Protecting aquatic organisms from chemicals: the harsh realities

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Tens of thousands of man-made chemicals are in everyday use in developed countries. A high proportion of these, or their transformation products, probably reach the aquatic environment. A considerable amount is known about the environmental concentrations of some of these chemicals (such as metals), especially the regulated ones, but little or nothing is known about the majority. In densely populated countries, most or all rivers will receive both diffuse (e.g. agricultural runoff) and point source (e.g. sewage treatment plant effluent) inputs, and hence be contaminated with complex, ill-defined mixtures of chemicals. Most freshwater organisms will be exposed, to varying degrees, to this contamination. The number of species exposed is in the thousands, and quite possibly tens of thousands. Little is known about whether or not these species are adversely affected by the chemicals present in their environment. Often it is not even known what species are present, let alone whether they are affected by the chemicals present. In a few high-profile cases (e.g. tributyl tin causing imposex in molluscs and oestrogens ‘feminizing’ male fish), chemicals have undoubtedly adversely affected aquatic species, occasionally leading to population crashes. Whether or not other chemicals are affecting less visible species (such as most invertebrates) is largely unknown. It is possible that only very few chemicals in the freshwater environment are adversely affecting wildlife, but it is equally possible that some effects of chemicals are, as yet, undiscovered (and may remain so). Nor it is clear which chemicals may pose the greatest risk to aquatic organisms. All these uncertainties leave much to chance, yet designing a regulatory system that would better protect aquatic organisms from chemicals is difficult. A more flexible and intelligent strategy may improve the current situation. Finally, the risk due to chemicals is put into context with the many other threats, such as alien species and new diseases that undoubtedly can pose significant risks to aquatic ecosystems.

Keywords: aquatic organisms; chemicals; exposure; regulation of chemicals

1. Introduction

The realization that chemicals accidently or intentionally released into the environment could adversely affect wildlife is a relatively recent phenomenon. The acute and chronic toxicities of chlorinated pesticides to raptors documented

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in the 1960s and 1970s (reviewed in Walker 2006), and of the anti-foulant tributyl tin (TBT) to molluscs documented from the early 1970s (reviewed in Fent 1996) represent early examples of adverse environmental effects of chemicals in terrestrial and aquatic environments, respectively. Examples such as these raised awareness, and led to the development of ecotoxicology as a bonafide scientific discipline. Of course, gross pollution, particularly arising from disposal of untreated domestic and industrial wastes into rivers, had been occurring in industrialized countries for a century or more before specific ecotoxicological effects of chemicals were documented (see, for example, the story of pollution of the River Thames in England, as told in Wheeler (1979)). Once gross pollution was controlled, and to a reasonable extent eliminated, by the treatment of liquid wastes in sewage treatment plants (STPs) before release of effluent into rivers (or the marine environment), river water quality in urban areas improved considerably. As a consequence, aquatic organisms returned, and biodiversity improved considerably (e.g. Wheeler 1979). Although, even in highly developed countries (e.g. the UK), not all rivers have yet recovered fully from the gross pollution of the past, nevertheless, in general, river water quality is much improved, and aquatic biodiversity has increased (in some rivers considerably).

Despite these encouraging developments, recent changes in society have the potential to neutralize these improvements, and even potentially reverse them. These changes include a sustained increase in the size of the human population (there are currently 2.5 times the number of people in the world than there were when I was born!), an increased use of water (which is mostly returned to rivers after use), and an increased use and number of synthetic chemicals in widespread use. The consequences of these, and other, changes for some countries are profound. Some countries, such as the UK, are now very densely populated with people expecting a high standard of living (which is sustained by the use of synthetic chemicals), leading to a situation where water is re-used. That is, it is abstracted from rivers, purified, used by people or industry, and then disposed of as wastewater to a STP, where it is ‘cleaned’ before being discharged (as effluent) back into a river, only to be abstracted a second (or subsequent) time further downstream. This situation is beautifully exemplified by the River Thames in southern England. Over 12.5 million people live within the catchment of this river, each using approximately 200 l of water per day. Their wastewater goes to 352 STPs, all of which discharge their effluents into the main body of the river, or its tributaries (Williams et al. 2008). The flow of some of these tributaries (if they have major STPs on them) can consist primarily of effluent—a value of over 90 per cent is reached for some stretches in times of low or no rainfall, and even the main river is effluent dominated. This means that probably just about all aquatic organisms living in the River Thames and its tributaries will be exposed to whatever chemicals are present in effluent, with the degree of exposure being location-dependent. It will be highest in locations where major STPs discharge effluent into relatively small rivers, such that little dilution of the effluent occurs (Williams et al. 2008).

The construction of STPs last century, and the delivery of almost all wastewater to them, very substantially reduced the incidence of gross pollution killing aquatic organisms. For example, large ‘fish kills’ on rivers in the UK are now relatively rare, although they still occur occasionally, often due to a failure at a STP. Now, most concern is instead focussed on potential chronic effects
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of chemicals present in STP effluent on aquatic organisms. These effects could be quite subtle, and hence not at all obvious, but nevertheless important. It is easy to spot dead fish floating on the surface, killed by gross pollution, but very much more difficult to know if biochemical or physiological changes are occurring in otherwise healthy fish due to exposure to STP effluent, let alone accurately estimate the consequences of those changes at both the individual and population levels.

In this article, I will explore how much we know, and how much we do not know, about chemical pollution of the freshwater environment in a typical well-developed country, and the effects this pollution has on freshwater aquatic organisms. If we want to protect aquatic organisms (plants as well as animals) from chemicals, we need to be able to adequately, if not completely, answer the following questions:

(i) What chemicals are present in the aquatic environment?
(ii) At what concentrations are these chemicals present, and where?
(iii) What species receive exposure to these chemicals?
(iv) What are the effects of this exposure?

The first two questions are concerned with defining exposure, and the last two concerned with (potential) effects of this exposure. Although defining exposure is usually thought to be the job of environmental chemists, and addressing effects of chemicals the job of biologists (ecotoxicologists), very little meaningful progress can be made unless both groups of environmental scientists work together; the more closely the better. Sadly, this still happens relatively infrequently, to the detriment of good science and the environment.

I conclude this article with a discussion of the relative success of chemicals regulation in improving the situation in the aquatic environment from its low point of perhaps 100 years ago. I also discuss some of the complex issues that the current regulatory framework does not deal with well, such as the ‘mixtures issue’. I suggest how regulation could be improved, in particular, by utilizing knowledge of mechanisms of action in ecotoxicity testing. I end with some philosophical musing on the need to put the threat to aquatic wildlife posed by chemicals into perspective alongside the many other threats it faces. Although doing so would be very challenging, I consider it very necessary, and a challenge that needs to be accepted if the limited resources available are to be used most effectively to protect aquatic wildlife from the myriad of threats it faces.

2. What do we know about exposure?

In this article, I address only exposure to man-made chemicals, or natural chemicals (such as nutrients) introduced directly or indirectly into the aquatic environment by man’s activities. I do not address the issue of natural chemicals present due to natural processes, such as chemicals washed from the land into rivers, or minerals washed from rock into the freshwater environment. The water in even the purest river or stream, one completely unaffected by man, will contain a myriad of natural chemicals. Very few of these, however, are toxic in the manner of some (but by no means all) man-made chemicals.
We have seen that most ‘unnatural’ chemicals found in the freshwater environment in many countries (especially the developed ones) get there because STPs discharge large amounts of effluent into this environment. So, what do we know about what chemicals are present in this effluent, and at what concentrations? The answer appears to be ‘very little’ (Reemstsma et al. 2006). We know quite a lot about some chemicals, or groups of chemicals (e.g. metals), but little or nothing about the vast majority of chemicals likely to be present in STP effluent. It is easy to see why this is so when one realizes that a vast number of man-made chemicals are in everyday use in modern society, pretty much all of which are likely to be present in STP effluent. It is unclear precisely how many man-made chemicals are in regular use, and perhaps the figure can never be determined precisely (because, for example, it is constantly changing). Nevertheless, numbers such as 30 000 to 100 000 are often stated (Boxall et al. 2004; Schwarzenbach et al. 2006; Matthiessen & Johnson 2007). Whatever the exact number, it is a very large one, which is the key point. Nor it is known what proportion of these reach the aquatic environment: is it 1 per cent, or 99 per cent? It seems likely that the majority do, given the almost ubiquitous use of water (which is then disposed of as wastewater) in the processes and tasks of everyday life. Thus, it seems reasonable to conclude that a very large number (probably tens of thousands) of man-made chemicals arrive at STPs. But how many of these leave the STPs, and enter the freshwater environment?

Unsurprisingly, this number is not known either. STPs are designed to reduce, or even eliminate completely, the toxicity of wastewater before it reaches the environment. They remove some chemicals (in the sludge, which is then usually disposed of to land or incinerated), and degrade many others (Lester & Edge 2001; Johnson & Sumpter 2001; Ternes et al. 2004). Different chemicals will be removed to different degrees: some are almost completely degraded, and hence the concentrations of these chemicals in effluents are a very low percentage of the concentrations in influent (the natural oestrogen 17\(\beta\)-oestradiol serves as one of the very many potential examples; Kanda & Churchley (2008)), whereas others are very recalcitrant, and effluent concentrations are similar to influent concentrations (the pharmaceutical carbamazepine serves as an example; Okuda et al. (2008)). Other chemicals can be produced in the STP because they are intermediates in the biodegradation of chemicals entering the STP. Nonylphenol (NP) is one such chemical, whose presence in STP effluents is a consequence of the incomplete biodegradation of NP polyethoxylates, a class of non-ionic surfactants, in STPs (Giger et al. 1984). It is possible, even likely, that there are many chemicals in STP effluents (that therefore enter the aquatic environment) which were not present in influents to STPs. Thus, although STPs probably remove completely some chemicals, and markedly reduce the concentrations of very many others, they also add some new ones!

The question ‘how many man-made chemicals are present in the aquatic environment?’ is made all the more difficult to answer because many organic chemicals can exist in a number of different forms. I will use NP again as my example. To a biologist (like me), NP is one chemical—NP—even if I had to learn that only one form of it, namely 4-tert-NP, is widely used. But 4-NP is not one chemical at all; in fact it could consist of 211 isomers, because the nonyl group can be, and is, branched in many different ways (Guenther et al. 2006). In fact, many of these isomers can exist as two or four enantiomers (mirror images of
one another), leading to the remarkable fact that 4-NP can exist in 550 different structures (Guenther et al. 2006). Thus, perhaps 4-NP should not be considered as one chemical, but as 550! None of this is pedantic, because different isomers of NP vary very considerably in oestrogenic activity (Gabriel et al. 2008), and also degrade in the environment at different rates (Gabriel et al. 2008). This suggests that the biological effects of NP on aquatic organisms are likely to be isomer-specific. All of this means that we will probably never know how many chemicals are present in STP effluents, and therefore how many end up in the freshwater aquatic environment.

Obviously, it is not only the presence of a chemical that is important, but also its concentration. Despite some opinions to the contrary (e.g. Calabrese 2005; Vom Saal & Welshons 2006), most (eco)toxicologists think that the higher the concentration of a chemical, the greater the effect it will have (if it has any effect, of course). However, the concentration of a chemical that causes concern varies greatly, depending on the chemical. For example, for a very biologically potent chemical, such as ethinyl oestradiol (EE2), a concentration as low as a few nanograms per litre would be of considerable concern because it would prevent fish from reproducing (Caldwell et al. 2008), whereas much higher (μg l⁻¹) concentrations of non-toxic chemicals (such as EDTA or linear alkylbenzene) would not be of concern. Thus, the concentration of a chemical needs to be put into context.

The concentration of any chemical in the aquatic environment will be very spatially variable, and may also vary temporally. For point sources of chemical pollution, such as effluents, often only the concentration of a chemical in the effluent is determined; there may be few, if any, measurements of river concentrations of the chemical, despite the fact that these are the important ones. The latter will depend on the degree of dilution of the effluent in the receiving source (which can be very variable), and the rate of degradation of the chemical (or loss by other means). The concentration of a chemical 100m downstream of an STP could easily be very different (much higher) than the concentration 5km downstream.

As far as chemicals entering rivers in effluents (probably the majority of chemicals in developed countries), the situation can be summarized as follows. The majority of chemicals likely to be present have not even been identified. In other words, we do not know that they are present. If this seems improbable, think of human pharmaceuticals. Twenty years ago, none had been reported to be present in the aquatic environment. Ten years ago, about a dozen (or less) had been positively identified (though usually only in effluents, not rivers). Today, about 100 have been identified (Batt et al. 2008; Kasprzyk-Hordern et al. 2008), most of which were probably there 20 and more years ago. As about 3000 different pharmaceuticals are in everyday use, presumably many more await ‘discovery’ in effluents. Knowledge of river concentrations, for all chemicals, is scant, and often non-existent. It has recently been cogently argued that modelling, rather than measuring, aquatic concentrations of chemicals of interest may well be an efficient way forward (Johnson et al. 2008).

Besides the chemicals entering rivers at point sources (i.e. in effluents), an appreciable number will enter as diffuse sources. Many of these will be agrochemicals, including nutrients (used as fertilizers) and biocides (pesticides, fungicides and herbicides). More is known about the aquatic concentrations of
these chemicals than most chemicals that enter at point sources. This is primarily a consequence of the known toxicity of many of these chemicals. For example, pyrethroid insecticides are extremely toxic to many aquatic invertebrates (Hill 1989). Because of this, concentrations of many nutrients and agricultural biocides are monitored routinely in national monitoring programmes. Thus, for these chemicals, exposure concentrations are well known.

In answer to the question posed at the beginning: What do we know about exposure? The general answer has to be 'surprisingly little'. Only for relatively few groups of chemicals (such as the agrochemicals) is there both temporal and spatial information of the required quality. For most chemicals entering at point sources, the information needed for intelligent decision-making is not available (metals may be one exception, because concentrations in effluents of receiving waters are monitored to conform to legislation). Finally, when discussing chemicals, it should be mentioned that everything I have said above leads to the conclusion that aquatic organisms are exposed (probably throughout their lives) to very complex, very ill-defined, mixtures of man-made chemicals whose composition is probably highly variable and continuously changing; even the weather will influence exposure greatly! This makes carrying out environmentally relevant ecotoxicology extremely challenging.

3. What species are exposed?

We have seen that the vast majority of the freshwater environment in the UK (and probably many other countries) is contaminated with a very large number of chemicals, many man-made. This must mean that the vast majority of aquatic organisms are exposed to these contaminants. Hence, the answer to the question 'what species are exposed' is probably 'essentially all of them'. But how many species is this, and what do we know about their responses to the chemical contamination of their environment?

The UK has one of the most studied fauna and flora of any country. For hundreds of years, amateurs and professionals alike have been studying its fauna and flora, and recording their observations. Thus, one might think that a fairly precise answer to the question 'how many species of aquatic organisms are there in the UK' would be readily available, but this appears not to be the case. However, the total number of species, in all habitats (terrestrial as well as aquatic) in England has recently been estimated (table 1).

Of the approximate 40000 species of invertebrates, around 4000 live in freshwater, which provides an indication of the degree of biodiversity in the freshwater environment in the UK. There will, of course, be countless species of microscopic animals (e.g. protozoa and bacteria) that are not included in the list above. However, despite what seems like quite high numbers of species living in the aquatic environment (such as 4000 species of invertebrates), in fact England has a fairly impoverished fauna and flora compared with many other countries. This is because the UK is an island, that it is relatively small and that it is in a temperate region of the world. If it was an island of comparable size but located in the tropics, it would have a much more diverse fauna and flora. Nevertheless, the key point is that thousands of freshwater species are probably exposed to a large number of ‘unnatural’ chemicals that
Table 1. Estimated number of species in the UK\textsuperscript{a}.

<table>
<thead>
<tr>
<th>species group</th>
<th>estimated number of native species</th>
<th>estimated number of non-native species</th>
</tr>
</thead>
<tbody>
<tr>
<td>birds</td>
<td>550</td>
<td>10</td>
</tr>
<tr>
<td>mammals (terrestrial)</td>
<td>64</td>
<td>20</td>
</tr>
<tr>
<td>amphibians</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>reptiles (terrestrial and marine)</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>freshwater fish</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>invertebrates (terrestrial and freshwater)</td>
<td>40,000</td>
<td>474</td>
</tr>
<tr>
<td>vascular plants (terrestrial and freshwater)</td>
<td>2744</td>
<td>1,846</td>
</tr>
<tr>
<td>bryophytes</td>
<td>935</td>
<td>18</td>
</tr>
<tr>
<td>lichens</td>
<td>&gt;1,700</td>
<td>n.a.</td>
</tr>
<tr>
<td>fungi</td>
<td>12,000–20,000</td>
<td>198</td>
</tr>
<tr>
<td>marine mammals</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>marine fish</td>
<td>&gt;300</td>
<td>0</td>
</tr>
<tr>
<td>marine plants/algae</td>
<td>&gt;900</td>
<td>25</td>
</tr>
<tr>
<td>marine invertebrates</td>
<td>&gt;7,000</td>
<td>39</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Taken with permission from Natural England (2008) where the sources of the original data can be found.

get into the freshwater environment through Man’s activities. Some of these species, such as most of the fish, live in the water itself, but many others will live on, or in, the sediment. Many will spend their entire lives in freshwater, but some will spend part of their lives in the marine environment (e.g. eels, salmon, lampreys), and others some of their lives in the terrestrial environment (e.g. amphibians, many insects). Some are sessile, others quite mobile. They demonstrate a wide variety of lifestyles: some animals are at the bottom of the food chain, filtering their food from the water, whereas others are predators, at the very top of the food chain. All these factors will have profound effects on the degree of exposure to chemicals experienced by these different aquatic organisms.

If this exposure to chemicals was adversely affecting some of our aquatic species, would we know? Given that the fauna and flora of the UK is extremely well studied (by international standards), one would assume that we would, but in fact this is not so—certainly the vast majority of aquatic species in the UK, including (perhaps surprisingly) most of the protected ones (these are usually rare, endemic or their populations are declining rapidly), are not systematically monitored (Natural England 2008). This means that, if the populations of these species changed, this may well not be apparent. If there are no baseline data, a population change is extremely difficult to detect (Sumpter & Johnson 2005). Hence, if a chemical, or mixture of chemicals, adversely affected a species, or a group of species, this fact may not be detected. The accidental discoveries of the adverse effects of TBT on molluscs (see later) and oestrogenic chemicals on fish (Sumpter & Johnson 2008) attest to the fact that detecting effects of chemicals, even on obvious species, is not easy; chance may play the greatest role in these discoveries (Sumpter & Johnson 2005).
The apparently intractable problem of so many chemicals potentially affecting so many different aquatic organisms would be appreciably simplified if all the organisms in one group (e.g. all the fish, or all the molluscs) responded in the same manner to a particular chemical or even group of chemicals. Although the information available to examine this possibility is by no means as extensive as one would like it to be, it does appear that, in general (there will almost inevitably be exceptions), groups of similar organisms do behave in similar ways to similar chemical exposures. For example, various oestrogenic chemicals are of comparable potency, and cause the same effects, in a variety of species of fish (Sumpter & Jobling 1995; Sumida et al. 2003; Denny et al. 2005), and TBT causes imposex in over a 100 species of prosobranch molluscs (Fioroni et al. 1991). There may well be subtle differences, caused by a variety of factors, between similar species, but the underlying message seems to be that phylogenic groups of aquatic organisms should be expected to respond in a similar manner when exposed to the same chemical, or some mixture of chemicals. This situation presumably reflects similar molecular, biochemical and physiological processes within a group of closely related organisms (i.e. all fish have oestrogen receptors that, when activated by an oestrogenic chemical, lead to the synthesis of vitellogenin).

However, the other side of the coin is that different groups of organisms may (at least theoretically) respond in different ways to a chemical, or one (or more) group may respond, whereas others do not. For example, pyrethroid insecticides are extremely toxic to fish and aquatic arthropods (a group of invertebrates), whereas these chemicals are of low toxicity to birds and mammals. Even within the aquatic arthropods, sensitivity varies by at least 100-fold (Maund et al. 1998). Similarly, fish are much more sensitive to EE2 than are invertebrates (Caldwell et al. 2008), often by many orders of magnitude. I am not (yet) aware of a chemical causing one effect, via one mechanism of action, in one species or group of species, and another, quite different, effect, via another mechanism of action, in another species, or group of species, but it does not seem implausible to me that such a situation could occur.

In summary, many different organisms, from many different taxa, live in, or are to some degree dependent on, the freshwater environment. Most, if not all, of these organisms will receive exposure to the myriad of chemicals contaminating freshwaters, especially rivers that receive inputs of effluents from STPs. For most chemicals, there is little or no evidence that this exposure causes harm. However, populations of most aquatic organisms are not regularly monitored, and hence detecting any adverse effects of chemicals is difficult, if not impossible.

### 4. Lessons from tributyl tin

This is one of the best documented examples of endocrine disruption. The story is very well known (see Matthiessen & Gibbs (1998), for a thorough review), and will not be repeated here. TBT got into the aquatic environment because it was widely used as an antifoulant on the hulls of ships. Abundant field and laboratory data causally link TBT with an irreversible sexual disruption of female molluscs known as ‘imposex’. This phenomenon has been reported from around the world, and has led to local extinctions of some species of molluscs (because imposex individuals cannot reproduce). Many lessons can be learnt from this example of
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a chemical adversely affecting aquatic wildlife, while others probably still remain to be teased out by future research. Some lessons relevant to this article include the following:

(i) The effect of TBT was not predicted, it was discovered by accident.

(ii) It took almost 35 years to discover the mechanism of action (MoA) of TBT. For many years, TBT was thought to be an aromatase inhibitor (thereby depressing oestrogen concentrations while simultaneously elevating androgen concentrations, leading to masculinization of females). This theory was challenged when TBT, and other organotins, were shown to be strong agonists of the retinoid X receptor (RXR) nuclear receptor, a receptor known to be involved in the regulation of various developmental processes, including male sexual development (Nishikawa et al. 2004). There is now compelling evidence that organotins function as powerful agonists for the RXR, and possibly other nuclear receptors, rather than act as aromatase inhibitors (Nakanishi 2008; Sternberg et al. 2008).

(iii) TBT affects entire ecosystems, and not just molluscs. The vast majority of research on the ecotoxicity of TBT has been conducted on molluscs, although organisms at other trophic levels are known to be sensitive to TBT. Given the dramatic effects of TBT on molluscs, many of which are grazers feeding on algae and macrophytes, it is perhaps surprising that the possible effects of TBT on plant communities, and other associated animal communities, have been so little studied. However, a recent comprehensive study (Sayer et al. 2006) has provided the first evidence that TBT can be associated with a dramatic loss of both macrophytes (the submerged vegetation) and the diverse animal communities usually associated with this vegetation. In other words, TBT can alter the entire aquatic ecosystems. This should not come as a surprise, given the complex inter-relationships between, and dependencies on, all the animal and plant species that make up any ecosystem; perturb one species, and it is likely you will perturb all other species, until a new equilibrium (a new ecosystem) is reached. Indirect effects can be much more profound than direct effects.

(iv) Even with today’s knowledge and experience of ecotoxicology, the effect of TBT on molluscs would not be detected in regulatory tests. Despite continuing improvements in the methodologies of testing chemicals to assess whether or not they could be of environmental concern, currently no tests are in use that would detect the effect that TBT has on molluscs (Matthiessen 2008), although the acute toxicity of TBT to many organisms would be detected by many of the ecotoxicity tests in use currently.

General ‘lessons’ such as these (see also Sumpter & Johnson 2005) probably apply to many other chemicals of, or potential, environmental concern. Further research on TBT is likely to produce more surprises, from which it may (ultimately) be possible to deduce more lessons. One key unresolved issue can be formulated as the following question:

(i) why are molluscs so specifically affected by TBT?

Or more generally by the following question:

(ii) does TBT act specifically (through the RXR?) on all species, or only on molluscs?
Put another way, is there something unique about molluscs that makes them so susceptible to TBT, or does the chemical adversely affect many different groups of aquatic organisms? If the main MoA of TBT and other organotins is via nuclear receptors such as the RXR and peroxisome proliferator activated receptor \( \gamma \) (Nakanishi 2008), then any organism possessing these receptors could, potentially, be adversely affected if exposed to these chemicals. These nuclear receptors are ubiquitous throughout the animal kingdom (in both vertebrates and invertebrates), and they play important roles in regulating the rate of transcription of many genes. It is therefore possible that TBT could adversely affect just about any organism. It is likely that all aquatic species receive exposure to TBT, because it is widespread in both the freshwater and marine environments (Fent 1996; Matthiessen & Gibbs 1998). Exposure could be either direct via the water and/or sediment, or indirect, through the food web.

An alternative explanation for the apparent unique susceptibility of molluscs to TBT is that they receive the highest level of exposure of any groups of aquatic organisms, and hence show the most dramatic effects. Given the lifestyle of many molluscs, and the propensity of TBT to bind to particulate matter, this is entirely possible, although unproven currently.

5. How successful has regulation of chemicals been?

This is also a difficult question to answer with any degree of certainty. It can be cogently argued that regulation has, in general, been very successful. Regulation has reversed the gross pollution of rivers in industrialized countries that was so apparent less than 100 years ago. Despite the fact that many more chemicals are in use today than were even only 50 years ago, and that much greater volumes of (treated) wastewater enter rivers now than was the case in the past (a consequence of larger populations, using much more water, in just about all countries), both the chemical and biological qualities of rivers in most developed countries have improved recently (Environment Agency 2004; Natural England 2008). This is not the case, however, in countries that are currently developing rapidly, such as China (Zimmerman et al. 2008), where regulations lag behind development, and chemical pollution of freshwaters is a major problem. Today, in developed countries, regulation permits contamination of the aquatic environment, as long as this contamination does not cause significantly adverse effects on aquatic biodiversity. It would probably be technically impossible, and certainly extraordinarily expensive, to remove all chemicals from wastewater in STPs, so that only effluent of the quality of ‘pure’ water was discharged into rivers, and hence it is accepted that effluent will contaminate the receiving waters. That effluent has to meet environmental quality standards (EQSs) for a growing list of substances (e.g. many metals, some solvents, many pesticides, some plasticizers), in order that it does not adversely impact the biodiversity of the receiving water. The general improvement in river water quality in many developed countries, and the consequent improvement in biodiversity, attest to the fact that legislation has been effective. Although the toxicity of some chemicals evaded detection, and their use led to serious consequences (e.g. TBT), the number of such chemicals that we are aware of is very small, and hence they represent a minute proportion of the total number of chemicals in everyday use. If this argument is correct, then
regulation has been remarkably successful. However, one always needs to keep in mind the fact that, for the vast majority of aquatic organisms, we have absolutely no idea whether or not they have been impacted by chemical pollution of their environment. Hence, it may be premature to conclude that the current chemicals present in rivers (at the concentrations present today) pose little or no threat to aquatic wildlife.

It is widely accepted that the current regulatory framework is not perfect. One problem is that chemicals are tested individually to determine their ecotoxicity, yet are present in the environment as one component of complex chemical mixtures. Current regulations deal with the ‘mixtures issue’ either through the use of safety factors or, if enough information is available on the potencies of similarly acting chemicals, through toxic equivalency factors. As an example of the latter, if the steroid oestrogens, such as oestradiol, oestrone and EE2 are assigned relative biological potencies of 1, 0.3 and 10, and are considered to act additively (Brian et al. 2005), then an EQS of 1 oestradiol equivalent per litre could be sent by a regulatory authority (Jobling et al. 2006) in order to protect fish from oestrogens, which could be made up by any combination of the three oestrogens, such as 0.5 ng l\(^{-1}\) oestradiol, 1.0 ng l\(^{-1}\) oestrone and 0.02 ng l\(^{-1}\) EE2. It is not going to be easy to translate recent theoretical advances in mixtures toxicity (Kortenkamp 2007) into practical legislation that better protects aquatic wildlife. The only other way to address the mixtures problem is to assess the toxicity of whole effluents, and legislate on that basis, as is done already.

The problem of determining the effects of complex mixtures of chemicals is exemplified by the situation of the eel (*Anguilla anguilla*). Probably as a consequence of their lifestyle (they live in the sediment), eels can contain a high body burden of contaminants; so far about 100 chemicals have been identified in freshwater eels, including polychlorinated biphenyls (PCBs), organochlorine pesticides, many metals, brominated flame retardants, many volatile organic pollutants, endocrine disruptors, dioxins, perfluorooctane sulphonic acids, metallothioneins and polycyclic aromatic compounds (Belpaire & Goemans 2007). However, despite these eels being heavily contaminated with chemicals, it is unknown if this contamination is detrimental to the health of the fish, and nor is it at all easy to know how that issue could be answered one way or the other.

A second important issue that current chemicals legislation does not deal with well (if at all) is indirect effects. As exemplified with TBT (see above), if one taxon is adversely affected by a chemical, or mixture of chemicals, present in the aquatic environment, then it is extremely likely that many other taxa, both up and down the food web, will also be affected. Populations of some might increase, because numbers of their predators decline, whereas populations of others might decrease, due to them having a reduced good supply. The effects, both direct and indirect, of EE2 on phytoplankton and zooplankton serve to illustrate how even a single chemical can have complex effects at many trophic levels of an ecosystem (Hense et al. 2008; Schramm et al. 2008). One approach to address this deficiency in current chemical regulations would be to incorporate knowledge of community ecology into risk assessments of chemicals (Rohr et al. 2006). Indirect effects of chemicals are often overlooked (Fleeger et al. 2003), yet there is growing evidence that they are both common and complex; more so than the direct effects. An
improved understanding of species interactions and functions within ecosystems, gained through community ecology, and the latter’s integration into ecotoxicology, is needed if accurate predictions of the effects of chemicals on ecosystems (which should be the goal) are to be made.

A final problem of current regulatory practice is that each chemical is usually tested (to determine its ecotoxicity) on only a few taxa (three is normal: one plant, one invertebrate and one fish), whereas many more taxa will be exposed to that chemical when it is present in the aquatic environment. As discussed above, it is unclear how protective to all aquatic organisms such a strategy is.

It is easy to be critical of the current regulatory strategy used to control the concentrations of chemicals in the aquatic environment (because of its failures), but could a better, yet still practical, regulatory system be devised that would be more protective of the environment? My personal opinion is only with great difficulty, if at all. However, this opinion does not mean that we cannot learn from what we know now, to improve on where we are presently. Perhaps, the two biggest advances that could be made would be for the regulatory regime to be more flexible (e.g. Walker 2006) and to use known MoA of chemicals in ecotoxicity testing (e.g. Segner 2006; Ankley et al. 2007). If chemicals have known MoAs (such as pharmaceuticals), it seems sensible to use this information when designing ecotoxicology tests, in order to maximize the chances of detecting any potential adverse effects they might have on aquatic organisms. However, despite continuing improvements in regulatory practice, based on what we know now, we probably still could not have predicted the consequences of TBT use. Nor were the devastating effects of diclofenac, when used as a veterinary pharmaceutical, on vultures (about 40 million have been killed by the drug; Oaks et al. (2004) predicted. Although, admittedly, an example from the terrestrial, rather than aquatic, environment, a lot of general applicability can be learnt from this example about unexpected routes of exposure to chemicals, and unexpected sensitivity of one group of organisms to a particular chemical. On the other hand, the effects of EE2 on fish (reviewed in Caldwell et al. 2008) could, and should, have been predicted. I consider it likely that it will prove possible to predict the ecotoxicological effects of some chemicals (synthetic progestogens may well be an example; Sumpter (2005)), but that some surprises still await discovery. These are likely to come from both chemicals already in use, and from new ones not yet present in the aquatic environment.

There is one final angle on regulation that is often forgotten, yet needs to be factored in. Regulations are usually viewed as necessary to protect aquatic organisms from the threats they face; that is, society addresses its ethical responsibility to maintain a healthy aquatic environment rich in biodiversity through regulation (of chemicals in this example). However, these regulations also aim to protect consumers (usually, but not exclusively, human) of fish and other aquatic organisms from the adverse effects of chemicals. A tragic example of how things can go wrong began in Japan in 1950s. Metallic mercury released from a paper factory on the shore of Minamata Bay in Japan was methylated in the sediments by bacteria, forming methyl mercury, which is much more bioavailable than elemental mercury. It passed rapidly up the food chain, accumulating in high concentrations in fish and shellfish, causing mass deaths of these organisms. Contaminated fish were eaten by local people, who suffered mercury poisoning. About 100 people, mainly the physiologically weak, died, and many more suffered...
severe adverse effects, such as permanent paralysis, from the mercury poisoning. Foetuses of pregnant mothers were affected, suffering developmental problems, leaving a very long legacy (Kudo & Miyahara 1991; Harada 2005). Similar issues arose when salmon and other fish species in the Great Lakes of North America became heavily contaminated with polycyclic aromatic hydrocarbons and PCBs in the middle of the last century, although, in this case, people were not directly killed by ingesting contaminated fish and, in fact, the evidence for the contamination adversely affecting consumers of the fish is not particularly strong (Leatherland 1998). These examples serve to illustrate why it is important to protect aquatic organisms from chemicals; it is not just for their own sake (though this is surely a strong enough reason on its own), but also because, once animals are contaminated, contamination will inevitably reach people one way or another, and may cause public health problems.

6. Getting things in perspective

If the aim of environmental scientists is to understand and protect the environment, then they should focus their attentions, and use the resources available to them, in the most effective manner. One way to try and accomplish this is to focus primarily on the major threats facing the environment. In the case of the freshwater environment, currently perceived threats include habitat degradation (or even complete loss), alien species, new or introduced diseases and parasites, and climate change, as well as chemicals. Ranking these threats, in order to concentrate the efforts of environmental scientists on the biggest threats, is extremely difficult. As far as I know, nobody has tried to do so; it may be an impossible task. However, a few examples are well documented where threats have been real, and led to major effects on aquatic biodiversity. These examples include the following:

(i) Displacement of native crayfish by an American crayfish.

The non-native signal crayfish (*Pciastacus leniusculus*) escaped from farms and is now thriving in many rivers in the UK. It has rapidly and very effectively displaced the native white-clawed crayfish (*Austropotamobius pallipes*), primarily by carrying the fungal crayfish plague, to which the native species (but not the alien species) is highly susceptible (Alderman 1993).

(ii) Rapidly declining wild salmon populations due to a disease connected to fish farming.

In British Columbia, Canada, some wild populations of pink salmon are declining very rapidly, while others are not. The cause of the declines appears to be a parasite (sea lice) that wild juvenile salmon catch from caged salmon maintained on fish farms (Krkosek *et al*. 2007). If parasite outbreaks continue, a 99 per cent collapse of some pink salmon populations is predicted, leading to local extinctions.

(iii) Loss of native water voles (*Arvicola terrestris*) due to predation by introduced mink (*Mustela vison*).

American Mink, reared on farms for their fur in the UK (and some other countries) inevitably escaped. The species is now widespread over the entire UK. Mink predate water voles, so populations of the latter have collapsed, and a once common species has disappeared completely from many rivers (Jefferies 2003).
More examples could be provided of how the freshwater biodiversity of the UK has been changed dramatically in the recent past, and is continuing to change. That process has been going on for thousands of years. It is no coincidence that the best documented examples of man’s effects on aquatic biodiversity (such as those above) involve highly visible species, such as fish and mammals. Less visible species (such as small invertebrates) have probably also been dramatically affected by man’s intentional or unintentional interference with the freshwater environment, but these cases are much less well documented—many probably remain unseen and unknown.

As stated earlier, chemicals (or, more accurately, untreated wastewater) had profound effects on aquatic biodiversity in the past; they probably posed the single biggest threat. Now, however, it is much less clear how significant a threat chemicals pose to aquatic biodiversity in a well-developed country such as the UK, and hence where one would place chemicals in a list that ranked current threats. Nevertheless, I think it would be very foolish to downplay the threat that chemicals can pose to freshwater biodiversity; that would be a dangerous move. What we need to do is learn from the mistakes of the past, and apply those lessons to current and future issues concerning chemicals in the aquatic environment (Sumpter & Johnson 2005). For example, the loss of otters (Lutra lutra) from much of their range in many countries, a consequence of them eating fish (such as eels—see above) contaminated with chemicals has taught us a lot about how recalcitrant chemicals can move through a food chain, and accumulate in top predators, leading to adverse health effects (Mason & MacDonald 1993). But what chemicals, or group of chemicals, pose the greatest threat to aquatic biodiversity currently, and hence merit the most attention? Despite most attention being placed on micro-organic contaminants, such as endocrine disruptors, pharmaceuticals and flame retardants, could it be that nutrients represent the worse case of chemical contamination of the environment?

As discussed above, levels of organic and industrial pollution of freshwaters have, in general, been decreasing steadily in recent decades, at least in developed countries, partly as a result of the decline in heavy industries, and partly due to investment in sewage treatment (Mainstone et al. 2008; Natural England 2008). Improving sewage treatment also permits some inorganic pollutants, such as phosphates, to be controlled. In contrast, control of diffuse sources of pollution, particularly from agriculture, is still in its infancy. Thus, as controls on point sources pollutions have taken effect, the contribution to chemical pollution from agriculture has become more apparent. For example, the main cause of the unfavourable condition of many freshwaters (both rivers and lakes) currently classified as Sites of Special Scientific Interests (i.e. they are of high conservation status) in the UK is eutrophication (Natural England 2008). Surveys conducted by the UK Environment Agency suggest that a surprisingly high percentage of river stretches in England (perhaps as high as 50%) contain unacceptably high concentrations of nutrients such as phosphate and nitrate (Environment Agency 2008). Eutrophication alters growth of plants, in particular stimulating growth of algae. The consequences are felt throughout the food chain, from invertebrates to fish and mammals. Other indirect effects of eutrophication include oxygen depletion in both the water and the sediment, which will, in turn, affect all biodiversity. Thus, in many countries, ‘old-fashioned’, unsexy chemicals may be having a greater adverse effect on aquatic biodiversity than those organic

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chemicals receiving a lot of attention from scientists currently, such as endocrine disruptors, flame retardants and perfluorinated compounds (the latter are used primarily as surfactants and surface protectors). However, we still know very little about the ecotoxicological effects of these, and many other, micro-organic pollutants, and hence it is essentially impossible to put into context the worldwide aquatic distribution of these persistent chemicals. We do not know if a situation as devastating as the almost complete loss of vultures in southeast Asia due to diclofenac poisoning (Oaks et al. 2004) will occur in the future in the aquatic environment. Reducing inputs of chemicals into the freshwater environment is probably the best strategy to minimize the chances of such an event occurring. Further improvements in STP processes, and educating farmers in order to reduce diffuse pollution from agriculture, would seem to be sensible strategies. Such strategies will become all the more important if the human population continues to grow, there is increasing use of synthetic chemicals and global climate change reduces the flow of rivers at certain times of the year.

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