicals, ones that have been highlighted as a potential concern based on the ecotoxicity and environmental data collated for this study include Cu, Zn, EE2 and triclosan (Figure 7-9A).

Based on the median ecotoxicity data and the 50th percentile effluent concentrations, the chemicals of greatest concern present and measured in effluent are Cu, Zn, EE2 and triclosan, with risk ratios of 0.1, 0.07, 0.05 and 0.02, respectively (Figure 7-9B). Based on the median ecotoxicity data and the 95th percentile average effluent concentrations, the chemicals of greatest concern are Cu, EE2, Zn and triclosan, with risk ratios of 0.29, 0.14, 0.13 and 0.08, respectively (Figure 7-9C). Regardless of the source of the environmental data, the same chemicals occur in the top chemicals of concern. Even when using the 95th percentile sewage effluent concentration and the median effect concentration, none of the risk ratios calculated exceed 1.

7.5.2. Conclusion to risk-ranking based on sewage effluent concentrations

The study goes on to compare the average concentrations for each chemical with either existing or proposed EQS standards. Chemicals where the effluent concentration exceeds the EQS or PNEC values in over 50% of STPs are highlighted in Table 7-8.

Table 7-8 - Chemicals of concern based on Gardner et al (2012)

Chemical Class	Chemicals have been prioritised for further consideration on the basis of their concentrations in effluent. These exceeded their EQS or PNEC values in over 50% of the STPs. *chemicals in red are also included in this study
Metals	Zinc
Pharmaceutical	Erythromycin, oxytetracycline, ibuprofen, propranolol, fluoxetine and diclofenac. Steroids — EE2, E2.
Organics	PAHs — fluoranthene, benzo[a]pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene and indeno(1,2,3-cd) pyrene. BDEs — 47 and 99 TBT, Triclosan

The concern around chemicals entering the aquatic environment has initiated research and the introduction of sophisticated tertiary treatments to STPs, thus removing/eliminating chemicals which would otherwise enter into the aquatic environment. However, there are concerns around the introduction of sophisticated tertiary treatments, primarily because they are expensive to build and maintain. It is presumed that the implementation of the advanced treatments and the cleaning up of sewage effluent will improve the ecological quality. What must be remembered is that sewage effluent will also be a major driver in river biodiversity and local ecosystems. The past problems related to highly contaminated effluent have largely been dealt with by increased awareness and implemented legislation.

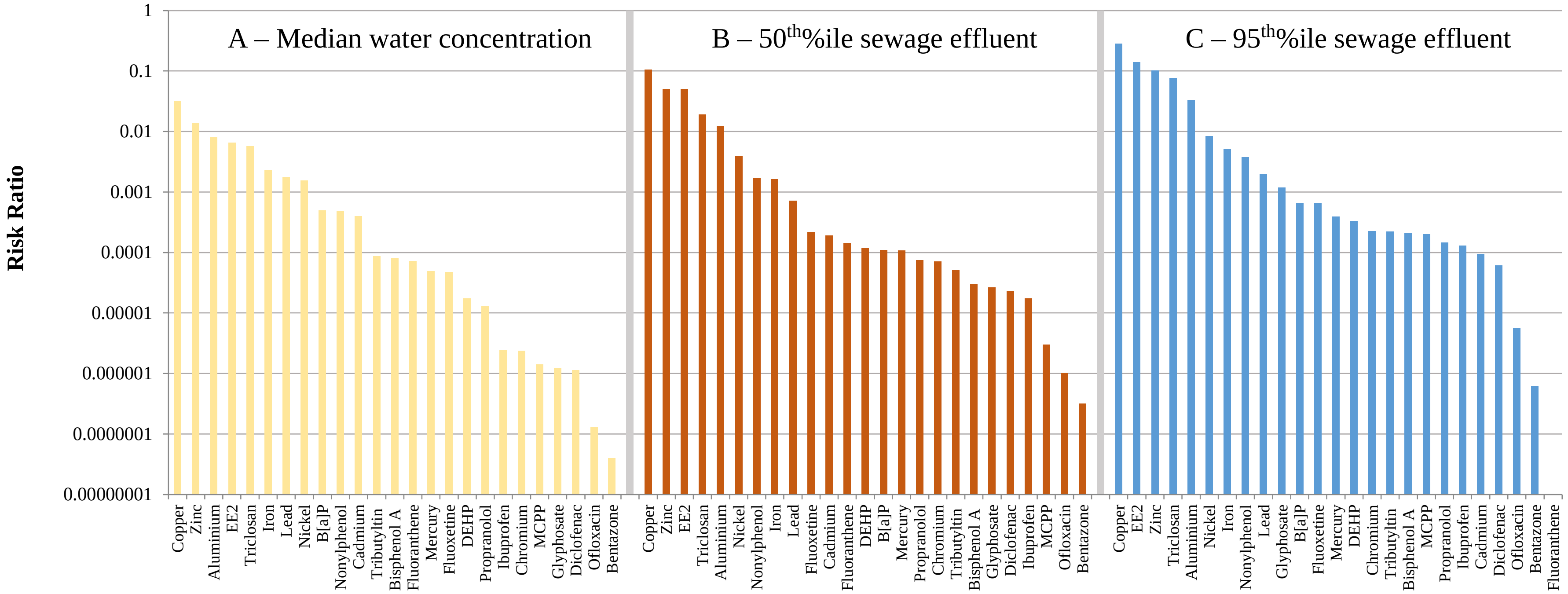


Figure 7-9 Risk Ratios for 25 chemicals based on median effect concentration and (A) median water concentration, (B) 50th%ile sewage effluent concentration and (C) 95th %ile sewage effluent concentration.

7.6. CONCLUSIONS

In this chapter, the literature based chemical risk-ranking was tested both experimentally and through the questioning of external experts.

The experts challenged the ranking by suggesting the same result would occur if it was based only on the toxic concentration, measured concentration, number of available data on ecotoxicity or presence. It turned out all these factors were influential but only in a weak sense. There is further analysis which could be completed on the data and the risk-rankings, to investigate and develop the method further (this will be discussed in the final chapter). This chapter has allowed the data driving the rankings to be tested, with no strong driver being identified behind the ultimate risk-ranking results.

The relative risk-ranking of a selection of the 73 chemicals was compared to relative risk derived from experimental work conducted within the project. Whilst this was primarily a training exercise, it supported the relative ranking.

The comparison of the final project risk-ranking with the risk-ranking based on sewage effluent concentrations identified some of the same chemicals as of concern. The comparison showed that, even when using sewage effluent concentrations, the risks did not exceed 1 for any chemical, although this is based on the median ecotoxicity value rather than a higher percentile. This issue will be discussed further in the final chapter with regards to the degree of concern that chemicals pose compared to the risk of other environmental stressors.

8. FINAL DISCUSSION

8.1. INTRODUCTION

Freshwater is an extremely important resource which is under threat globally from a multitude of stressors [10]. There is a consensus, at least in developed societies, that social and economic development should not come at the expense of the environment. Understanding which stressor is of greatest concern is a challenge to environmental scientists, industry and government. How to tackle the multiple stressors and where to place research efforts or funding to best conserve and/or preserve freshwater environments are difficult questions to answer [11, 250]. Chemicals are just one of the many environmental stressors which threaten aquatic organisms. Understanding their impact on aquatic organisms is an ongoing challenge facing scientists and policy makers alike [251-254]. Their production and consumption, globally, is not likely to decrease, therefore the number of chemicals potentially entering the freshwater environment is likely to increase. Chemicals enter the freshwater environment via direct or indirect routes. Established processes are in place to reduce the contamination of freshwater from some chemicals [255]. However, the removal of all chemicals from sewage effluent is currently not feasible, due to time and financial limitations, as well as the unpredictable contamination via indirect sources. Further, some chemicals are very resistant to degradation. So where should we focus our efforts and why? Ultimately the aim is to be able to preserve the quality of water bodies from the influence of industry, agriculture, urban development and recreational use etc [256].

8.2. THE OBJECTIVE

The objective of this study was to compare and rank different chemicals, which are already on the market, against one another on the basis of risk in order to understand which is of potentially the greatest threat to freshwater organisms. It is important to note this is a different task from traditional risk assessment which has as its aim the absolute avoidance of risk to all wildlife. Thus, a traditional risk assessment will only focus on the data showing effects at the very lowest concentrations. Nevertheless, most attempts to do risk assessment and prioritise chemicals take some form of exposure versus hazard. In the literature, there are 1,000's of papers which, via different approaches, identify, prioritise, rank or list chemicals of concern to the aquatic environment. These methods range from simple assessments to complex processes. Assessing risk on the basis of exposure versus hazard is logical and so it might be assumed that there would be consensus between scientists and regulators worldwide on the chemicals that we should do our utmost to study, manage and control. There is currently no scientific consensus on the best method to assess hazardous chemicals; there are advantages and disadvantages in most approaches [73]. A traditional risk assessment process is designed from the outset to be precautionary, and considerable emphasis is placed on the lowest reported effect concentrations and highest reported/predicted river water concentrations. This is done to ensure there is a significant margin of safety between the concentration of a chemical found in the environment and the concentration at which it is known to have an effect. Globally, however, different approaches are used by regulators to derive protective water quality guidelines. For example, in the EU, when calculating a water quality guideline concentration for a chemical (usually an EQS), safety factors of 100, 50 or 10 are applied. Safety factors are used in risk assessment if there are concerns over insufficient data for a chemical. They are also a means to account for the uncertainty involved in extrapolating from either from one species to others, short to long exposure times, acute to chronic effects, chronic to ecosystem effects, and effects in one ecosystem to those in another. However, concerns have been raised about the over-precautionary nature of their approach based on the deviation of EQS values derived using large safety factors, which can lead to unachievable targets and high cost expenditures [257]. In Australia and New Zealand, safety factors are not applied to the 5% hazardous concentration (HC5) derived from a species sensitivity distribution for a chemical [236].

The approach focused on reported effects to understand which chemical is of the greatest concern based on ‘typical’ values, rather than precautionary values. The reason for this approach being to remove the onus on the data that occurs at the extremes – i.e. the lowest effect concentrations and the highest reported or predicted river water concentrations, thus giving weight to the typical value at which a chemical causes an effect and the typical concentration at which it is found in the environment. For this study the median of the ecotoxicity dataset was compared with the median water concentration with the proximity of the two indicating the degree of risk. In this way, whilst even the more doubtful studies purporting to show effects at low concentration, or surprisingly high water concentrations are included, these do not have an excessive influence on the median (as they might on the mean, or even more the extreme percentiles, such as 5%ile, 95% etc). Thus, the chemicals selected as being of high risk imply a danger to a very wide range of organisms in a very wide range of locations. Therefore, in an attempt to create a fair and reliable chemicals risk-ranking protocol, as much ecotoxicology and water data as possible was included. The approach which was developed for this project may not be to everyone’s taste, it was not developed via the traditional ‘avoidance of risk PNEC’ route but it is defendable. Its intention was to be a thought provoking, transparent assessment of the data we have on chemicals which have been suggested as posing a threat to aquatic ecosystems.

8.3. DISCUSSION

8.3.1. Chemicals of concern

This study has identified copper, zinc, triclosan and EE2 as the highest ranked chemicals, based on a refined interpretation of the collated ecotoxicity data and environmental data. With regards chemical classes, metals and pesticides have dominated the higher rankings. These chemicals, and others included in this study, have been investigated (using different approaches) and identified by others as chemicals of potential concern, both within the UK, the EU and globally. Guillén et al (2012) provide a summary of different approaches to chemical prioritisation [55]. Von der Ohe (2011) assessed the risk of 500 contaminants as potential river basin specific pollutants (RBSP) under the WFD, on the four river basins of Danube, Elbe, Scheldt and Llobregat. The study highlighted the exceedance of PNEC values for 38% of the chemicals at a minimum of one site (a risk ratio greater than 1). The frequency of exceedance, however, for some chemicals was up to 88%. The chemical class appearing as the greatest concern was pesticides [63]. As previously mentioned, Gardner et al (2012) highlighted concern within the UK for zinc, fluoranthene, B[a]P, TBT, triclosan, ibuprofen, fluoxetine, diclofenac and EE2 based on sewage effluent concentrations [82]. Rule et al (2006) investigated the occurrence of priority substances in crude influent entering STPs in England. Metals were found to occur in all wastewaters, while pesticides were often below the limit of detection [258]. Margot et al (2015) discuss the fate of pollutants and their likelihood to enter the aquatic environment [255]. Lopez et al (2013), established risk indexes based on PEC/(PNEC or EQS). Based on this approach significant risks were reported for zinc, copper, nickel and barium for the inorganics, while for organics terbuthylazine, diazinon, MCPA, chlorpyrifos and lindane were considered of concern. Lopez et al (2013) clearly state the concern and limitations of using PNEC values and emphasize that the use of PNEC values could cause risk assessment to be very conservative [256]. Based primarily on a chemicals exposure categorization, Gotz et al (2010) identified potential relevant microcontaminants for monitoring purposes in Switzerland. This included atenolol, benzotriazole, diclofenac, sulfamethoxazole, BPA, nonylphenol, simazine and terbuthylazine [259]. Kuzmanovic et al (2015) prioritised 200 organic pollutants for four Iberian rivers using a ranking index (RI) based on measured environmental concentrations and EC50 values. Chlorpyrifos, chlorfenvinphos, diazinon, dichlofenthion, prochloraz, ethion carbofuran, diuron, nonylphenol and octylphenol were highlighted as being of concern. Kuzmanovic et al (2015) emphasised the need to understand the risk of chemicals both at a regional and local scale [65]. Tsaboula et al (2016), using MEC_{max}/PNEC, identified pesticides considered to be candidates for the RBSP based on findings from the Pinios River Basin. The pesticides which ranked highest based on level of environmental risk included linuron, terbuthylazine, methomyl, imidacloprid and pendimethalin [237]. There is a certain amount of consideration that needs to go into why pesticides, notably insecticides tend to feature so highly in these assessments. They are designed from the outset to be toxic, if not very toxic, so the hazard they represent is clear. What is less clear is exposure. The major source is diffuse (agriculture) but they are not used everywhere all the time, so the major exposure is episodic. Thus, the question is should we prioritise chemicals which would be rarely if ever encountered by most aquatic wildlife? Is this a higher risk to a slightly less toxic chemical which is a more common presence in water?

Research projects and papers (such as the ones mentioned above) were conducted based on their own specific objectives. Ecotoxicity data will have been collated and considered based on parameters specific to that project. Environmental data, whether measured or modelled, will have been based on a specific country, region or river basin. As a consequence of this selectivity, it would not be likely to get an identical risk-ranking from one published report to another. However, the simple approach used here has identified some of the same chemicals of concern as other studies, using different data and a different approach.

8.3.2. Factors to consider with risk-ranking approach

As with any approach, given the inevitable limitation of time and resources compromises have to be made. The risk-ranking exercise reported here was limited to only 73 chemicals which were selected as of high concern from the different chemical groups. The methodology can be used only for chemicals for which there is information on their effects. Similarly, it can only deal with chemicals whose environmental concentrations have been measured or modelled.

The data: For individual chemicals there was a varying amount of data available, with regards to both the ecotoxicity data and the data on a chemical's occurrence in the environment. This approach has tried to be holistic with regards to the inclusion of data. To reflect the current state of knowledge and take advantage of all the available data, any species or end-point was considered. Often the potential risk of a chemical is based on end-points such as NOEC or LOEC data, which has its own benefits and limitations [260]. However, the use of only specific (well-known) end-points might exclude important effects which may be harmful. This approach was also inclusive with regards the test organisms included in the study. Often only specific test organisms, (i.e. OCED guideline species) are used in risk assessments. A lot of the data included in this study will be based on the standard test species. However, some other species have been found to be more sensitive than the OECD test species to some chemicals. It was deemed appropriate to include data from all species, as they are all potentially exposed to chemicals in the freshwater environment [34].

The ranking is only as reliable as the amount and quality of the data will allow. The approach was developed to be as unbiased as possible with regards the collating of data from the literature. The input of new data into the dataset for each chemical may alter its ranking position, particularly where little currently exists, such as for the pharmaceuticals. More sensitive end-points may be reported, or the concentrations found in the environment may increase or decrease, thus altering the median values and therefore the ultimate risk ratio.   However, it should be noted that a median is much less likely to change significantly than a ranking based on a PNEC which is dominated by only a few references of effects at low concentrations. Ideally, the ranking would be based on equal amounts of data for each chemical, with comparable tests completed so that the comparison is made on an equal basis. This is not possible for a lot of chemicals, as some have only recently been the subject of investigation.

In this risk-ranking approach the quality of the information is currently not assessed, and it may influence the ranking of a chemical. This would have been one moderating factor which could have been included, thus only including studies which meet very strict standards, such as those suggested by Harris et al (2014) [75]. However, many ecotoxicology studies do not meet the highest standards, so by dismissing such information it may lead to few or no values remaining in your database! Also the interpretation of quality can bring its own bias [254].

Exposure via the water: This approach considered chemical exposure via water. There are two problems with this approach where hydrophobic pollutants are concerned; firstly, the realistic route of exposure in the wild would largely be via contaminated food and sediment (not water), and secondly, the water concentration of the chemical, due to its high partition coefficient, would be very low and difficult to measure. Thus, both the hazards and presence of such POPs may be underestimated.

An important aim underlying the project was to reflect the average or typical situation of British lowland rivers. This should work well for chemicals which are ubiquitous and come from the domestic population via sewage treatment plants. However, it is less clear how to handle the very toxic but rarely encountered insecticides used in agriculture?

This project has relied on water measurements reported in the open scientific literature , as well as modelled values for pharmaceuticals, information from the EA WIMS database and Waterbase database. The number of data points varies from 1->1,000. An advantage of the WIMS data is that it is collected from across the country and thus provides a fair reflection of typical UK water concentrations. This is in contrast to the scientific literature where monitoring may have focused on problem ‘hot-spots’. However, there could be a number of problematic issues in comparing the environmental data from one chemical to another:

- How to deal with values that are <LOD? In this project, they were all used and recorded as ½ the LOD?
- In the data how many values for each chemical are below a LOD? If these are more than 50% then they could be having a big impact on the median and the risk-ranking?

Moderating factors: It was originally thought that the simple approach of using all the ecotoxicity and environmental data to do risk-ranking was far too simple and introducing a range of moderating factors would change the result. These moderating filters included excluding metals ecotoxicity data outside neutral pH, using only recent UK environmental data, excluding lethal or sub-lethal ecotoxicity data or only including chemicals with a high bioconcentration factor. Throughout these changes copper and zinc remained at or near the top of the risk-rankings and this seemed to be an unequivocal message. The moderating factors included in this approach were chosen so that filtering of the data could be done based on clear, definable boundaries. As detailed in Chapter 6, even with this intention, there were still factors, such as the categorisation of reported effects on growth for algae, which were not clear-cut.

8.4. CONCLUSION

Which chemical is of greatest to concern to freshwater organisms? If a fish could vote, which chemical would he or she choose as the biggest threat? These are the key questions to answer if we are to best protect aquatic organisms from chemicals. The occurrence and potential threat of chemicals is a known problem, but science is often driven by external factors, and thus the answers to some of the large, cross-disciplinary questions have not been answered. The hype and attention following a trend can be misleading or can be justified. Current examples are the high degree of concern over nanoparticles and microplastics. The phrase ‘emerging contaminants’ suggests a never-ending issue of anxiety. Yet, the missing ingredient is context. Nano zinc oxide maybe toxic but is it the same or more dangerous than other contaminants of the water environment? If we don’t compare chemicals by using a standard approach using all the resources which we have, how can we compare the effects of them? The benefit of the approach applied here is it uses all the data available, and gives that context as we compare relative risk. In the majority of cases we don’t know if any of these chemicals are actually harming wildlife in rivers. But the dramatic difference in risk and hence potential impacts on wildlife revealed by this analysis of data seems to make a complex situation very much simpler, because it identifies the chemicals for which control would be appropriate. This project did not aim to be a risk assessment, but rather a means of comparing the potential threat of a chemical in relation to another chemical, regardless of its class. If the motivation for studying chemicals is protecting the environment, then basing their potential risk on all the available information reporting effects seems sensible.

The approach developed here essentially started from a blank sheet of paper. Its main principles, achievements and conclusions were:

- Stay within the data (no use of complex scoring systems)
- Rely on as wide a dataset as possible (not simply the lowest effect concentrations)
- Find the fairest and most robust method to compare chemicals
- Collated from the literature >4,000 ecotoxicity data points for a range of aquatic species for 73 chemicals
- Collated >300,000 environmental data points from the literature and databases for 73 chemicals
- Read and used >1000 papers to understand chemical risk and collated the data for analysis

- Ranked metals as the chemical class of greatest concern, followed by pesticides – based on the risk ranking approach developed.
- Highlight that pharmaceuticals (generally) do not rank highly compared to other chemical classes.
- Highlight the challenges and limitations of chemical risk assessment, depending on the approach used, the data and an individual’s subjectively.
- Discussed and presented the method and results to scientific, industry and government experts.

8.5. FUTURE WORK

Although it was not the focus of the risk-ranking carried out here, there is another way the data could have been used and interpreted. It will be noted that for more than half of the test chemicals some of the measured river data exceeded some of the reported effect concentrations. This implied actual harm could be occurring in some rivers. So, a next stage would be to find some way of quantifying this overlap and giving it a simple value, much like the risk-ranking ratio. This degree of overlap could be a final and decisive factor in which should be our priority chemicals.

It would be of interest to explore further whether chemicals such as copper and zinc would turn out to be the highest risk-ranked chemicals of concern in other river networks in Europe, China/Asia and America?

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10. APPENDIX

10.1.1. Regulations

Outline of the regulations related to the freshwater environments

The WFD was established to ensure that the EU aquatic environments are preserving, protecting and improving the quality of the environment – based on a precautionary principal and on the principles that preventive action should take place and that environmental damage should be rectified.

The WFD is complemented by other, more specific, EU laws. Detailed below is a list of the legislations which are in place to a) manage and protect water resources and b) actions in place specifically related to the discharge of substances into water resources.

EU water resources protection plan

Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: A Blueprint to Safeguard Europe's Water Resources [COM(2012)673]

Water protection and management General framework

- Urban waste water treatment
  Directive 91/271/EEC – urban wastewater treatment

  It aims to protect the environment in the European Union (EU) from the adverse effects (such as eutrophication Eutrophication: enrichment of water by nutrients causing, among other things, an accelerated growth of algae which disturb the balance of water organisms and the water quality.) of urban wastewater

- Flood-risk management in the EU

Directive 2007/60/EC of the European Parliament and of the Council of 23 October 2007 on the assessment and management of flood risks.

It sets out rules to halt deterioration in the status of European Union (EU) water bodies and achieve good status for Europe's rivers, lakes and groundwater by 2015.

- Good-quality water in Europe (EU Water Directive) water protection and management

  Directive 2000/60/EC – framework for Community action in the field of water policy

- Addressing water scarcity and droughts in the EU

  Communication (COM(2007) 414 final) – addressing water scarcity and droughts in the EU

  It recognises the major challenges caused by water scarcity and medium- or long-term droughts in the European Union (EU) and provides guidelines for addressing them.

Specific uses of water

- Drinking water — essential quality standards

  Directive 98/83/EC — quality of water intended for human consumption

  It sets standards for drinking water. It aims to protect public health from the adverse effect of any contamination by ensuring water for human consumption. Water for human consumption: water in its original state or after treatment intended for drinking, cooking, preparing food or other domestic purposes. It may be supplied from a tap, tanker, bottle or container.

- Water suitable for fish-breeding

  Directive 2006/44/EC lays down quality criteria applying to water-courses and lakes. Compliance with these criteria is essential in order to maintain or improve water quality and to safeguard fresh water fish species. Update: Regulation No EC 1137/2008

  The quality of fresh water is essential for aquatic life. In order to ensure that fish populations living in water-courses and lakes develop in a balanced way, the European Union (EU) lays down quality criteria applying to designated waters. Compliance with these criteria enables pollution to be reduced or eliminated, and various fresh water fish species to be maintained at balanced levels.

- Bathing water quality

  Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC.
  **Directive 2013/64/EU amends Directive 2006/7/EC*

  This Directive enables water monitoring and management measures to be improved, and information to be made available to the public.

- Quality of shellfish waters

  Directive 2006/113/EC on the environmental quality of shellfish waters

  In this way, it seeks to safeguard certain shellfish from the harmful effects of discharges of pollutants into the seas.

Discharges of substances

- Community strategy concerning mercury

  Communication from the Commission of 28 January 2005: "Community Strategy concerning Mercury" [COM(2005) 20 final – Official Journal C 52 of 2 March 2005].

- Protection of groundwater against pollution

  Directive 2006/118/EC on the protection of groundwater against pollution and deterioration (Groundwater Directive)

- Safer detergents for European consumers

  Regulation (EC) No 648/2004 on detergents

- Protection of the aquatic environment against discharges of dangerous substances (until 2013)

  Directive 2006/11/EC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community.

- Environmental quality standards applicable to surface water

  Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC

- Industrial emissions

  Directive 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control).

- Fighting water pollution from agricultural nitrates

Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources

- Tackling threats posed by chemicals (Stockholm Convention)
  Council Decision 2006/507/EC of 14 October 2004 concerning the conclusion, on behalf of the European Community, of the Stockholm Convention on Persistent Organic Pollutants.

REACH

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC

REACH applies to substances manufactured or imported into the EU in quantities of 1 tonne or more per year. Its aims are:

To provide a high level of protection of human health and the environment from the use of chemicals.

To make the people who place chemicals on the market (manufacturers and importers responsible for understanding and managing the risks associated with their use.)

To allow the free movement of substances on the EU market.

To enhance innovation in and the competitiveness of the EU chemicals industry.

To promote the use of alternative methods for the assessment of the hazardous properties of substances e.g. quantitative structure-activity relationships (QSAR) and read across.

The Derivation of Environmental Quality Standards

Under the EU legislation, the UK and other member states have an obligation to derive environmental quality standards for chemicals which pose a threat to human and environmental health.

As knowledge has increased, the method to approach and derive an EQS has changed,

Figure 10-1 and Figure 10-2 detail the process of EQS derivation and the current assessment or safety factors applied to the available data to take account of uncertainly.

Compliance with the EQS should permit reduction in the costs of treating surface waters used for drinking water production, as well as improve the health of the aquatic environment and thus the health of organisms living in these waters and of livestock drinking these waters. The EQS must be respected in order to achieve good surface water chemical status.

Substances or groups of substances identified as priority pollutants on account of the substantial risk they pose to or via the aquatic environment (Directive (2008/105/EC)) have established EQS.

A threshold for the average concentration of the substance concerned calculated from measurements over a one-year period. The purpose of this standard is to ensure protection against long-term exposure to pollutants in the aquatic environment;

A maximum allowable concentration of the substance concerned, i.e. the maximum for any single measurement. The purpose of this standard is to ensure protection against short-term exposure, i.e. pollution peaks.

Under the WFD (Directive 2000/60/EC) priority substances are defined: currently there are 45 in total (Table 10-2): 33 were specified by Decision 2455/2001/EC, and a further 12 by amending Directive 2013/39/EU. Seven of the original 33 priority substances have had updated EQS based on updated scientific information. (Directive 2013/39/EU).

An established Watch List enables Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises (Directive 2013/39/EU). The first watch list established in 2014 include three pharmaceutical substances (Diclofenac, 17-beta-estradiol (E2) and 17-alpha-ethinylestradiol (EE2)). The watch list is to be updated every 2 years, with the caveat that a continuous watch list monitoring period for any individual substance may not exceed four years.

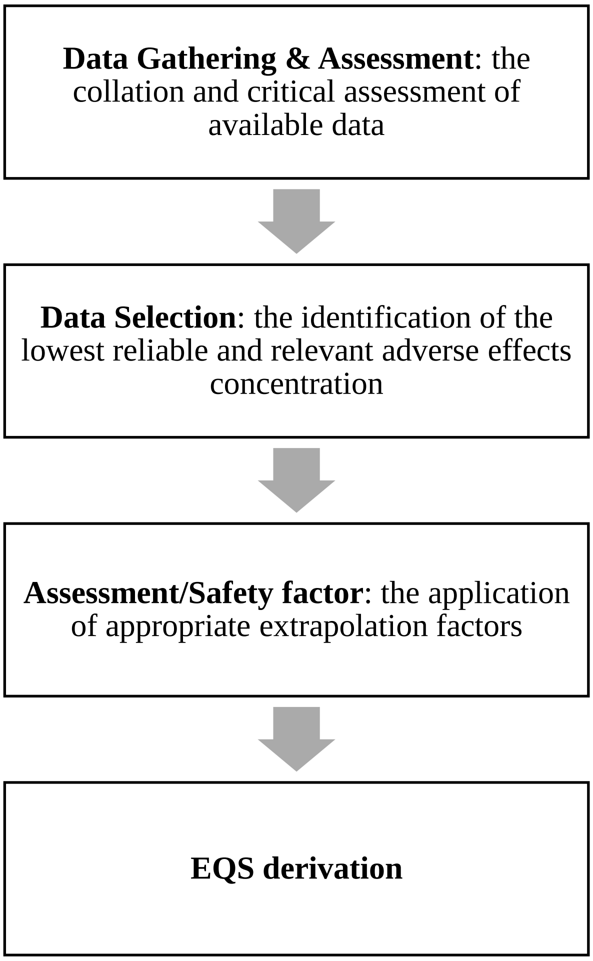


Figure 10-1 Derivation of Environmental Quality Standards

Available data	**Assessment/ Safety factor**
At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae) (i.e. base set)	1000
One long-term EC10 or NOEC (either fish or Daphnia)	100
Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1
Field data or model ecosystems	Case by case review

Figure 10-2 Safety factors to be applied to aquatic toxicity data for deriving an EQS for freshwater

10.1.2. Methodology

Table 10-1 Papers collated from the Web of Knowledge, using two searchers to test publication collection method

Search Terms – copper + toxicity + water + laboratory → **most recent**	
[261]	Assessment of toxicity in waters due to heavy metals derived from atmospheric deposition using Vibrio fischeri.
[262]	Calcium nitrate addition to control the internal load of phosphorus from sediments of a tropical eutrophic reservoir: Microcosm experiments
[263]	Toxicity of metal-ethylenediaminetetraacetic Acid solution as a function of chemical speciation: an approach for toxicity assessment.
[264]	Copper oxide nanoparticles can induce toxicity to the freshwater shredder Allogamus ligonifer
[265]	Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm Eisenia fetida?
[266]	Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice
[267]	Ecotoxicological assessment and evaluation of a pine bark biosorbent treatment of five landfill leachates
[268]	Selection for Cu-tolerant bacterial communities with altered composition, but unaltered richness, via long-term Cu exposure."
[269]	Effects of copper on growth, radial oxygen loss and root permeability of seedlings of the mangroves Bruguiera gymnorrhiza and Rhizophora stylosa
[270]	Biomarkers of metal toxicity and histology of Perna viridis from Ennore estuary, Chennai, south east coast of India
[91]	Toxicity of Copper to Early-life Stage Kootenai River White Sturgeon, Columbia River White Sturgeon, and Rainbow Trout
[271]	Electronic access summary for "Metal (Pb, Cd, and Cu)-induced reactive oxygen species accumulations in aerial root cells of the Chinese banyan (Ficus microcarpa).
[272]	Poly-alpha,beta-DL-Aspartyl-L-Cysteine: A Novel Nanomaterial Having a Porous Structure, Special Complexation Capability for Pb(II), and Selectivity of Removing Pb(II).
[273]	Use of the Multispecies Freshwater Biomonitor to assess behavioral changes of Poecilia reticulata (Cyprinodontiformes: Poeciliidae) and Macrobrachium lanchesteri (Decapoda: Palaemonidae) in response to acid mine drainage: laboratory exposure
[274]	Heat stress effects on toxicity of copper and oxytetracycline on the marine protozoa Euplotes crassus in a climate change perspective
[275]	Possible environmental impacts of recycled glass used as a pavement base material.
[276]	Evaluation of the toxic effects of arsenite, chromate, cadmium, and copper using a battery of four bioassays
[277]	Subcellular distribution and toxicity of cadmium in Potamogeton crispus L
[278]	Interactive effects of phosphorus and copper on Hyalella azteca via periphyton in aquatic ecosystems
[279]	Environmental hazard of oil shale combustion fly ash
Search Terms - copper + toxicity + water + laboratory → **highest citations**	
[280]	Mulligan, C. N., R. N. Yong, et al. (2001). "Surfactant-enhanced remediation of contaminated soil: a review

[281]	Glutathione, glutathione-dependent and antioxidant enzymes in mussel, mytilus-galloprovincialis, exposed to metals under field and laboratory conditions - implications for the use of biochemical biomarkers
[282]	Technical basis and proposal for deriving sediment quality criteria for metals
[283]	Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals
[284]	A biotic ligand model predicting acute copper toxicity for Daphnia magna: The effects of calcium, magnesium, sodium, potassium, and ph
[285]	Aquatic insects and trace-metals - bioavailability, bioaccumulation, and toxicity
[286]	Comparative strategies of heavy-metal accumulation by crustaceans - zinc, copper and cadmium in a decapod, an amphipod and a barnacle
[287]	Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa
[288]	The allium test - an alternative in environmental-studies - the relative toxicity of metal-ions
[289]	Acute and chronic toxicity of copper to 4 species of daphnia
[290]	Toxicity of single walled carbon nanotubes to rainbow trout, (Oncorhynchus mykiss): Respiratory toxicity, organ pathologies, and other physiological effects.
[291]	Waste-water treatability potential of some aquatic macrophytes - removal of heavy-metals
[292]	Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations
[293]	Bioavailability and toxicity of dietborne copper and zinc to fish
[292]	Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations
[294]	A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment
[295]	Extrapolation of the laboratory-based oecd earthworm toxicity test to metal-contaminated field sites
[296]	Inflammatory effects of coarse and fine particulate matter in relation to chemical and biological constituents.
[297]	Evidence for iron, copper and zinc complexation as multinuclear sulphide clusters in oxic rivers.
[298]	A field-study of metal toxicity and accumulation by benthic invertebrates - implications for the acid volatile sulfide (avs) model.

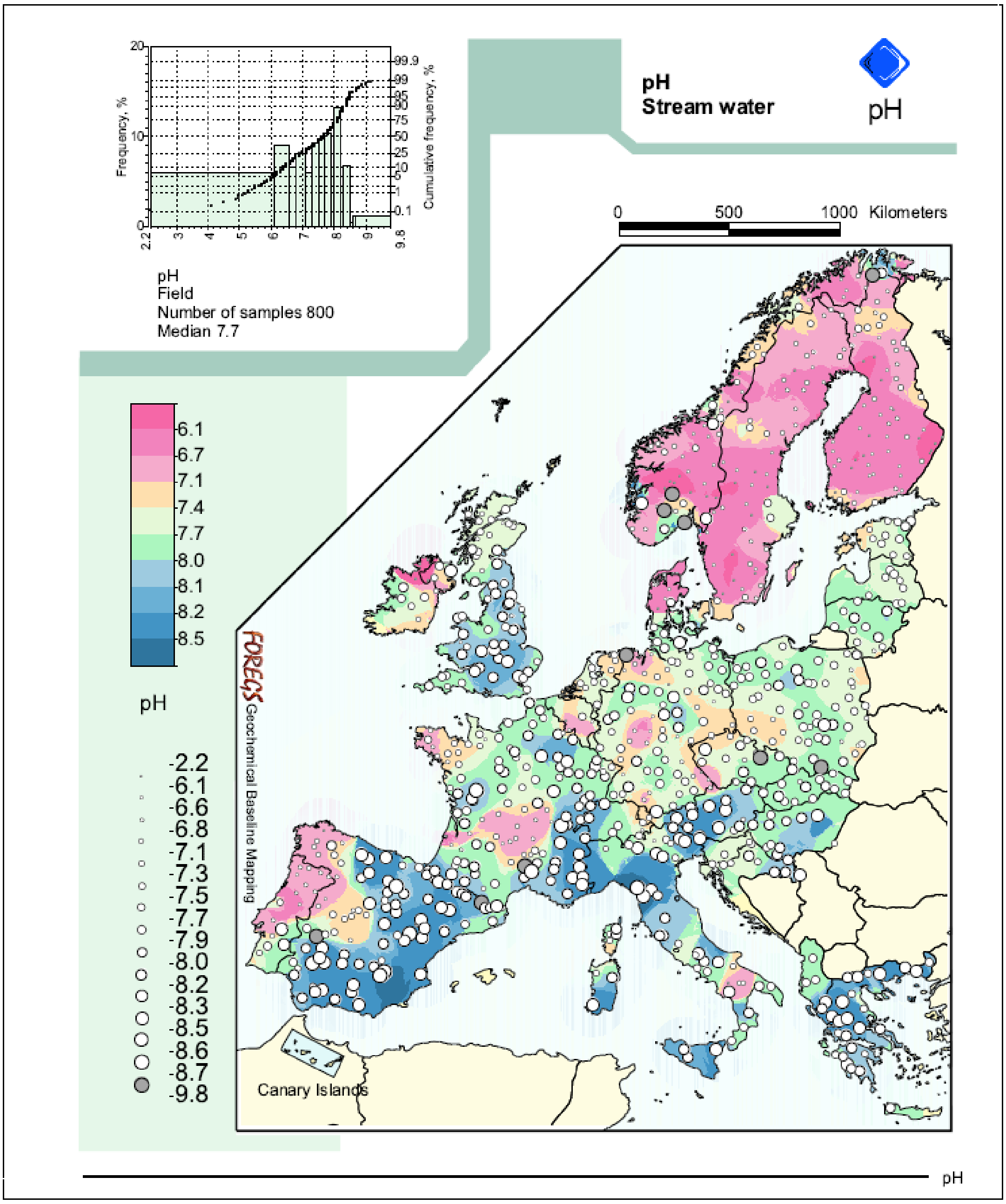


Figure 10-3 pH of stream water across Europe – FOREGS project [87]

Table 10-2 Priority Substances under the Water Framework Directive (WFD)

Name of priority substance	Identified as priority hazardous substance
Alachlor	
Anthracene	X
Atrazine	
Benzene	
Brominated diphenyletheriv	X
Pentabromodiphenylether (congener numbers 28, 47, 99, 100, 153 and 154)	
Cadmium and its compounds	X
Chloroalkanes, C10-13 iv	X
Chlorfenvinphos	
Chlorpyrifos (Chlorpyrifos-ethyl)	
1,2-Dichloroethane	
Dichloromethane	
Di(2-ethylhexyl)phthalate (DEHP)	
Diuron	
Endosulfan	X
Fluoranthene	
Hexachlorobenzene	X
Hexachlorobutadiene	X
Hexachlorocyclohexane	X
Isoproturon	
Lead and its compounds	
Mercury and its compounds	X

Naphthalene	
Nickel and its compounds	
Nonylphenols	X
(4-nonylphenol)	X
Octylphenols	
(4-(1,1',3,3'-tetramethylbutyl)-phenol)	
Pentachlorobenzene	X
Pentachlorophenol	
Polyaromatic hydrocarbons	X
(Benzo(a)pyrene)	X
(Benzo(b)fluoranthene)	X
(Benzo(g,h,i)perylene)	X
(Benzo(k)fluoranthene)	X
(Indeno(1,2,3-cd)pyrene)	X
Simazine	
Tributyltin compounds	X
(Tributyltin-cation)	X
Trichlorobenzenes	
Trichloromethane (chloroform)	
Trifluralin	

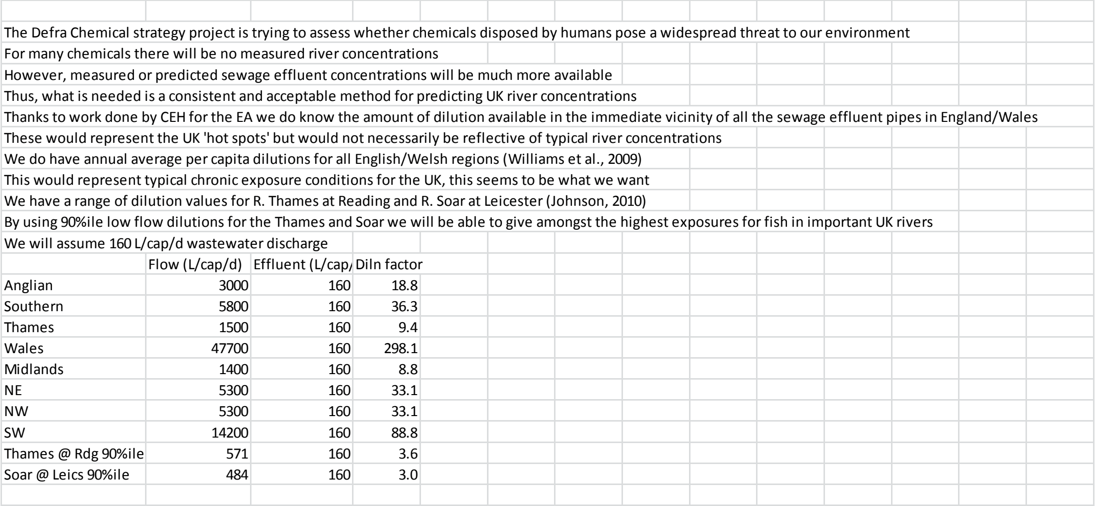
Source: http://ec.europa.eu/environment/water/water-framework/priority_substances.htm

Table 10-3 Solubility limits for the 15 POPs considered in this project.

POP	Solubility Limit (source [94])
BDE 209	In water, <1.0x10^{-4} mg/L at 25 deg
B[a]P	In water, 1.62x10^{-3} mg/L at 25 deg C
DDE	In water, 0.04 mg/L at 25 deg C
Dibutyltin	Insoluble in cold water; hydrolyzed in hot water. In water, 3 ppm at room temperature.
Dichlorobenzene	In water, 79 mg/L at 25 dec C (1-4 DCB) In water, 125 mg/L at 25 deg C (1-3 DCB) In water, 156 mg/L at 25 deg C (1-2 DCB)
Fluoranthene	Virtually insoluble (0.20-0.26 mg/L) in water
Hexachloro-butadiene	In water, 3.20 mg/L at 25 deg C
Lindane	In water, 7.3 mg/L at 25 deg C
PCB	
PCB 52	Solubility in water is extremely low; soluble in oils and organic solvents
PCB 153	Solubility in water is extremely low; soluble in oils and organic solvents
PCB 180	Solubility in water is extremely low; soluble in oils and organic solvents
PFOS	In water, 3.2x10^{-3} mg/L at 25 deg C (est)
Trichlorobenzene	In water, 30 mg/L at 25 deg C In water, 49.0 mg/L at 25 deg C (1,2,4 TCB) In water, 18 mg/L at 25 deg C. (1,2,3 TCB) In water, 6.01 mg/L at 25 deg C (1,3,5 TCB)
Trichloro-methane	In water, 7.95x10^{+3} mg/L at 25 deg C

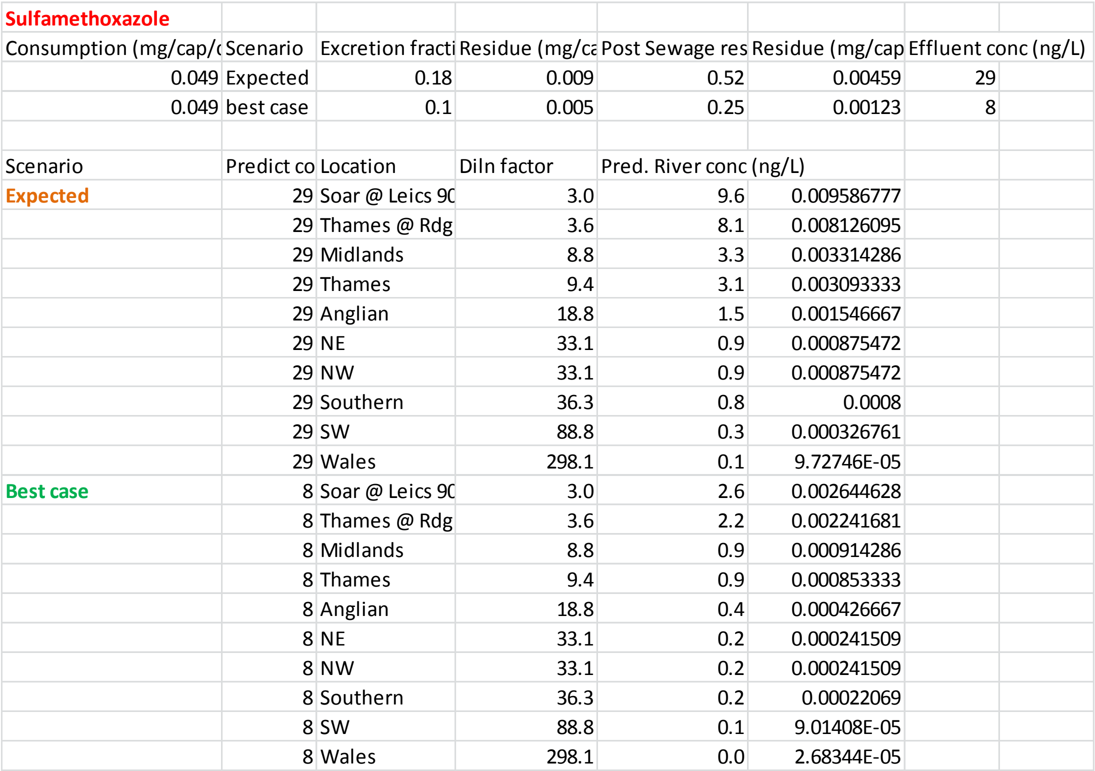
Table 10-4 Example pharmaceutical river water predications based on a) consumption data and b) sewage effluent concentrations.

The pharmaceutical predictions provide data for three scenarios; the expected, a best case and a worst case. By using the data for all three scenarios the predicted environmental data used to generate a median value for the risk ranking calculations, incorporates the extreme scenarios as well as the most likely. Thus, attempting to not overpredict or underpredicted the concentrations of the pharmaceutical in the UK environment.



The Defra Chemical strategy project is trying to assess whether chemicals disposed by humans pose a widespread threat to our environment
For many chemicals there will be no measured river concentrations
However, measured or predicted sewage effluent concentrations will be much more available
Thus, what is needed is a consistent and acceptable method for predicting UK river concentrations
Thanks to work done by CEH for the EA we do know the amount of dilution available in the immediate vicinity of all the sewage effluent pipes in England/Wales
These would represent the UK 'hot spots' but would not necessarily be reflective of typical river concentrations
We do have annual average per capita dilutions for all English/Welsh regions (Williams et al., 2009)
This would represent typical chronic exposure conditions for the UK, this seems to be what we want
We have a range of dilution values for R. Thames at Reading and R. Soar at Leicester (Johnson, 2010)
By using 90%ile low flow dilutions for the Thames and Soar we will be able to give amongst the highest exposures for fish in important UK rivers
We will assume 160 L/cap/d wastewater discharge

	Flow (L/cap/d)	Effluent (L/cap/	Diln factor
Anglian	3000	160	18.8
Southern	5800	160	36.3
Thames	1500	160	9.4
Wales	47700	160	298.1
Midlands	1400	160	8.8
NE	5300	160	33.1
NW	5300	160	33.1
SW	14200	160	88.8
Thames @ Rdg 90%ile	571	160	3.6
Soar @ Leics 90%ile	484	160	3.0



Sulfamethoxazole						
Consumption (mg/cap/c	Scenario	Excretion fracti	Residue (mg/ca	Post Sewage res	Residue (mg/cap	Effluent conc (ng/L)
0.049	Expected	0.18	0.009	0.52	0.00459	29
0.049	best case	0.1	0.005	0.25	0.00123	8
Scenario	Predict co	Location	Diln factor	Pred. River conc (ng/L)		
Expected	29	Soar @ Leics 90	3.0	9.6	0.009586777	
	29	Thames @ Rdg	3.6	8.1	0.008126095	
	29	Midlands	8.8	3.3	0.003314286	
	29	Thames	9.4	3.1	0.003093333	
	29	Anglian	18.8	1.5	0.001546667	
	29	NE	33.1	0.9	0.000875472	
	29	NW	33.1	0.9	0.000875472	
	29	Southern	36.3	0.8	0.0008	
	29	SW	88.8	0.3	0.000326761	
	29	Wales	298.1	0.1	9.72746E-05	
Best case	8	Soar @ Leics 90	3.0	2.6	0.002644628	
	8	Thames @ Rdg	3.6	2.2	0.002241681	
	8	Midlands	8.8	0.9	0.000914286	
	8	Thames	9.4	0.9	0.000853333	
	8	Anglian	18.8	0.4	0.000426667	
	8	NE	33.1	0.2	0.000241509	
	8	NW	33.1	0.2	0.000241509	
	8	Southern	36.3	0.2	0.00022069	
	8	SW	88.8	0.1	9.01408E-05	
	8	Wales	298.1	0.0	2.68344E-05	

Diclofenac my predictions based on NHS prescriptions and assumptions on excretion & sewage removal

Consumption (mg/cap/d)	Scenario	Excretion	Residue (mg/cap/d	Post Sewage fra	Residue (mg/cap/	Effluent conc (ng/L)
0.957	Expected	0.095	0.091	0.78	0.07091	443
0.957	best case	0.02	0.019	0.18	0.00345	22
0.957	worst case	0.23	0.220	1	0.22011	1376

Diclofenac based on Gardner et al 2012

Concentration reported in UK for 162 STP effluents of Gardner et al 2012 median 260 ng/L 95%ile 700 ng/L and 5%ile 90 ng/L

Scenario	Predict co	Location	Diln facto	Pred. River conc (ng/L)
Expected	260	Soar @ Le	3.0	86.0
(from me	260	Thames @	3.6	72.9
	260	Midlands	8.8	29.7
	260	Thames	9.4	27.7
	260	Anglian	18.8	13.9
	260	NE	33.1	7.8
	260	NW	33.1	7.8
	260	Southern	36.3	7.2
	260	SW	88.8	2.9
	260	Wales	298.1	0.9
95%ile eff	700	Soar @ Le	3.0	231.4
(worst cas	700	Thames @	3.6	196.1
	700	Midlands	8.8	80.0
	700	Thames	9.4	74.7
	700	Anglian	18.8	37.3
	700	NE	33.1	21.1
	700	NW	33.1	21.1
	700	Southern	36.3	19.3
	700	SW	88.8	7.9
	700	Wales	298.1	2.3
5%ile	90	Soar @ Le	3.0	29.8
(best case	90	Thames @	3.6	25.2
	90	Midlands	8.8	10.3
	90	Thames	9.4	9.6
	90	Anglian	18.8	4.8
	90	NE	33.1	2.7
	90	NW	33.1	2.7
	90	Southern	36.3	2.5
	90	SW	88.8	1.0
	90	Wales	298.1	0.3

Table 10-5 List of chemicals which do and don't have an overlap in the effect and environmental data

Chemicals WITH an overlap between the effect and environmental data	Chemicals WITHOUT an overlap between the effect and environmental data
AES	Alkylsuphate
Aluminium	Aspirin
Arsenic	Atenolol
Benzo [a] pyrene	BDE-209
Cadmium	Bentazone
Carbamazepine	Benzotriazoles
Carbofuran	Beta-HCH
Chlorpyrifos	Bisphenol A
Chromium	DEHP
Copper	Dichlorobenzene
DDE	Fluoranthene
Diazinon	Glyphosate
Dibutyltin	HCBD
Diclofenac	MCPA
EE2	MCPP
Fluoxetine	Metaldehyde
Ibuprofen	Methomyl
Imidacloprid	Metolachlor
Iron	Metoprolol
LAS	Nano AgO
Lead	Nano ZnO
Lenacil	Naproxen
Lindane	Ofloxacin
Linuron	Paracetamol
Malathion	PCB 153
Manganese	PCB 194
Mercury	PFOS
Nickel	Pirimicarb
Nonylphenol	Propranolol
Octylphenol	Simazine
PCB 180	Silver
PCB 52	Sulfamethoxazole
Pendimethalin	Trichlorbenzene

Permethrin	Trichloromethane
Sucralose	
Terbuthylazine	
Tributyltin	
Triclosan	
Zinc	

10.1.3. *Risk ratio summary tables for tier one and tier two*

Table 10-6 Risk ratios and rankings for the 73 chemicals studied based on tier one

	T1 Median Risk Ratio & Ranking		T1 5th%ile Risk Ratio & Ranking		T1 Fish Risk Ratio & Ranking		T1 Invertebrates Risk Ratio & Ranking		T1 Algae & Aquatic Plants Risk Ratio & Ranking	
Metals & Nano-metals										
Aluminium	2.42x10-1	1	2	1	0.44	1	4.37x10-2	2	0.4	1
Arsenic	3.09x10-4	9	7.84x10-3	10	7.42x10-5	10	4.17x10-4	3	2.17x10-4	10
Cadmium	4.55x10-4	8	6.02x10-2	6	3.33x10-3	6	1.71x10-4	11	1.13x10-3	8
Chromium	6.74x10-5	11	1.11x10-2	9	6.95x10-6	13	6.91x10-4	7	6.97x10-4	9
Copper	5.00x10-2	2	0.822	2	3.85x10-2	4	5.58x10-2	1	5.23x10-2	3
Iron	9.88x10-3	4	0.449	3	8.43x10-2	2	5.27x10-3	4	5.62x10-2	2
Lead	1.23x10-3	7	3.65x10-2	8	1.64x10-3	7	3.98x10-4	10	1.61x10-4	11
Manganese	4.13x10-3	5	4.8x10-2	7	6.75x10-3	5	3.91x10-3	5	2.74x10-3	7
Mercury	5.43x10-5	13	5.1x10-3	11	5.95x10-5	11	2.87x10-5	14	8.20x10-5	12
Nickel	1.65x10-3	6	0.107	5	9.52x10-4	8	3.53x10-3	6	4.79x10-3	6
Silver	1.14x10-4	10	1.58x10-3	13	1.03x10-4	9	4.40x10-4	8	1.83x10-5	13
Zinc	3.01x10-2	3	0.413	4	4.22x10-2	3	7.01x10-3	3	3.08x10-2	4
Nano Ag	1.12x10-5	12	3.88x10-4	14	3.05x10-6	14	4.36x10-5	13	2.88x10-9	14
Nano ZnO	6.30x10-5	14	3.51x10-3	12	4.79x10-5	12	1.71x10-4	12	5.26x10-3	5
Pharmaceuticals										
Aspirin	2.48x10-8	13	4.6x10-6	12	4.86x10-10	13	2.77x10-8	13	1.7x10-8	13
Atenolol	5.12x10-7	8	1.03x10-5	11	4.91x10-6	7	4.12x10-8	7	1.05x10-7	11
Carbamazepine	1.42x10-6	6	7.04x10-3	6	8.71x10-7	8	1.74x10-6	5	9.51x10-7	5
Diclofenac	1.13x10-7	7	1.3x10-2	4	1.97x10-5	4	5.73x10-7	6	2.94x10-7	8
EE2	7.30x10-3	1	7.46x10-2	2	1.63x10-2	1	5.27x10-8	11	7.74x10-8	12
Fluoxetine	4.72x10-5	2	5.68E-02	3	9.43x10-5	3	6.25x10-5	1	1.11x10-4	1
Ibuprofen	2.41x10-6	5	2.63x10-1	1	6.13x10-4	2	2.32x10-6	4	4.48x10-7	6
Metoprolol	6.8x10-8	12	2.11x10-6	13	2.09x10-8	11	8.50x10-8	10	1.19x10-7	10
Naproxen	2.62x10-7	9	3.56x10-4	10	2.46x10-8	10	2.62x10-7	8	2.90x10-7	9
Ofloaxcin	1.34x10-7	10	1.19x10-4	9	2.57x10-9	12	4.87x10-8	12	4.21x10-7	7
Paracetamol	3.47x10-6	4	1.02x10-2	5	4.7x10-7	9	1.08x10-5	3	4.55x10-6	4
Propranolol	1.28x10-7	3	2.5x10-4	8	5.64x10-6	6	1.5x10-5	2	3.42x10-5	2
Sulfamethoxazole	1.13x10-7	11	2.97x10-4	7	8x10-6	5	1.13x10-7	9	1.21x10-5	3
Persistent Organic Pollutants										
B[a]P	5x10-4	1	5x10-2	1	3.13x10-4	1	5x10-4	2	3.38x10-4	1
BDE 209	2.32x10-5	8	3.81x10-3	6	2.32x10-5	5	1.86x10-2	1	x	x
Dichlorobenzene	1.6x10-6	13	1.66x10-5	12	2.36x10-6	11	4.46x10-6	10	3.09x10-7	5
DDE	8.08x10-5	2	5x10-3	5	1.67x10-8	15	1.29x10-4	3	x	x
Dibutlytin	3.96x10-5	7	6.86x10-3	4	1.11x10-5	9	5.54x10-5	8	2.5x10-4	2
Fluoranthene	7.13x10-5	3	1.25x10-3	8	2.30x10-4	2	7.14x10-5	5	1.43x10-7	7
Hexachlorobutadiene	1.2x10-5	9	1.31x10-4	10	1.25x10-5	8	1.15x10-5	9	x	x
Lindane	4.29x10-5	6	1.5x10-2	2	2.14x10-5	6	1.15x10-4	4	1x10-6	4
PCB 153	1.95x10-6	11	3.9x10-3	15	3.9x10-9	10	7.22x10-7	13	x	x
PCB 180	7.04x10-5	4	7.69x10-3	3	7.0x10-5	3	6.67x10-6	6	x	x
PCB 194	3.68x10-7	15	1.23x10-5	14	1.7x10-7	14	4.64x10-6	14	x	x
PCB 52	5.88x10-5	5	1.87x10-3	7	4.3x10-5	4	6.60x10-6	7	x	x
PFOS	3.47x10-6	10	1.04x10-3	9	2.08x10-5	7	2.60x10-6	11	2.16x10-7	6

Trichlorobenzene	1.79x10-6	12	1.56x10-5	13	1.72x10-6	12	1.92x10-6	12	2.27x10-6	3
Trichloromethane	4.95x10-7	14	3.73x10-5	11	6.67x10-7	13	1.72x10-7	15	1.14x10-7	8
Pesticides										
Bentazone	3.96x10-8	21	1.03x10-6	21	4.17x10-9	20	3.49x10-8	17	2.48x10-7	11
Beta- HCH	9.2x10-7	18	1.87x10-6	19	9.12x10-7	12	x	x	x	x
Carbofuran	8.93x10-5	7	1.21x10-2	4	2.97x10-5	6	2.00x10-3	3	3.18x10-6	7
Chlorpyrifos	1.86x10-3	2	8.06x10-2	1	1.90x10-5	8	1.43x10-2	1	1.55x10-6	10
Diazinon	5.76x10-5	10	6.06x10-3	8	1.66x10-5	9	5.08x10-4	6	1x10-7	17
Glyphosate	1.2x10-6	17	3.45x10-5	16	5.18x10-7	13	2.27x10-6	13	1.98x10-6	8
Impidacloprid	2.48x10-6	14	1.21x10-2	5	8.87x10-9	19	1.76x10-5	9	3.91x10-8	18
Lenacil	1.25x10-4	5	4.72x10-4	12	x	x	x	x	1.25x10-4	2
Linuron	1.00x10-4	6	1.24x10-3	11	2.91x10-5	7	2.38x10-5	8	9.94x10-5	3
Malathion	1.94x10-4	4	3.00x10-2	2	8.3x10-5	5	1.49x10-3	4	2.31x10-7	13
MCPA	1.51x10-7	19	6.28x10-6	17	1.16x10-7	15	6.00x10-8	16	1.80x10-7	14
MCPP	1.41x10-6	16	4.17x10-6	18	5.50x10-8	16	1.41x10-6	14	1.37x10-7	15
Metaldehyde	1.33x10-7	20	1.36x10-6	20	1.39x10-7	14	1.29x10-7	15	1.32x10-7	16
Methomyl	2.06x10-3	1	1.92x10-2	3	1.10x10-5	10	5.95x10-3	2	2.31x10-7	12
Metolachlor	5.x10-5	11	3.36x10-4	13	1.54x10-6	11	4.76x10-6	12	7.39x10-5	4
Pendimethalin	1.03x10-5	12	6.25x10-3	7	6.25x10-3	1	8.55x10-6	10	8.36x10-6	5
Permethrin	2.42x10-4	3	8.85x10-3	6	1.67x10-4	3	6.90x10-4	5	x	x
Pirimicarb	2.78x10-6	13	1.67x10-4	14	3.21x10-8	17	5.00x10-6	11	2.08x10-8	19
Simazine	2x10-6	15	4.45x10-5	15	9.09x10-9	18	5.56x10-9	18	1.96x10-6	9
Terbuthyalzine	7.52x10-5	9	5.82x10-3	9	3.81x10-5	4	x	x	1.75x10-3	1
Tributyltin	8.66x10-5	8	3.72x10-3	10	1.69x10-4	2	1.50x10-4	7	4.59x10-6	6
Surfactants and Others										
AES	9x10-4	3	1.34x10-2	5	9.47x10-4	2	1.04x10-3	3	6.34x10-4	3
Alkyl sulfonate	1.43x10-5	9	4.47x10-4	10	3.81x10-5	8	4.82x10-6	10	5.88x10-6	7
Benzotriazole	4.68x10-5	7	8.27x10-4	8	4.15x10-5	7	1.14x10-5	8	3.19x10-4	4
Bisphenol A	8.11x10-5	6	1.3x10-3	7	1.51x10-4	5	4.13x10-5	7	8.11x10-5	6
DEHP	1.74x10-5	8	7.31x10-4	9	1x10-6	10	2x10-4	5	1.54x10-6	9
LAS	5.5x10-3	2	7.88x10-2	2	7x10-3	1	3.39x10-3	1	1.05x10-2	2
Nonylphenol	4.81x10-6	4	2.69x10-2	3	6.25x10-4	3	2.82x10-4	4	1.52x10-4	5
Octylphenol	1.32x10-4	5	5.76x10-3	6	1.83x10-4	4	6.11x10-5	6	x	x
Sucralose	5.3x10-6	10	1.41x10-2	4	2.21x10-6	9	9.7x10-6	9	3.79x10-6	8
Triclosan	5.73x10-3	1	1.03x10-1	1	1.32x10-4	6	2.25x10-3	2	2.89x10-2	1

Table 10.6 continued – Risk ranking for the 73 chemicals studied based on tier one

	T1 Median Risk Ratio & Ranking	T1 5^{th}%ile Risk Ratio & Ranking	T1 Fish Risk Ratio & Ranking	T1 Invertebrates Risk Ratio & Ranking	T1 Algae & Aquatic Plants Risk Ratio & Ranking
1	Aluminium	Aluminium	Aluminium	Copper	Aluminium
2	Copper	Copper	Iron	Aluminium	Iron
3	Zinc	Iron	Zinc	BDE 209	Copper
4	Iron	Zinc	Copper	Chlorpyrifos	Zinc
5	EE2	Ibuprofen	EE2	Zinc	Triclosan
6	Triclosan	Nickel	LAS	Methomyl	LAS
7	LAS	Triclosan	Manganese	Iron	Nano ZnO
8	Manganese	Chlorpyrifos	Pendimethalin	Manganese	Nickel
9	Methomyl	LAS	Cadmium	Nickel	B[a]P
10	Chlorpyrifos	EE2	Lead	LAS	Manganese
11	Nickel	Cadmium	Nickel	Triclosan	Terbuthylazine
12	Lead	Fluoxetine	AES	Carbofuran	Cadmium
13	AES	B[a]P	Nonylphenol	Malathion	Chromium
14	B[a]P	Manganese	Ibuprofen	AES	AES
15	Nonylphenol	Lead	B[a]P	Chromium	Benzotriazoles
16	Cadmium	Malathion	Fluoranthene	Permethrin	Dibutyltin
17	Arsenic	Nonylphenol	Octylphenol	Diazinon	Arsenic
18	Permethrin	Methomyl	Tributyltin	B[a]P	Lead
19	Malathion	Lindane	Permethrin	Silver	Nonylphenol
20	Octylphenol	Sucralose	Bisphenol A	Arsenic	Lenacil

21	Lenacil	AES	Triclosan	Lead	Fluoxetine
22	Silver	Diclofenac	Silver	Nonylphenol	Linuron
23	Linuron	Carbofuran	Fluoxetine	DEHP	Mercury
24	Carbofuran	Imidacloprid	Arsenic	Cadmium	Bisphenol A
25	Tributyltin	Chromium	PCB 180	Nano ZnO	Metolachlor
26	Bisphenol A	Paracetamol	Mercury	Tributyltin	Propranolol
27	DDE	Permethrin	Nano ZnO	DDE	Silver
28	Terbuthylazine	Arsenic	PCB 52	Lindane	Sulfamethoxazole
29	Fluoranthene	PCB 180	Benzotriazoles	Fluoranthene	Pendimethalin
30	PCB 180	Carbamazepine	Terbuthylazine	PCB 180	Alkylsuphate
31	Chromium	Dibutyltin	Alkylsuphate	PCB 52	Tributyltin
32	Nano ZnO	Pendimethalin	Malathion	Fluoxetine	Paracetamol
33	PCB 52	Diazinon	Carbofuran	Octylphenol	Sucralose
34	Diazinon	Terbuthylazine	Linuron	Dibutyltin	Carbofuran
35	Mercury	Octylphenol	BDE 209	Nano AgO	Trichlorbenzene
36	Metolachlor	Mercury	Lindane	Bisphenol A	Glyphosate
37	Fluoxetine	DDE	PFOS	Mercury	Simazine
38	Benzotriazoles	BDE 209	Diclofenac	Linuron	Chlorpyrifos
39	Lindane	Tributyltin	Chlorpyrifos	Imidacloprid	DEHP
40	Dibutyltin	Nano ZnO	HCBD	Propranolol	Lindane
41	BDE 209	PCB 52	Dibutyltin	HCBD	Carbamazepine
42	DEHP	Silver	Methomyl	Benzotriazoles	Ibuprofen
43	Alkylsuphate	Bisphenol A	Sulfamethoxazole	Paracetamol	Ofloxacin
44	Propranolol	Fluoranthene	Chromium	Sucralose	Dichlorobenzene

45	Nano AgO	Linuron	Propranolol	Pendimethalin	Diclofenac
46	HCBD	PFOS	Atenolol	Pirimicarb	Naproxen
47	Pendimethalin	Benzotriazoles	PCB 153	Alkylsuphate	Bentazone
48	Paracetamol	DEHP	Nano AgO	Metolachlor	Methomyl
49	Sucralose	Lenacil	Dichlorobenzene	Dichlorobenzene	Malathion
50	PFOS	Alkylsuphate	Sucralose	PFOS	PFOS
51	Pirimicarb	Nano AgO	Trichlorbenzene	Ibuprofen	MCPA
52	Imidacloprid	Naproxen	Diazinon	Glyphosate	Fluoranthene
53	Ibuprofen	Metolachlor	Metolachlor	Trichlorbenzene	MCPP
54	Simazine	Sulfamethoxazole	DEHP	Carbamazepine	Metaldehyde
55	PCB 153	Propranolol	Beta-HCH	MCPP	Metoprolol
56	Trichlorbenzene	Pirimicarb	Carbamazepine	PCB 153	Trichloromethane
57	Dichlorobenzene	HCBD	Trichloromethane	Diclofenac	Atenolol
58	Carbamazepine	Ofloxacin	Glyphosate	PCB 194	Diazinon
59	MCPP	Simazine	Paracetamol	Atenolol	EE2
60	Glyphosate	Trichloromethane	PCB 194	Naproxen	Imidacloprid
61	Diclofenac	Glyphosate	Metaldehyde	Trichloromethane	Pirimicarb
62	Beta-HCH	Dichlorobenzene	MCPA	Metaldehyde	Aspirin
63	Trichloromethane	Trichlorbenzene	MCPP	Sulfamethoxazole	Nano AgO
64	PCB 194	PCB 194	Pirimicarb	Metporolol	
65	Atenolol	Atenolol	Naproxen	MCPA	
66	Naproxen	MCPA	Metoprolol	EE2	
67	Sulfamethoxazole	Aspirin	DDE	Ofloxacin	
68	MCPA	MCPP	Simazine	Bentazone	

69	Metaldehyde	PCB 153	Imidacloprid	Aspirin	
70	Ofloxacin	Metoprolol	Bentazone	Simazine	
71	Metpprolol	Beta-HCH	Ofloxacin		
72	Bentazone	Metaldehyde	Aspirin		
73	Aspirin	Bentazone			

Table 10-7 Risk ratios and rankings for the 73 chemicals studied based on tier two

	T2 Median Risk Ratio & Ranking		**T2 5th 5ile Risk Ratio & Ranking**		**T2 Lethal Risk Ratio & Ranking**		**T2 Sub-lethal Risk Ratio & Ranking**		**BCF & Ranking**	
Metals & Nano-metals										
Aluminium	8.02x10-3	4	1.44x10-1	6	7.07x10-3	5	2.6x10-2	3	215	28
Arsenic	2.35x10-4	18	6.67x10-3	28	1.07x10-4	20	5.71x10-7	10	4	52
Cadmium	3.97x10-4	16	6.09x10-2	11	2.27x10-4	14	1.91x10-3	13	1866	14
Chromium	2.37x10-6	44	4.06x10-4	42	1.64x10-6	40	2x10-5	34	2	61
Copper	3.11x10-2	1	6.59x10-1	1	3.24x10-2	1	3.61x10-2	1	1359	18
Iron	2.25x10-3	8	1.74x10-1	5	9.10x10-4	9	1.37x10-2	5	50	36
Lead	1.77x10-3	11	3.56x10-2	14	1.39x10-3	8	1x10-2	8	511	24
Manganese	2.8x10-3	7	2.5x10-2	16	2.34x10-3	6	3.49x10-3	12	10.6	47
Mercury	4.85x10-5	28	1.47x10-2	19	2.84x10-5	27	1x10-4	23	6000	8
Nickel	1.52x10-3	12	1.13x10-1	7	1.41x10-3	7	3.84x10-3	11	1367	29
Silver	4.55x10-4	15	6.33x10-3	29	4.76x10-4	11	7.34x10-5	25	1233	19
Zinc	1.37x10-2	3	2.62x10-1	4	1.37x10-2	3	1.37x10-2	6	2623	12
Nano Ag	1.21x10-5	36	3.88x10-4	43	2.24x10-5	29	3.05x10-6	44		
Nano ZnO	6.30x10-5	2	3.51x10-3	34	6.94x10-5	23	4.79x10-5	31		
Pharmaceuticals										
Aspirin	2.26x10-8	64	4.15x10-6	59	1.44x10-8	41	1.65x10-7	60	3	52
Atenolol	3.44x10-7	54	1.03x10-5	56	4.12x10-7	49	1.66x10-7	57	3	53
Carbamazepine	1.42x10-6	47	7.04x10-3	26	8.15x10-7	45	1.83x10-6	49	10.5	48
Diclofenac	1.13x10-6	50	1.29x10-2	21	5.70x10-7	47	1.72x10-6	50	121.5	30
EE2	6.50x10-3	5	6.5x10-2	10	1.07x10-8	60	1.2x10-2	7	610	22
Fluoxetine	4.72x10-5	29	5.68x10-2	12	1.16x10-5	33	9.35x10-5	24	63	33
Ibuprofen	2.41x10-6	43	2.63x10-1	3	3.96x10-7	50	5.08x10-6	41	58	34
Metoprolol	6.80x10-8	61	2.11x10-6	60	2.72x10-8	59	1.54x10-7	61	1	62
Naproxen	2.75x10-7	55	3.56x10-4	44	1.82x10-7	51	5.38x10-7	55	28	39
Ofloaxcin	1.30x10-7	60	1.19x10-4	49	4.35x10-8	58	2.44x10-6	46	3	56
Paracetamol	5.50x10-6	33	1.02x10-2	23	6.10x10-6	34	2.28x10-6	47	3	57
Propranolol	1.34x10-5	35	2.50x10-4	46	1.34x10-5	31	1.17x10-5	36	107	31
Sulfamethoxazole	2.55x10-7	56	2.97x10-4	45	6.50x10-8	55	5.23x10-6	40	3	59
Persistent Organic Pollutants										
B[a]P	5.00x10-4	13	5.00x10-2	13	6.25x10-4	10	3.13x10-4	16	3891	10
BDE 209										
Dichlorobenzene										
DDE	8.08x10-5	23	5.00x10-3	32	1.29x10-4	19	2.24x10-5	33	160000	3
Dibutlytin	3.96x10-5	32	6.86x10-3	27	3.28x10-5	26	6.49x10-5	27	53	35
Fluoranthene	7.13x10-5	24	1.25x10-3	36	7.11x10-5	22	7.14x10-5	26	1738	15
Hexachlorobutadiene	1.20x10-5	37	1.31x10-4	48	6.00x10-6	35	5x10-5	30	50119	5
Lindane	4.29x10-5	31	1.50x10-2	18	2.14x10-5	30	6x10-5	29	692	21
PCB 153	5.20x10-8	62	1.04x10-66	64	5.20x10-8	57	6.93x10-8	64	60832	4
PCB 180	2.54x10-5	57	2.77x10-3	53	1.43x10-5	52	1.31x104	53	1780000	1
PCB 194	3.68x10-7	53	1.23x10-5	55	1.11x10-7	54	6.04x10-6	38	320000	2
PCB 52	2.56x10-5	33	8.12x10-4	40	1.21x10-5	32	1.38x10-4	21	39811	6
PFOS	4.07x10-6	41	1.22x10-3	38	1.28x10-6	43	4.07x10-6	42	2796	11
Trichlorobenzene	1.79x10-6	46	1.56x10-5	54	1.67x10-6	39	3.57x10-6	43	1700	16
Trichloromethane	4.95x10-7	52	3.73x10-5	51	6.33x10-7	4	1.65x10-7	59	6	51
Pesticides										
Bentazone	3.96x10-8	63	1.03x10-6	63	2.15x10-8	60	3.23x10-7	56	21	40

Beta- HCH	9.20x10-7	51	1.87x10-6	61	9.12x10-7	44	1.5x10-6	51	515	23
Carbofuran	8.93x10-5	21	1.21x10-2	22	9.26x10-5	21	1.87x10-5	35	12	45
Chlorpyrifos	1.86x10-3	10	8x10-2	9	8.33x10-3	4	2.x10-4	17	1374	17
Diazinon	5.76x10-5	27	6x10-3	31	5.58x10-5	24	6.04x10-5	28	63	32
Glyphosate	1.20x10-6	49	3.45x10-5	52	5.38x10-7	48	1.98x10-6	48	3	55
Impidacloprid										
Lenacil										
Linuron	1.00x10-4	19	1.24x10-3	37	1.62x10-6	41	1.17x10-4	22	48	37
Malathion	6.49x10-5	25	1.00x10-2	24	1.39x10-4	18	8.33x10-7	54	20	41
MCPA	1.51x10-7	58	6.28x10-6	57	6.00x10-8	56	1.66x10-7	58	8	50
MCPP	1.41x10-6	48	4.17x10-6	58	1.41x10-6	42	1.41x10-6	52	9	49
Metaldehyde	1.33x10-7	59	1.36x10-6	62	1.33x10-7	53	1.32x10-7	62	11	46
Methomyl	2.06x10-3	9	1.92x10-2	17	2.01x10-4	16	6.33x10-3	9		
Metolachlor										
Pendimethalin	1.03x10-5	38	6.25x10-3	30	5.00x10-6	36	1.07x10-5	37	5100	9
Permethrin	2.42x10-4	17	8.85x10-3	25	2.27x10-4	13	1.03x10-3	15	2202	13
Pirimicarb	2.78x10-6	42	1.67x10-4	47	2.78x10-6	37	1.12x107	63	16	43
Simazine	2.00x10-6	45	4.45x10-4	50	5.56x10-9	63	2.71x10-6	45	14.6	44
Terbuthyalzine										
Tributlytin	9.04x10-5	20	3.89x10-3	33	1.55x10-4	17	4.44x10-5	32	11200	7
Surfactants and Others										
AES										
Alkyl sulfonate										
Benzotriazole	4.68x10-5	30	8.27x10-4	39	2.34x10-5	28	1.52x10-4	19	4370	60
Bisphenol A	8.11x10-5	22	1.30x10-3	35	4.38x10-5	25	1.48x10-4	20	40.5	38
DEHP	1.74x10-5	34	7.31x10-4	41	2.20x10-6	38	2x10-4	18	741	20
LAS	2.20x10-2	2	3.15x10-1	2	1.54x10-2	2	3.23x10-2	2		
Nonylphenol	4.81x10-4	14	2.69x10-2	15	2.57x10-4	12	1.56x10-3	14	166	28
Octylphenol									302	26
Sucralose	5.30x10-6	56	1.41x10-2	20			5.3x10-6	39	3	57
Triclosan	5.80x10-3	6	1.05x10-1	8	2.05x10-4	15	2.05x10-2	4	500	25

Table 10-8 Metal (Cu, Zn and Mn) bioavailability predications and risk rankings

Metal (Cu, Zn and Mn) bioavailability predictions: based on dissolved metal concentrations the screening tool takes into account the pH, DOC and Ca of a water body and predicts the bioavailable fraction of the metal. Risk ratios can then be calculated based on the ecotox median value calculated for this study and the bioavailable metal concertation.

Tool – WCA Metal Bioavailability Screening Tool Version 28.0 created Nov 2011.

	Bioavailable Copper Concentration (µg l-1)	Bioavailable Zinc Concentration (µg l-1)	Bioavailable Manganese Concentration (µg l-1)	
Thames	0.11677576	2.347208727	13.52993687	
Trent	0.080248361	2.249063855	9.186408851	
Calder	0.04476298	2.014938627	6.131316996	

Ecotox median	Environment median			
5.3	1.66			
473	6.5			
7115	19.9			

		Risk Rankings based on Ecotox median & BLM environment data		
Risk Ranking (this study)		Copper	Zinc	Manganese
3.13E-01	Thames	2.20E-02	4.96E-03	1.90E-03
1.37E-02	Trent	1.51E-02	4.75E-03	1.29E-03
2.80E-03	Calder	8.45E-03	4.26E-03	8.62E-04

Chemical	**Risk Ratio (medians)**
Copper	0.031144465
Copper BLM	0.022033162
Aluminium	0.020462963
Copper BLM	0.0151412
Zinc	0.013742072
Copper BLM	0.008445845
EE2	0.007303371
LAS	0.00655308
Triclosan	0.005732484
Zn BLM	0.004962386
Zn BLM	0.004754892
Zn BLM	0.004259913
Manganese	0.002796908
Iron	0.002252778
Methomyl	0.002057613
Mn BLM	0.001901607
Chlorpyrifos	0.001862197
Lead	0.001769912
Nickel	0.001408333
Mn BLM	0.001291133
Mn BLM	0.000861745
B[a]P	0.0005
Nonylphenol	0.000480769
Silver	0.000454545

Table 10-9 Median BCF values reported for T2 chemicals

(* indicates chemicals with a BCF > 500 which went through to risk ranking)

Chemical		Median BCF value	Source
Metals and Nano metals			
	Aluminium	172	[299] [94]
	Arsenic	2.5	[94]
*	Cadmium	1,116	[300]
	Chromium	125	[94]
*	Copper	1,493	[300]
	Iron	50	[17]
*	Lead	518	[300]
	Manganese	14	[301] [302]
*	Mercury	5,000	[300] [299]
	Nickel	80	[303] [304]
*	Silver	1,233	[300]
*	Zinc	3,957	[300]
	Nano Ag	N/A	
	Nano ZnO	N/A	
Pesticides			
	Bentazone	50	[305] [94]
*	Beta-HCH	573	[306] [95] [94]
	Carbofuran	13.6	[307] [305] [95]
*	Chlorpyrifos	1,000	[305] [94] [95]
	Diazinon	36.6	[306] [307] [305] [94]
*	DDE	16,000	[306] [244] [95]
	Glyphosate	3	[305] [95]
*	Lindane	692	[306] [244] [305] [95]
	Linuron	48	[305] [94] [95]
	Malathion	20	[95] [305] [308] [307] [309]

Chemical		Median BCF value	Source
	MCPA	7.6	[305] [95]
	Mecoprop	9.4	[305] [95]
	Metaldehyde	11	[305]
	Methomyl	2.3	[305] [95]
*	Pendimethalin	5,100	[305]
*	Permethrin	1,906	[306] [305] [95] [94]
	Pirimicarb	24	[305] [94] [95]
	Simazine	15	[305] [94] [95]
*	Tributlytin	5,006	[305] [94]
Other Persistent Organic Pollutants			
*	B[a]P	3,891	[310] [311] [312] [313] [314] [315] [316] [317] [318] [306] [95]
	Dibutlytin	57	[94]
*	Fluoranthene	1,738	[319] [95]
*	Hexachlorobutadiene	11,959	[95] [94]
*	PFOS	2,950	[320] [321] [322] [323] [324]
*	Trichlorobenzene	1,700	[306] [244] [95]
	Trichloromethane	7	[306] [244] [95]

Chemical		Median BCF value	Source
Pharmaceuticals			
	Atenolol	N/A	
	Aspirin	3	[94]
	Carbamazepine	10.5	[94] [325] [326]
	Diclofenac	335	[327] [328] [329] [330] [331] [326] [332]
*	EE2	635	[50] [94]
	Fluoxetine	63	[333] [334] [335]
	Ibuprofen	58	[336] [241] [325] [331] [337] [326] [94]
	Metoprolol	1	[94] [235]
	Naproxen	28	[337] [331] [326] [94]
	Ofloaxcin	3	[94]
	Paracetamol	3	[94]
	Propranolol	107	[235] [338]
	Sulfamethoxazole	3	[94]
Surfactants and similar			
	Benzotriazole	2.5	[94]
	Bisphenol A	90	[94]
*	DEHP	750	[94] [339]
	LAS	N/A	
	Nonylphenol	112	[94] [340] [341]
	Sucralose	3	[94]
*	Triclosan	500	[94] [342] [343]

10.1.4. Experimental Graphs

- Metal and pharmaceutical experiments - *Pseudokirchneriella subcapitata*
- Metals and pharmaceutical experiments - *Daphnia magna*

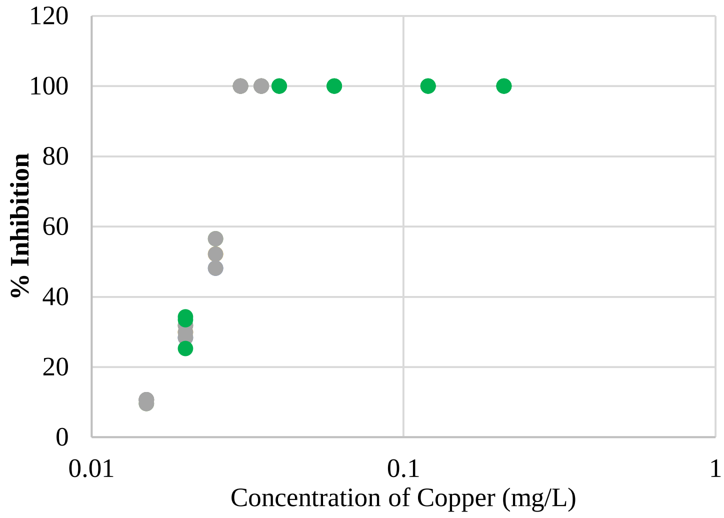


Figure 10-4 Percentage inhibition of algal growth following 72h exposure to copper (mg/L)

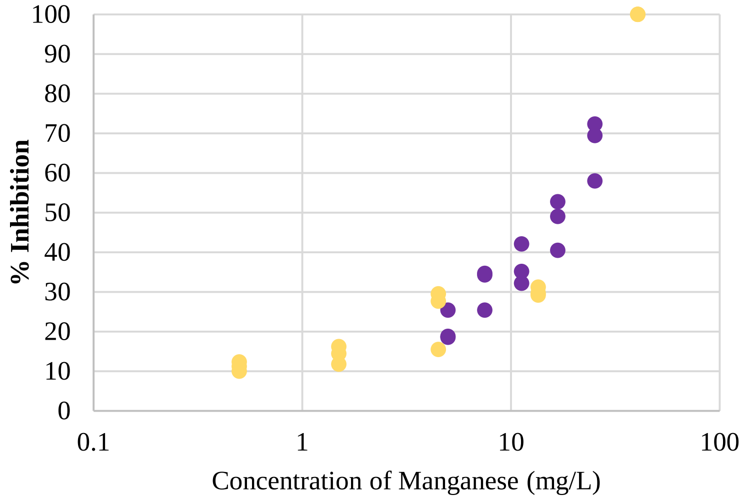


Figure 10-5 Percentage inhibition of algal growth following 72h exposure to manganese (mg/L)

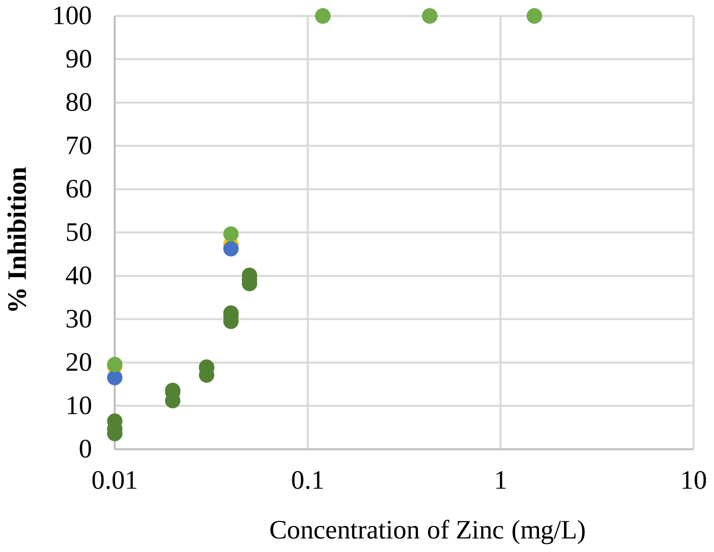


Figure 10-6 Percentage inhibition of algal growth following 72h exposure to zinc (mg/L)

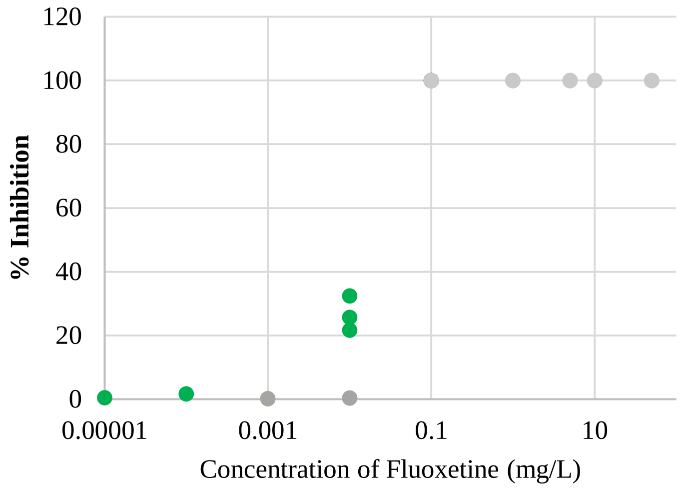


Figure 10-7 Percentage inhibition of algal growth following 72h exposure to fluoxetine (mg/L)

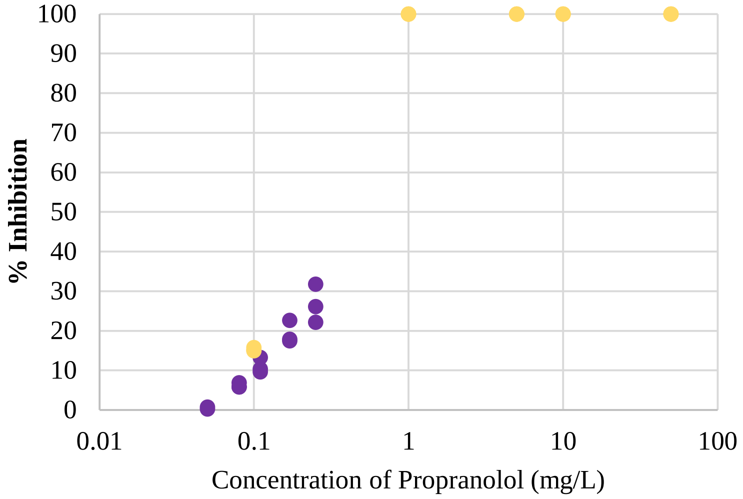


Figure 10-8 Percentage inhibition of algal growth following 72h exposure to propranolol (mg/L)

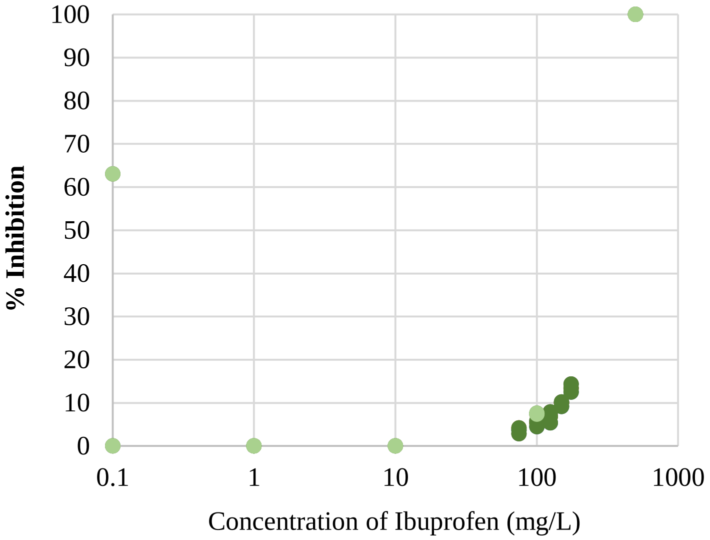


Figure 10-9 Percentage inhibition of algal growth following 72h exposure to ibuprofen (mg/L)

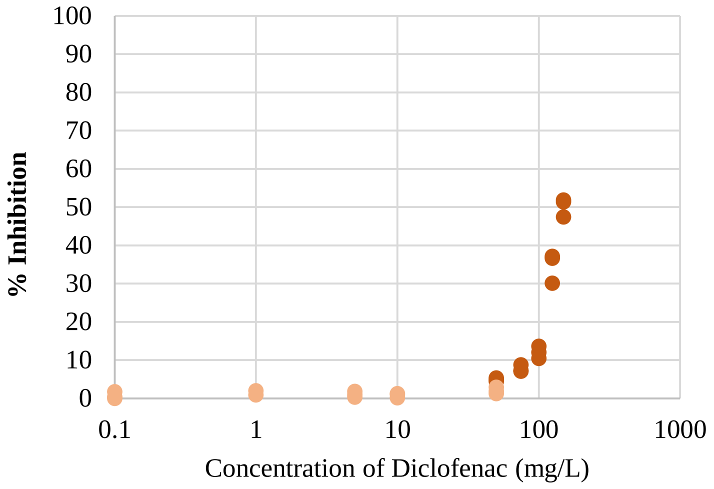


Figure 10-10 Percentage inhibition of algal growth following 72h exposure to diclofenac (mg/L)

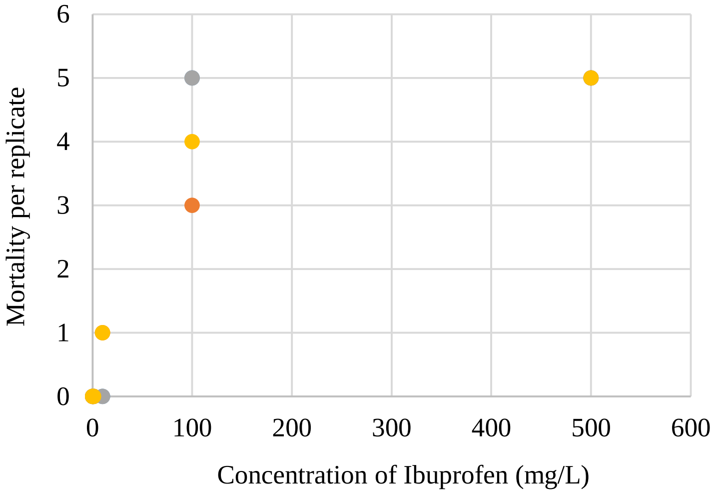


Figure 10-11 Mortality per replicate of Daphnia magna following 48h exposure to ibuprofen (mg/L)

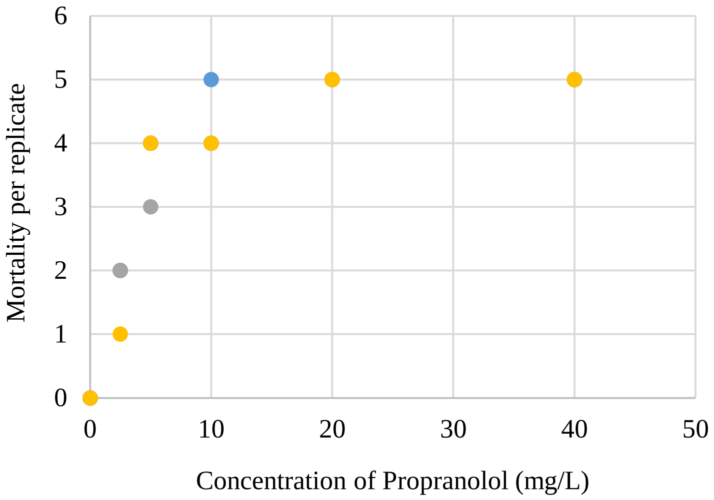


Figure 10-12 Mortality per replicate of Daphnia magna following 48h exposure to propranolol (mg/L)

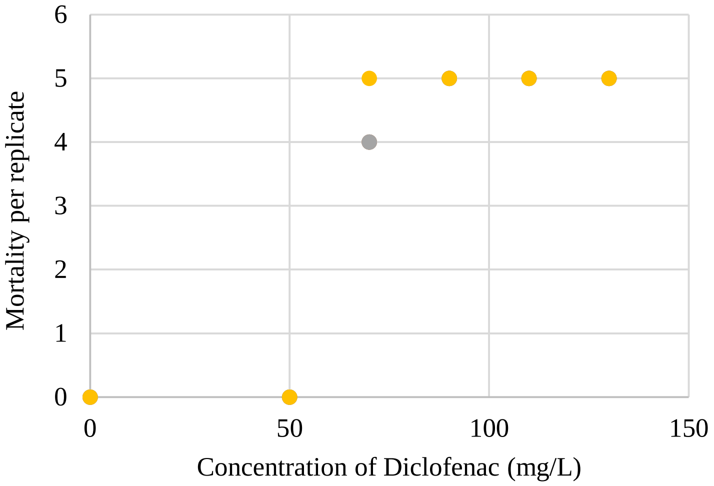


Figure 10-13 Mortality per replicate of Daphnia magna following 48h exposure to diclofenac (mg/L)

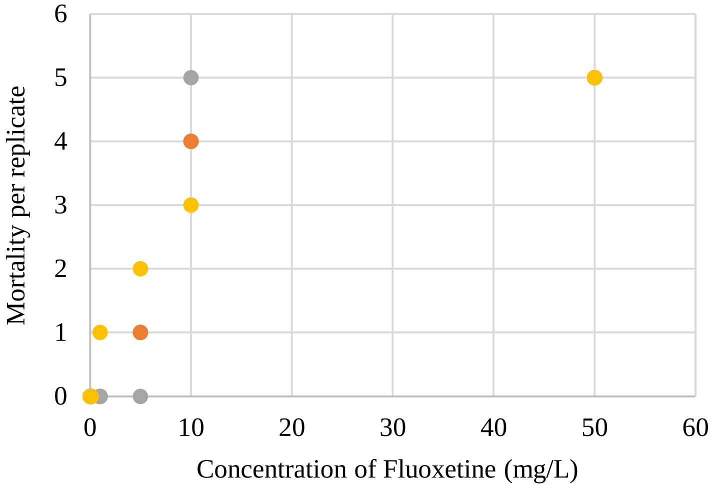


Figure 10-14 Mortality per replicate of Daphnia magna following 48h exposure to fluoxetine (mg/L)

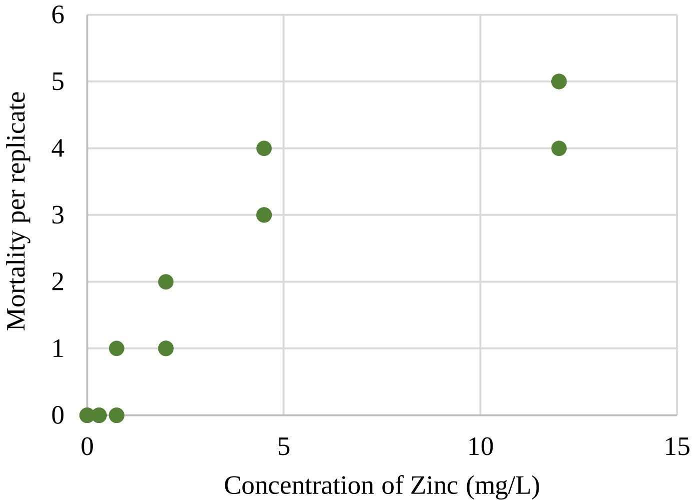


Figure 10-15 Mortality per replicate of Daphnia magna following 48h exposure to zinc (mg/L)

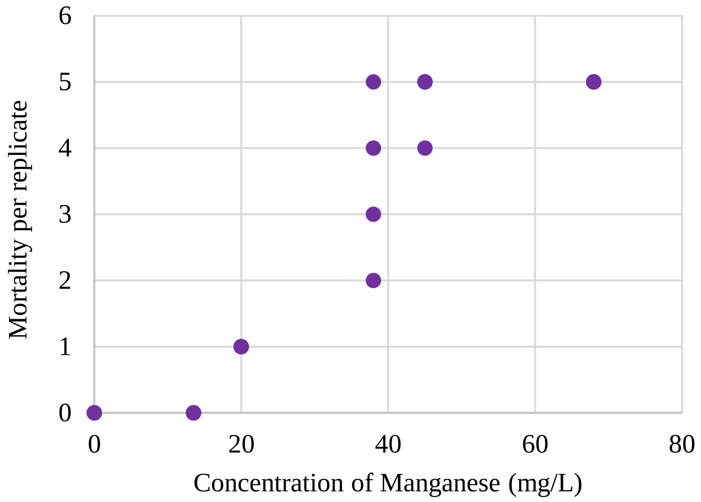


Figure 10-16 Mortality per replicate of Daphnia magna following 48h exposure to manganese (mg/L)

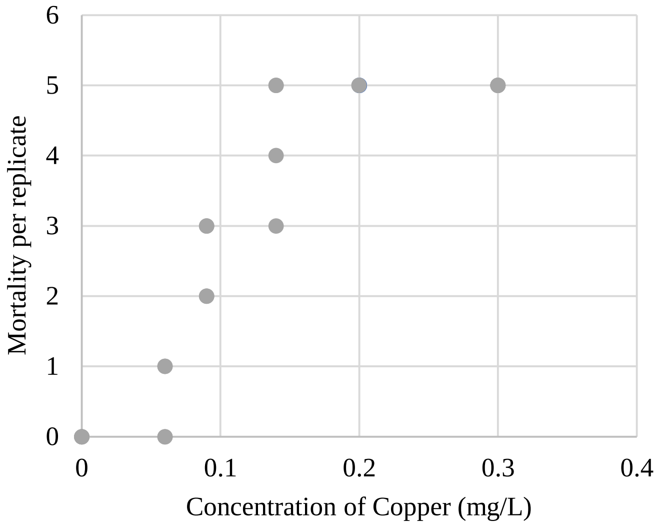


Figure 10-17 Mortality per replicate of Daphnia magna following 48h exposure to copper (mg/L)

10.1.5. Comparison of lethal and sub-lethal risk ratios

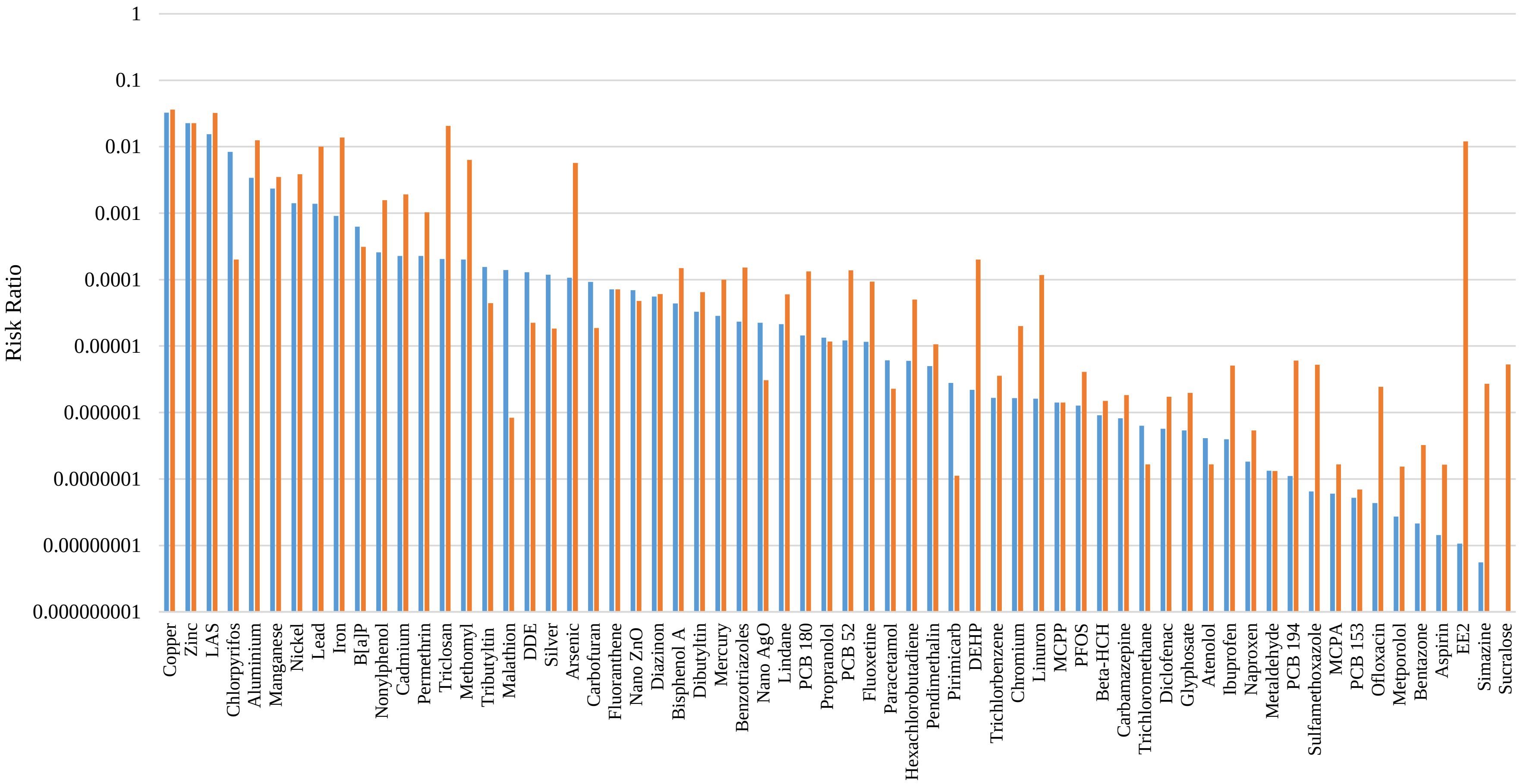


Figure 10-18 Comparison of lethal and sub-lethal risk ratios for chemicals based on tier two moderations

10.1.6. Publications

Table 10-10 Publications related to this project

Paper	
1.	**Donnachie, Rachel L**.; Johnson, Andrew C.; Moeckel., Claudia; Pereira, M. Gloria.; Sumpter, John P. 2014 *Using risk-ranking of metals to identify which poses the greatest threat to freshwater organisms in the UK*. Environmental Pollution, 194. 17-23. 10.1016/j.envpol.2014.07.008
2.	**Donnachie, Rachel L**.; Johnson, Andrew C.; Sumpter, John P. 2016 *A rational approach to selecting and ranking some pharmaceuticals of concern for the aquatic environment and their relative importance compared with other chemicals* [in special issue: Pharmaceuticals in the environment] Environmental Toxicology and Chemistry, 35 (4). 1021-1027. 10.1002/etc.3165
3.	Johnson, A. C., **R. L. Donnachie**, J. P. Sumpter, M. D. Jürgens, C. Moeckel and M. G. Pereira. 2017.*An alternative approach to risk rank chemicals on the threat they pose to the aquatic environment*. Science of The Total Environment 599–600: 1372-1381.
4.	Sumpter, J.P.; **Donnachie, R.L**.; Johnson, A.C. 2014 *The apparently very variable potency of the anti-depressant fluoxetine*. Aquatic Toxicology, 151. 57-60. 10.1016/j.aquatox.2013.12.010