NATIONAL REPORT

MIXTURES OF CHEMICALS IN WATER: IMPLICATIONS FOR CHEMICAL REGULATION AND ENVIRONMENTAL POLICY

Dr Jayne V Brian, Professor John P Sumpter *Institute for the Environment, Brunel University, UK*

Scientists have shown that mixtures of chemicals can act together to reduce the reproductive capacity of fish even if each individual chemical in the mixture is present at a concentration that does not cause an adverse effect. This evidence demonstrates that legislation based on the assessment of single chemicals may not be sufficiently protective and suggest that there is a need for a review of existing environmental policy.

Introduction

Adequate environmental protection is 'essential for ensuring the quality of life of current and future generations'.[1] However, efforts to ensure that our water resources are managed in a sustainable manner are compromised by inputs of anthropogenic chemicals. Government regulatory agencies are legally required to assess the risk that these chemicals pose to wildlife and to human health,[2] but this presents a formidable challenge. The majority of chemicals enter the environment as complex mixtures (eg effluents from wastewater treatment), whereas most chemical regulation is based on maximum acceptable exposure thresholds, which are set for individual chemicals, using data derived from single-substance toxicity tests. A safety factor is generally applied to account for uncertainties that might arise in the extrapolation of laboratory data to the field (eg differences in species sensitivity and/or effects on different life stages, as well as the possibility of interactive effects). However, it does not take any formal account of mixture effects that might occur on release into the environment.[3] This is cause for concern in light of growing evidence that chemicals can act together in combination, even at low and individually ineffective concentrations. Much of this evidence stems from the analysis of chemicals that have been associated with the disruption of normal endocrine function in a wide range of aquatic wildlife.[4]

Endocrine disrupting chemicals (EDCs) do not exert their effects in the traditional sense by causing acute toxicity. Instead, they interfere with endocrine function by mimicking the actions of hormones, blocking their effects or by interfering with their synthesis or excretion. A plethora of chemicals with endocrine disrupting properties has been identified. These include natural and synthetic steroid hormones, such as those used in the manufacture of the birth control pill, as well as a multitude of highly diverse chemicals, including some commonly found in detergents, cosmetics, plastics and pesticides. The sheer numbers of these chemicals, combined with their ability to exert effects at levels that are well below the limits of detectable effects in standard toxicity tests, poses a considerable challenge for environmental regulators. Some of the issues surrounding the regulation of EDCs under the Water Framework Directive (WFD)[5] were raised recently in this journal.[6] Matsuno discusses the issue of mixtures of chemicals to some extent, pointing out that safety standards based on the effects of single chemicals may underestimate the effects of exposure to mixtures of EDCs in real life exposure situations, due to the potential for combined effects. Here, we will build on the previous text using recent data on estrogenic mixture effects in fish as a means of highlighting some of the regulatory and legal implications of our research.

Mixture effects: the evidence

There is now unequivocal evidence that certain classes of EDC have the capacity to act in combination, at very low concentrations, to produce significant mixture effects. Of particular note are studies demonstrating the effects of multi-component mixtures of estrogenic chemicals on the reproductive physiology of fish.[7] This

1 Europa 'Overviews of the European Union Activities: Environment' http://europa.eu/pol/env/overview_en.htm. Last visited July 2007.

2 W R Munns 'Assessing Risks to Wildlife Populations from Multiple Stressors: Overview of the Problem and Research Needs' (2006) 11 Ecology and Society Art No 23.

3 P Mattheissen, I Johnson 'Implications of Research on Endocrine Disruption for the Environmental Risk Assessment, Regulation and Monitoring of Chemicals in the European Union' (2007) 146 Environmental Pollution 9–18.

4 S Jobling, C R Tyler 'The Ecological Relevance of Chemically Induced Endocrine Disruption in Wildlife' (2006) 114 Environmental Health Perspectives 7–8 Supplement 2.

5 Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy OJ 2000 L 327. Text of the directive and information on the implementation timetable are available at http://www.defra.gov.uk/environment/water/wfd/index.htm.

6 R Matsuno 'The Regulation of Endocrine Disruptors under the Water Framework Directive' (2006) 17 Water Law 93–99.

7 K L Thorpe and others 'Assessing the Biological Potency of Binary Mixtures of Environmental Estrogens Using Vitellogenin Induction in Juvenile Rainbow Trout (*Oncorhynchus mykiss*)' (2001) 35 Environmental Science and Technology 2476–81; K L Thorpe and others 'Relative Potencies and Combination Effects of Steroidal Estrogens in Fish' (2003) 37 Environmental Science and Technology 1142–49; J V Brian and others 'Accurate Prediction of the Response of Freshwater Fish to a Mixture of Estrogenic Chemicals' (2005) 113 Environmental Health Perspectives 721–28.

laboratory-based evidence shows that groups of similarly acting chemicals, such as those with estrogenic activity, behave in an additive manner, such that significant effects can occur even when each chemical in the mixture is present at a low and individually ineffective concentration (see Figure 1). The realisation that each component contributes to the overall effect of the mixture, in a manner that is proportional to its potency and concentration, undermines the traditional risk assessment paradigm of there being a threshold level of exposure below which a chemical is not considered to pose a threat (the no observed effect concentration (NOEC)). This has important environmental implications with particular regard to the regulation of effluents, which may contain tens, hundreds, or even thousands of chemicals at concentrations that are deemed environmentally acceptable on the basis of their individual effects. This issue represents one of the major shortcomings of existing legislation and highlights the need for more integrated approaches in the implementation of environmental policy.

In order to illustrate the potential implications of these combined effects for wildlife populations, we recently carried out an investigation into the combined effects of estrogenic chemicals on the reproductive performance of laboratory fish.[8] Breeding pairs of the fathead minnow (*Pimephales promelas*) were exposed to five different chemicals at low-effect concentrations, both individually and as a mixture, and their influence on a suite of reproductive endpoints, including egg production, spawning frequency and the expression of male secondary sexual characteristics, were assessed. Data obtained from a series of experiments provided evidence of mixture effects on fitness and fecundity, demonstrating that these chemicals have the capacity to act together to adversely affect the reproductive performance of aquatic organisms, even when each component of the mixture is present at a concentration below the threshold of detectable effects.

Environmental implications

The results obtained from our investigation are consistent with our understanding of the effects of mixtures at the cellular and physiological level. However, they are of particular significance in that they demonstrate the risk of combination effects on reproductive endpoints, which may impact upon population-level processes. From this, we can infer that the exposure of wildlife to mixtures of chemicals at low, environmentally relevant concentrations could threaten population sustainability. Furthermore, these experiments, which focus on the response of fish to mixtures of estrogenic chemicals, simply provide a model system: the implications are far reaching. The principle that we have demonstrated is likely to apply to any class of chemical, any species and any effect. Hence, people, as well as wildlife, are at risk from combined effects. Indeed, there is growing evidence

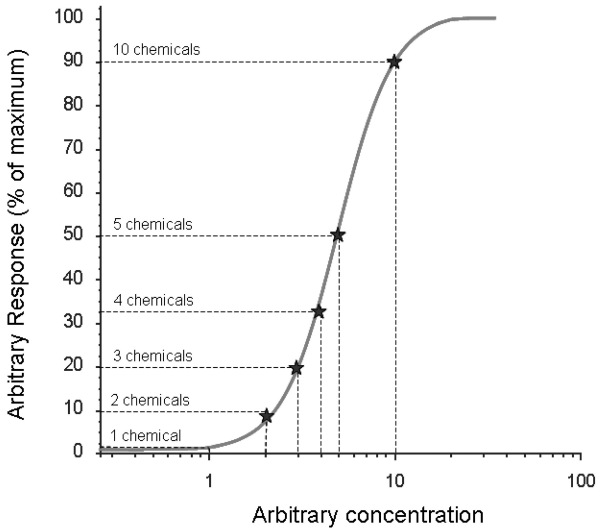

Figure 1: *Recent evidence shows that estrogenic chemicals act together additively, even at low and individually ineffective concentrations. According to the typical dose response curve shown here, this means that a mixture of ten chemicals that are each capable of producing a 1 per cent effect individually, can induce a near maximal response (90 per cent) when present in combination. This illustrates that there is no threshold concentration below which a chemical cannot be considered to pose a risk to the environment: even relatively small numbers of chemicals can act together to produce a significant overall response. The safety factors that are currently employed by regulators may not be sufficient to account for their combined activity.*

to suggest that this type of low-dose, combined exposure scenario is responsible for a wide range of endocrine-mediated effects in humans, such as those on the development, neurological function and immune status of children inhabiting contaminated regions.[9]

Growing recognition of the mixtures issue in recent years has prompted mammalian toxicologists and ecotoxicologists concerned with the risk assessment of chemicals in the environment to move rapidly from a substance-specific approach to a realisation of the need to take account of the effects of mixtures. Although we, the authors, are scientists with no legal training, we have come to realise that the mixtures issue raises a series of complex and challenging legal issues that may require regulators and policy makers to do the same. In the following section, we have highlighted some of these issues in an attempt to raise their profile. We hope that environmental lawyers will recognise that these legal problems merit more attention than they appear currently to receive.

Regulatory implications

Evidence of the potential for low-dose mixture effects in the environment begs the question of whether existing legislative measures, such as the WFD, can

8 J V Brian and others 'Evidence of Estrogenic Mixture Effects on the Reproductive Performance of Fish' (2007) 41 Environmental Science and Technology 337–44.

9 J G Koppe and others 2006 'Exposure to Multiple Environmental Agents and Their Effect' (2006) 95 Acta Paediatrica 106–13.

adequately protect aquatic wildlife from such combination effects. The implementation of this directive is dependent upon a system of Environmental Quality Standards (EQS), which are set for priority pollutants, on the assumption that there is an acceptable threshold concentration in accordance with the traditional risk assessment paradigm. This approach is likely to overlook the risk of mixture effects, by failing to consider the effects of groups of chemicals that act in combination as a whole entity. Instead of trying to determine the NOEC for each individual chemical and using this data to set a maximum permissible exposure level or EQS, there should be increased efforts to use our understanding of the way in which chemicals interact to develop legislation that can predict the risk of combined effects. In theory, this could be achieved using bio-mathematical models such as concentration addition (CA), which can accurately predict the combined effects of estrogenic chemicals.[10] However, in reality, the implementation of such a system is fraught with difficulty.

This additive approach has been used to assess the joint effects of substances with dioxin-like properties, which are expressed in terms of toxic equivalency factors (TEFs), but has only recently been considered for application to the regulation of EDCs. For example, it is utilised to some extent in the recent legislation concerning the registration, evaluation and authorisation of chemicals (REACH),[11] although only with regard to the regulation of preparations (ie products containing mixtures of chemicals). The Environment Agency (EA) of England and Wales has also incorporated this thinking into its proposed risk management strategy for estrogenic chemicals in effluents, having determined a safe level for the combined effects of the three main steroidal estrogens, which is equivalent to 1 ng/l of estradiol. However, this system has not yet been applied to the receiving waters in which the exposures take place. The implementation of such a scheme for surface waters would lead to improved environmental protection, by accounting for the total estrogenic activity of the chemicals present in the assessment of environmental risk. Nevertheless, it is also important to consider the potential influence of chemicals that exert their effects via different mechanisms of action, as well as the confounding effects of environmental factors that vary over space and time.

The current situation

Although the WFD does not specifically take account of the effects of mixtures, this piece of legislation is progressive in that it is the first European Community measure to bring EDCs under regulatory control.[12] It also takes a more integrative and holistic approach to the protection of the environment than previous legislation by combining the implementation of the EQS system with the assessment of ecosystem health. The latter is achieved through the routine monitoring of the structure and composition of the aquatic community for evidence of changes in biodiversity and population-level effects. This information is used to determine whether the environment is of a good ecological status. This combined approach will help to identify situations in which mixtures of chemicals may be acting together to cause effects that are overtly toxic. However, it is likely to be relatively insensitive to the subtle and chronic effects that are associated with endocrine disruption (eg behavioural and/or physiological alterations). Hence, there is a risk that adverse effects may only become apparent when the population is in decline, by which time it may be too late for the situation to be remedied.

Given that it is impractical to try to assess the effects of every possible mixture of chemicals and undesirable to implement draconian limits on their usage, there is a clear need to develop a workable solution that will ensure environmental protection against the effects of chemical mixtures. This will require greater cooperation between chemical manufacturers and consumers, as well as scientists and regulators. It is possible that a 'combined effort' such as this may promote the development of methods that can be used to incorporate the risk assessment of mixtures into existing environmental legislation more formally. This may lead to greater stringency with regard to the release of chemicals into the environment, which has obvious benefits in terms of protecting the health of humans and wildlife. However, there are also economic implications that must be considered. For example, some chemicals may be driven off the market, although this may be countered by an increase in the demand for replacement products. It is difficult to weigh up the pros and cons associated with such measures due to the difficulty in placing a value on an improvement in environmental quality? Further consideration should also be given to the legal implications of enforcing more formalised mixtures legislation, such as those associated with the issue of responsibility.

Responsibility: an issue for the future?

The issue of responsibility is relatively straightforward when legislation is based on a system of maximum permissible concentrations for single substances. For example, given a situation in which a chemical is accidentally released into a river and causes an adverse ecological effect (such as a fish kill), it is relatively easy to assign blame and take the appropriate legal action. In contrast, if the adverse effect is cause by a mixture of chemicals, it is more difficult to determine who should take responsibility. An example of this is provided by the widespread feminisation of male fish in British rivers. The EA has recently published an assessment of this phenomenon, concluding that a mixture of chemicals contributes to the estrogenic hazard of complex effluents, with the steroid estrogens (both natural and synthetic) being the dominant contributor to the overall estrogenic activity in most

10 Brian and others (n 6).

11 Council Regulation (EC) 1907/2006 of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, OJ 2006 L 396/1; Council Directive 2006/121/EC of 18 December 2006, amending Directive 67/548/EEC in order to adapt it to Regulation (EC) No 1907/2006 OJ 2006 L 396/850.

12 Matsuno (n 5).

locations.[13] Given that the EA is responsible for protecting the environment through the control of pollution and has special duties for the protection of fish, who, or what, should the Agency hold responsible?

The Agency could blame the usual 'bad guys' in industry for the manufacture and use of estrogenic chemicals – the pharmaceutical industry, in particular, could be held responsible for producing and selling the contraceptive pill. Should it also blame you and me for our production of natural hormones? Or should it blame the water industry for its failure to remove estrogenic chemicals from wastewater before it is discharged into our surface waters? It is easy to envisage a situation where nobody takes any, let alone enough, responsibility to do something about the problem. Each contributor to the problem could legitimately claim that 'my chemical is present at a concentration below that which causes effects on fish, so it isn't my fault: I cannot be held responsible for the problem'. So how do we then determine who should act to remove this threat to our wild fish stocks and who should bear the costs of remedial action? Unfortunately, we do not have the answers to this problem of apportioning responsibility for the combined effects of chemical mixtures. We can, however, envisage plenty of business for environmental lawyers for some time to come.

13 M Y Gross-Sorokin S D Roast and G C Brighty 'Assessment of Feminisation of Male Fish in English Rivers by the Environment Agency of England and Wales' (2006) 114 Environmental Health Perspectives 147–51 Supplement 1.