

# Chemical mixtures in aquatic environments

Understanding and preventing harm

***Chemical mixtures in aquatic environments:  
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# Abbreviations

**AI**

Artificial intelligence

**DNA**

Deoxyribonucleic acid

**eDNA**

Environmental DNA

**eRNA**

Environmental RNA

**MAF**

Mixture Assessment Factor

**NAMs**

New Approach Methodologies

**OECD**

Organisation for Economic Co-operation and Development

**PCB**

Polychlorinated biphenyl

**PFAS**

Per and polyfluoroalkyl substances

**REACH**

Registration, Evaluation, Authorisation, and Restriction of Chemicals

**RNA**

Ribonucleic acid

# Executive summary

Modern societies are reliant on chemicals for a range of uses including healthcare, manufacturing, agriculture and energy production. Yet, chemical pollution is a key contributor to biodiversity loss globally and a persistent threat to human health. Projections indicate large increases in global chemical production in the coming decades, which, without appropriate management, may further threaten the sustainability of our ecosystems.

In the UK, pollution of rivers, lakes and coastal areas is currently a topic of high public and political interest. The Government has made chemical pollution-related commitments via the Environmental Improvement Plan. While emissions of several well-known and regulated chemicals have declined, this does not reflect the full picture, as the concentrations of other emerging contaminants are increasing. The vast majority of anthropogenic chemicals currently present in the environment are not subject to monitoring and only a little is known about their potential impacts. Toxicity data are lacking for most chemicals and their biological effects are either unknown or not well understood.

Current approaches to chemical risk assessment have considerable shortcomings. This is true for individual chemicals, let alone when considering the additional complexities of mixture risk. Indeed, most chemicals are discharged into the environment as unintentional mixtures and/or co-exist once they enter the environment. The sheer number of chemicals in production has long overwhelmed our ability to assess their safety using traditional approaches. Therefore, it is important to leverage the latest science to better understand and deal with this inherent complexity and develop a more protective and efficient regulatory system.

There is clear evidence that unintentional mixtures, whether released as such or arising in the environment, can cause harm even when the constituent chemicals are present at levels deemed individually 'safe'. Yet chemical risk is generally assessed on a substance-by-substance basis, with mixture risk unaccounted for. This is a significant shortcoming.

Although most chemical mixtures are thought to behave additively (meaning their overall toxicity is the sum of the toxicities of the constituent chemicals), mixtures remain a complex regulatory challenge due to extensive toxicity data gaps, different regulatory frameworks for different chemical types and limitations in monitoring data. Moreover, estimates of the frequency of synergism (where combined toxicity is greater than that of constituent chemicals) are based on incomplete toxicity data for many chemicals and chemical combinations, particularly in environmentally relevant scenarios.

There is currently policy and regulatory interest in addressing chemical mixtures within the UK and the EU; both are exploring new possible risk management frameworks to better manage combination effects. In terms of scientific understanding, approaches for chemicals management are converging (including machine learning and 'omics' techniques), meaning that in the future more informed ways of predicting hazards associated with mixtures will be possible. Yet our understanding of the effects of mixtures is currently biased towards relatively well-studied environments, species and chemicals and it is likely that significant uncertainties will remain even as the science progresses. Dealing with this uncertainty will therefore remain an inherent aspect of any risk-based regulation relating to chemical mixtures and should not be used to justify inaction

# Recommendations

## RECOMMENDATION 1

Given the risk of harm due to the lack of current regulation and high uncertainty stemming from systemic data gaps, Defra and the Environment Agency should look to rapidly implement pragmatic and precautionary regulation for chemical mixtures in aquatic environments. As new evidence, data and methodologies become available and uncertainty decreases, the regulation should evolve towards a probabilistic and risk-based approach for different chemicals and/or chemical classes.

At present, chemical safety assessments in the UK are largely conducted on a single-chemical basis. Yet as this report has described, there is a weight of evidence – considering experimental, observational and mechanistic data – that suggests that even when chemicals are present individually, at potentially ‘safe’ concentrations, mixtures of environmental chemicals can have adverse effects on aquatic ecosystems, above and beyond the sum of their constituent parts. Innovative approaches are expanding our ability to predict environmental co-exposure and its potentially harmful effects. However, significant uncertainties and data gaps are likely to persist into the future.

In view of these uncertainties, it is not yet feasible to implement a fully informed probabilistic, risk-based, regulatory system for chemical mixtures. Given this and the risk of harm, we recommend that a precautionary approach, enshrined in UK law under the Environment Act 2021 (Section 17), be pragmatically applied in the interim with respect to environmental chemical mixtures. This should be with the aim of reducing the number of chemicals in the environment in the immediate term, recognising the risk of harm posed by chemical mixtures, as well as moving towards a more risk-based and probabilistic approach to mixture regulation as the evidence, data and tools become available. There are many ways this could be achieved (examples in Box 1) and options should be explored in detail along with a review of the current approach to individual chemical regulation and/or new legislation, risk-management and regulation specific to diffuse mixture effects.

## RECOMMENDATION 2

To capitalise on opportunities for faster, more accurate and cost-effective risk assessment, Defra and relevant UK agencies should become early adopters of innovative approaches for monitoring, managing and regulating chemical mixtures in the aquatic environment.

Innovative scientific approaches such as ‘new approach methodologies’ (NAMs), machine learning and ‘omics’ techniques, present potentially transformative opportunities for managing and regulating chemical mixtures in the environment. Given these opportunities and the possible cost-saving benefits, UK regulators and agencies (eg the Environment Agency, the Scottish Environmental Protection Agency and the Health and Safety Executive) should look to become early adopters of these approaches and work closely with researchers to operationalise these techniques for use in policymaking. For example, the Hazardous Substances Advisory Committee has recently published a paper outlining how NAM’s could be adopted into UK chemical regulation<sup>1</sup>. Over time, the adoption of new methods may allow a shift towards the development of probabilistic risk assessments for mixtures, leading to an estimate of the likelihood of harm, while accounting for uncertainties in data.

However, it is important to recognise that new approaches are unlikely to address the significant data gaps and biases in our current knowledge base in the short term. Many environmental chemicals remain poorly studied and our understanding of their effects varies across chemical classes, exposure scenarios and endpoints. This uneven distribution highlights the need for a dual strategy: embracing new techniques while simultaneously prioritising research efforts to fill critical knowledge gaps (see Recommendation 4).

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1 Department for Environment, Food and Rural Affairs. 2024 Recommendations for the Adoption of New Approach Methodologies (NAMs) in UK Chemical Regulation. See [https://assets.publishing.service.gov.uk/media/672cc07deee595f5288bdbdea/HSAC\\_Brief\\_on\\_NAMs\\_-\\_FINAL\\_20\\_Dec2023\\_PUBLICATION\\_VERSION.pdf](https://assets.publishing.service.gov.uk/media/672cc07deee595f5288bdbdea/HSAC_Brief_on_NAMs_-_FINAL_20_Dec2023_PUBLICATION_VERSION.pdf) (accessed 2 December 2024).

**RECOMMENDATION 3**

Address the fragmentation of current chemical monitoring data by creating a centralised, open-access hub. This might be achieved using the National Data Library that is currently being developed by the Department for Science, Innovation and Technology.

Current datasets related to chemical pollutants are stored in different repositories and some of these data are poorly curated and never published in open access databases. This makes it difficult to use these data for risk assessment without committing time and resources to obtaining the data and readying them for use, creating an immediate barrier for researchers, regulators and policymakers seeking to integrate datasets to understand chemical mixture risk. Establishing a data ‘hub’ would greatly aid mixture risk research and regulation and would minimise duplication of scientific effort.

Those establishing the data hub should consider mechanisms for encouraging industry participation via data sharing, while protecting commercial interests. The hub should be accompanied by guidance and reporting templates to support the use of these data for regulatory purposes – from collection and experimental procedures to the reporting of results. The ‘National Data Library’ of public sector data, announced in the 2024 Labour manifesto<sup>2</sup> and currently being developed by the Department for Science, Innovation and Technology, may be an appropriate means of delivering this.

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2 Labour Party UK. 2024 Change: Labour Party Manifesto 2024. See <https://labour.org.uk/wp-content/uploads/2024/06/Change-Labour-Party-Manifesto-2024-large-print.pdf> (accessed 6 December 2024).

**RECOMMENDATION 4**

UKRI should coordinate the development of an interdisciplinary research strategy that incentivises collaboration between funders, researchers, industry stakeholders and regulators, targeting critical evidence gaps with respect to the effects of chemicals and their mixtures.

Research into the effects of chemical mixtures does not currently target key evidence gaps, meaning significant uncertainty remains regarding the effects of mixtures in the environment. For example, published mixture studies are biased towards already relatively well-researched chemicals and species and few studies go beyond binary or tertiary mixtures (2 – 3 chemicals)<sup>3</sup>. Targeted research efforts could usefully focus on lesser-studied chemicals and validation of findings in real-world environments.

Researchers, funders, industry stakeholders and regulators should collaborate to develop an interdisciplinary research strategy to guide research efforts and funding to target key evidence gaps. This strategy should clearly delineate between fundamental research that will likely require state investment and ‘near market’ research that could be industry funded. Given novel chemicals will continue to be developed, this agenda-setting process must be iterative to adapt to new challenges and ensure that the latest research continually informs policy and regulation. In the short term, retrospective research that integrates diverse datasets might be an effective means of establishing strong correlations between mixtures and their impacts.

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3 Martin O *et al.* 2024 Ten years of research on synergisms and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies. *Environment international* 146, 106206.

**RECOMMENDATION 5**

To address current skills and expertise gaps, regulatory agencies such as the Environment Agency and Cefas should prioritise the development of a future pipeline of appropriately trained toxicologists and ecotoxicologists.

The UK needs to invest in toxicology and ecotoxicology expertise. Firstly, it is critical that the current workforce is supported to remain at the forefront of scientific innovation through continuous professional development. For example, to capitalise on the opportunities presented by machine learning or NAMs, regulators, contract research organisations and industry will need to develop skills and expertise so that they know how and when these techniques may be beneficially used and how to interpret and critically appraise the results.

Secondly, the UK currently faces a concerning decline in skilled applicants for toxicology and ecotoxicology roles and a limited number of specialised academic programmes<sup>4</sup>. These challenges are particularly acute given the increased regulatory burden following the UK's exit from the EU. Training programmes should equip emerging toxicologists and ecotoxicologists with a holistic skill set, including an understanding of the fast-moving science and familiarity with regulatory frameworks, to bridge the divide between scientific research and regulatory processes. In England, the Environment Agency and Cefas should work together as regulatory agencies to invest in and develop the pipeline of expertise required to deal with toxicology-related regulatory challenges.

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4 Judge S. 2022 Knowledge and skill requirements in regulatory toxicology in the UK and gaps in education provision: Phase 1: Education and skills gap project report to advisory group. See <https://www.thebts.org/wp-content/uploads/2022/10/BTS-Phase-1-Education-and-skills-gap-project-report.pdf> (accessed 2 December 2024).

## BOX 1

## Example policy options for implementing a pragmatic approach to chemical mixture risk

Illustrative and hypothetical examples of policy options that could be used to regulate chemical mixtures. These interventions aim to reduce the number of chemicals in the environment in the immediate term and achieve more risk-based and probabilistic approaches to chemical mixture regulation in the future. The options are indicative only and exclude direct financial controls such as taxes and levies, as well as behaviour-based interventions such as codes of conduct changes, supply chain scrutiny, labelling and advertisement.

### Detect and monitor to allow preventative action

Investing in the detection and monitoring of chemicals/chemical mixtures and their effects in aquatic environments could, if coupled with a dynamic regulatory response mechanism, allow regulators to take much swifter action when these are found to be present and causing harm. Persistent and bioaccumulative chemicals (like PFAS) could then be identified, phased out and/or tightly controlled before widespread contamination occurs. Techniques such as environmental DNA and biomarkers (see Chapter three), combined with analysis using machine learning, can be used to regularly monitor the environment and more quickly identify concerning exposure-effect relationships.

### Adapt the regulation and/or management of individual chemical contaminants

Minimising the number of contaminants entering the environment is the most effective way to reduce the exposure risk from mixture effects. There are several ways this could be achieved, some examples include:

- Moving towards a more circular economy for chemicals, aiming to reduce, reuse and recycle (advances in green chemistry have a lot to offer in this regard<sup>5</sup>);
- Tighter regulations to restrict or ban chemicals (especially persistent chemicals), based on potential hazards, not just proven harm;
- Target the existing risk assessment process, for example, by implementing a Mixture Assessment Factor (MAF)<sup>6</sup>. Applying a MAF lowers the acceptable concentration of a substance being discharged, providing a greater chemical 'safe space' to allow for mixture effects.

5 United States Environmental Protection Agency. Basics of Green Chemistry. See <https://www.epa.gov/greenchemistry/basics-green-chemistry#> (accessed 7 July 2025).

6 KEMI (2015) An additional assessment factor (MAF) – A suitable approach for improving the regulatory risk assessment of chemical mixtures? Report 5/15. See <https://www.kemi.se/publikationer/rapporter/2015/report-5-15-an-additional-assessmentfactor-maf---a-suitable-approach-for-improving-the-regulatory-risk-assessment-ofchemical-mixtures> (accessed 3 July 2025).

## BOX 1 (CONTINUED)

**Group contaminants based on shared properties for risk assessment**

This approach aims to group contaminants based on shared properties such as structures, modes of action or adverse effects – and these groupings could be used to assess and regulate mixture risk (New Approach Methodologies can be particularly helping for informing these groupings, see Chapter three). Grouping can be done in either a precautionary or more targeted way, depending on the amount of information available. An approach such as this could increase efficiency and help to prevent “regrettable substitution” (replacing one hazardous chemical with another similar one). Particularly problematic groups of persistent or rapid/acutely damaging chemicals could also be more heavily regulated on this basis. Regulators could choose to consider shared properties or mechanisms of action at relevant geographic scales, for example water catchment areas.

**Reduce the amount of known contaminant mixtures entering the environment**

Additional risk assessments could be performed for groups of chemicals that are known to enter the environment as chemical mixtures. For example, mixtures of chemicals emitted from single industrial, manufacturing or formulation sites. This approach could reduce exposure to known contaminant mixture risk before those mixtures become widespread in the environment. This is similar to how co-formulants are considered in pesticides legislation<sup>7</sup>.

**Implement regulation based on the level of risk associated with exposure**

This could target particularly vulnerable or important species or habitats and aim to minimise or remove the number of chemicals entering these environments. Such an approach would likely have to be catchment-based, recognising the interconnectedness of freshwater, estuarine and marine systems and consider vulnerable species from a more diverse range of phyla than are currently tested.

**Restrict chemicals shown to be harmful, either individually or as components of mixtures to only ‘essential’ uses and if no safer alternatives exist**

This could require an agreed definition of what an ‘essential use’ and ‘safer alternative’ is – but is an example of a pragmatic approach, where some degree of use is tolerated if the benefits are seen to outweigh the risks.

7 Health and Safety Executive. 2023. Register of unacceptable co-formulants for pesticide products and adjuvants in GB. See <https://www.hse.gov.uk/pesticides/active-substances/unacceptable-co-formulants.htm> (accessed 2 July 2025).

# Introduction

Chemicals play a critical role in modern society across a range of uses such as healthcare, manufacturing, agriculture and energy production. The production of chemicals globally is estimated to have increased 50-fold since 1950 and is projected to triple again between 2010 and 2050<sup>8</sup>. This increase in production has resulted in the pervasive presence of potentially hazardous chemicals in all ecosystems<sup>9</sup>.

The goal of a non-toxic chemical environment is not necessarily a chemical-free environment, but rather one where the harmful effects of chemicals are minimised. Examples of these harmful effects are evident across all types of ecosystems and species. In humans, per- and polyfluoroalkyl substances (PFAS) are linked to reproductive and developmental effects<sup>10</sup>, while exposure to phthalates (found in food packaging and personal care products) is associated with reproductive, developmental and neurological toxicity<sup>11</sup>.

Among aquatic species, polychlorinated biphenyl (PCB) exposure has been found to drive collapsing orca populations<sup>12</sup>, endocrine-disrupting pharmaceuticals have feminised fish<sup>13</sup> and PFAS have had a range of detrimental effects on invertebrates spanning DNA damage, developmental effects and behavioural changes<sup>14</sup>.

The United Nations has identified pollution as one of three components of the ‘triple planetary crisis’ along with climate change and biodiversity loss<sup>15</sup>. Against a backdrop of projected increases in chemical production, ecosystems face multiple environmental pressures which may exacerbate potential harms. For example, climate change is predicted to result in greater agriculture-related chemical pollution by driving increased agrochemical inputs<sup>16</sup>. Further, the changing distribution of populations of organisms caused by climate change may bring different species into contact with chemically contaminated areas<sup>17</sup>.

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- 8 Persson L *et al.* 2022 Outside the safe operating space of the planetary boundary for novel entities. *Environ. Sci. Technol.* 56, 1510–1521.
- 9 United Nations Environment Programme. 2019 Global Chemicals Outlook II: From legacies to innovative solutions. See <https://www.unep.org/resources/report/global-chemicals-outlook-ii-legacies-innovative-solutions> (accessed 26 November 2024).
- 10 United States Environmental Protection Agency. 2024 Our Current Understanding of the Human Health and Environmental Risks of PFAS. See <https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas> (accessed 16 June 2025).
- 11 Katsikantami, I *et al.* 2016 A global assessment of phthalates burden and related links to health effects. *Environment international* 97, 212-236.
- 12 Desforges JP *et al.* 2018 Predicting global killer whale population collapse from PCB pollution. *Science* 361, 1373-1376.
- 13 Matthee C, Brown AR, Lange A, Tyler CR. 2023 Factors determining the susceptibility of fish to effects of human pharmaceuticals. *Environmental science & technology* 57, 8845-62.
- 14 Ma T, Ye C, Wang T, Li X, Luo Y. 2022 Toxicity of per- and polyfluoroalkyl substances to aquatic invertebrates, planktons and microorganisms. *International Journal of Environmental Research and Public Health* 19, 16729.
- 15 United Nations Environment Programme, International Science Council. 2024 Navigating New Horizons: A global foresight report on planetary health and human wellbeing. See *Navigating New Horizons: A global foresight report on planetary health and human wellbeing* (accessed 26 November 2024).
- 16 Yang Y, *et al.* 2024 Climate change exacerbates the environmental impacts of agriculture. *Science* 385, eadn3747.
- 17 Sinclair T, Craig P, Maltby LL. 2024 Climate warming shifts riverine macroinvertebrate communities to be more sensitive to chemical pollutants. *Global Change Biol* 30, e17254.

Ecosystems increasingly face multiple, potentially compounding environmental stressors; in the aquatic environment, these include altered salinity, deoxygenation and acidification<sup>18</sup>.

In the UK, pollution of rivers, lakes and coastal areas is currently a topic of high public and political interest. The issue has gained significant attention due to reports of sewage discharges<sup>19</sup>, chemical contamination<sup>20</sup> and poor bathing water quality<sup>21</sup> across the country. Investments to address point source pollution (ie pollution from a single, identifiable source) may have led to gains for aquatic biodiversity in the UK. A study from 2023 found that stricter wastewater regulation had been associated with improvements in macroinvertebrate diversity in rivers over the last 30 years<sup>22</sup>.

However, it is important to note that such findings do not consider the different dimensions of biodiversity such as comparisons against historical baselines or preserving ecological functions. Moreover, findings suggest this biodiversity improvement has slowed more recently<sup>23</sup> and diffuse pollution (ie pollution from diverse, scattered sources such as agricultural and urban runoff) continues to pose an ongoing and largely unresolved challenge<sup>24</sup>.

The Government has made chemical pollution-related commitments via the Environmental Improvement Plan<sup>25</sup>. Goal 4 of the Plan sets out the importance of safely using and managing chemicals to minimise environmental and human health impacts<sup>26</sup>. Yet, based on 2022/23 data, the Office for Environmental Protection has reported that England is “off track and delayed” in achieving its chemicals-related objectives<sup>27</sup>.

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- 18 Hatje V *et al.* 2022 Emergent interactive effects of climate change and contaminants in coastal and ocean ecosystems. *Front Mar Sci* 9, 936109.
- 19 Hoog N. ‘Unacceptable’: how raw sewage has affected rivers in England and Wales – in maps. *The Guardian*. 12 September 2023. See <https://www.theguardian.com/environment/ng-interactive/2023/sep/12/unacceptable-how-raw-sewage-has-affected-rivers-in-england-and-wales-in-maps> (accessed 30 January 2024).
- 20 Parry L. ‘Alarming’ chemical levels found in East rivers. *BBC News*. 23 October 2024. See <https://www.bbc.co.uk/news/articles/clylyxj731yo> (accessed 5 February 2025).
- 21 Laville S. Record number of English bathing sites classified as having poor water quality. *The Guardian*. 26 November 2024. See <https://www.theguardian.com/environment/2024/nov/26/record-number-of-english-bathing-sites-classified-as-having-poor-water-quality> (accessed 5 February 2025).
- 22 Johnson AC, Jürgens MD, Edwards FK, Scarlett PM, Vincent HM, von der Ohe P. 2019 What works? The influence of changing wastewater treatment type, including tertiary granular activated charcoal on downstream macroinvertebrate biodiversity over time. *Environ Toxicol Chem* 38, 1820–1832.
- 23 Qu Y *et al.* 2023 Significant improvement in freshwater invertebrate biodiversity in all types of English rivers over the past 30 years. *Sci Total Environ* 905, 167144.
- 24 OECD. 2017 Diffuse Pollution, Degraded Waters: Emerging Policy Solutions. See [https://www.oecd.org/content/dam/oecd/en/publications/reports/2017/03/diffuse-pollution-degraded-waters\\_g1g75088/9789264269064-en.pdf](https://www.oecd.org/content/dam/oecd/en/publications/reports/2017/03/diffuse-pollution-degraded-waters_g1g75088/9789264269064-en.pdf) (accessed 26 November 2024).
- 25 Note that the Environmental Improvement Plan is primarily for England, however some aspects are reserved for the UK government, such as chemicals regulation.
- 26 Department for Environment, Food and Rural Affairs. 2023 Environmental Improvement Plan: First Revision of the 25 Environment Plan. See <https://assets.publishing.service.gov.uk/media/64a6d9c1c531eb000c64ffa/environmental-improvement-plan-2023.pdf> (accessed 26 November 2024).
- 27 Office for Environmental Protection. 2024 OEP response to consultation on UK REACH. See <https://www.theoep.org.uk/report/oep-response-consultation-uk-reach> (accessed 6 December 2024).

According to monitoring conducted under the Water Framework Directive in England, no rivers, coastal or estuarine waters monitored achieved “good” chemical status in 2019<sup>28</sup>. However, it must be noted that, this was largely driven by “ubiquitous, persistent, bio-accumulative and toxic” substances such as PFAS chemicals or mercury (which have only recently been included in these assessments)<sup>29</sup>.

There are still many other chemicals that are not included in statutory monitoring, with the number monitored an order of magnitude smaller than those likely present in the environment. This means that the true extent of chemical pollution in UK waters is still not well understood. As the Office for Environmental Protection notes, “While emissions of a few well-known and regulated chemicals have decreased, the large majority of chemicals are not monitored and their impacts are unknown”<sup>30</sup>.

To date, chemicals regulation has been based almost entirely on risk assessments of single substances<sup>31</sup>. Similarly, scientific studies exploring the effects of chemical pollutants on species and ecosystems have historically focused on single chemicals. Despite this focus, even on an individual-pollutant basis, many chemicals remain a major and largely unresolved environmental challenge. For example, pharmaceuticals can have devastating ecological effects when they enter aquatic environments via wastewater treatment plants and are regulated far less stringently for their environmental impacts compared to other chemicals<sup>32</sup>. Similarly, PFAS – a class of chemical contaminants that are highly persistent and many of which pose a threat to aquatic ecosystems – are only beginning to be addressed by regulatory frameworks, leaving major challenges in effectively managing their pervasive and enduring environmental effects<sup>33</sup>.

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28 State of the water environment indicator B3: supporting evidence. See <https://www.gov.uk/government/publications/state-of-the-water-environment-indicator-b3-supporting-evidence/state-of-the-water-environment-indicator-b3-supporting-evidence> (accessed 30 July 2025).

29 Environment Agency, Natural England. 2024 State of the water environment indicator B3: supporting evidence. See <https://www.gov.uk/government/publications/state-of-the-water-environment-indicator-b3-supporting-evidence/state-of-the-water-environment-indicator-b3-supporting-evidence> (accessed 6 December 2024).

30 Office for Environmental Protection. 2024 Progress in improving the natural environment in England 2022/2023. See <https://www.theoep.org.uk/report/government-remains-largely-track-meet-its-environmental-ambitions-finds-oep-annual-progress> (accessed 3 February 2025).

31 Drakvik E *et al.* 2020 Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ. Int.* 134, 105267.

32 Boxall AB. 2004 The environmental side effects of medication: how are human and veterinary medicines in soils and water bodies affecting human and environmental health? *EMBO Rep.* 5, 1110–1116.

33 University of Portsmouth. 2024 Plans to set legal limits on ‘forever chemicals’ after experts push for stricter regulation. See <https://ppl-ai-file-upload.s3.amazonaws.com/web/direct-files/attachments/17679693/7cfe85ad-c768-4a56-ab9a-26e605ebb280/paste.txt> (accessed 15 May 2025).

In contrast to the single-chemical focus of current regulatory settings, organisms in real-world environments are exposed to many different contaminants simultaneously. For decades, there has been widespread recognition among researchers that organisms are exposed across their entire life course to a myriad of combinations of environmental chemicals<sup>34</sup> and that these mixtures may pose greater risks than their individual chemical constituents alone<sup>35</sup>. This evidence base is strong and growing.

For reasons explored in this report, quantifying the additional risk posed by mixture effects is difficult. Gaps in toxicology data for many chemicals, combined with limitations in statutory monitoring, mean that drawing firm conclusions about the contribution of unintentional mixtures to the overall challenge of environmental pollution is challenging. However, laboratory- and field-based testing demonstrates that there is significant theoretical risk posed by mixtures even at levels that might be deemed 'safe' for individual chemical components. Despite these challenges, addressing chemical mixture risk in the environment is not something that can continue to be overlooked.

Due to these potential risks, there has been a recent growth in policy interest in unintentional mixtures. In the EU, the 2020 Chemicals Strategy for Sustainability explicitly addresses chemical mixtures through several key initiatives aimed at improving risk assessment and regulatory frameworks<sup>36</sup>. The Strategy includes a commitment to assess how best to introduce "mixture assessment factors" under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and to strengthen provisions for considering combination effects in other relevant legislation. Similarly, in the UK, the Environment Agency's Chief Scientist's Group produced a 2022 report evaluating potential approaches to mixture risk assessment under UK REACH<sup>37</sup>.

Scientific advances are gradually improving our ability to understand and predict the distribution and effects of complex chemical mixtures. For example, modelled data that predict mixture exposure can potentially be validated using environmental monitoring data and machine learning techniques. This will increasingly help with the development of more accurate predictive models<sup>38</sup>. Another example is that by combining environmental DNA data with chemical monitoring data it is easier to establish links between mixtures and their impacts on whole ecological communities<sup>39</sup>. Eventually, these advances will have important applications for environmental managers and regulators.

34 Drakvik E *et al.* 2020 Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ. Int.* 134, 105267.

35 Heys KA, Shore RF, Pereira MG, Jones KC, Martin FL. 2016 Risk assessment of environmental mixture effects. *RSC Adv.* 6, 47844-47857.

36 European Commission. 2020 Chemicals Strategy for Sustainability: Towards a Toxic-Free Environment. See <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52020DC0667> (accessed 15 May 2025).

37 Environment Agency. 2022 Evaluation of the potential approaches to risk assessment of unintentional chemical mixtures for future UK REACH assessments. See [https://assets.publishing.service.gov.uk/media/62fdafd1d3bf7f06eb72b6f6/Evaluation\\_of\\_the\\_potential\\_approaches\\_to\\_risk\\_assessment\\_of\\_unintentional\\_mixtures\\_for\\_future\\_UK\\_REACH\\_assessments\\_-\\_report.pdf](https://assets.publishing.service.gov.uk/media/62fdafd1d3bf7f06eb72b6f6/Evaluation_of_the_potential_approaches_to_risk_assessment_of_unintentional_mixtures_for_future_UK_REACH_assessments_-_report.pdf) (accessed 29 November 2024).

38 Drakvik E *et al.* 2020 Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ. Int.* 134, 105267.

39 Flynn AS *et al.* 2025 Assessing the relationship between sedimentary microbial community structure and pollutants in stormwater wetlands using eDNA metabarcoding. *Environ. DNA* 7, e70096.

It is important to note that these advances are unlikely to rapidly overcome critical knowledge gaps. Predicting the ecological effects of chemicals will remain an unresolved challenge for many years to come. Despite decades of research, our ability to accurately forecast the distribution, persistence and harmful impacts of single chemicals – let alone complex mixtures of chemicals – is still limited. This means it is crucial that in the interim, precautionary regulatory measures are used to safeguard environmental and human health and that the absence of data is not used to justify inaction.

Given the advancing science and live regulatory debates, the Royal Society brought together leading scientists from across the UK to explore the current evidence base regarding the biological effects of unintentional mixtures. This includes chemical mixtures created unintentionally by simultaneous point-source discharge, as well as through co-occurrence in the environment once discharged. While chemical mixtures impact all environmental compartments, including air and terrestrial ecosystems, this work focused specifically on aquatic environments.

This report summarises findings from a workshop held in October 2024, discussions with experts, as well as an evidence review commissioned by the Royal Society and delivered by the UK Centre for Ecology and Hydrology (available as supplementary information on the Royal Society's website<sup>40</sup>). These findings draw on expertise from marine, estuarine and freshwater scientists, as well as a range of disciplines such as ecologists, chemists and mathematical modellers to present an evidence-based, independent view.

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40 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).



# Chapter one

## The current approach to risk assessment under UK REACH

### Left

Surface pollution on the Leeds  
Liverpool Canal in Lancashire.  
© iStock.com / Alan Morris.

# The current approach to risk assessment under UK REACH

While this report focuses on the challenge of unintentional mixtures, the current system for assessing risks from individual chemicals has shortcomings. These foundational weaknesses make addressing the challenge of mixtures particularly complex. Indeed, even on an individual chemical basis, ecosystems are not yet adequately protected against known and potential chemical hazards.

UK REACH is the primary chemicals regulation in Great Britain, however there are a range of other regulatory frameworks that relate to chemical safety (see Box 2). Discussion in this section is most relevant to those chemicals registered under UK REACH, as some other classes of chemicals (eg pesticides and biocides) are already subject to more stringent risk assessment processes before being placed on the market. Nonetheless, given that chemicals regulated separately will co-occur in the environment, a joined-up approach between these different regulatory frameworks will be essential for addressing mixture risk<sup>41</sup>.

Under UK REACH, where any chemical registered has an annual supply level above 10 tonnes and has a hazard identified, a risk assessment is required to demonstrate that the substance does not pose an unacceptable risk to human health and the environment<sup>42</sup>. Assessments to determine ‘safe’ concentrations of chemicals in the environment do not currently account for interactions between chemicals, the exception to this being formulated products which contain multiple active ingredients (ie are intentional mixtures for which the composition is known)<sup>43</sup>. This means that substances emitted at thresholds deemed ‘safe’ by regulators, may still have negative effects on organisms when they are encountered as mixtures (see Chapter two).

Despite these regulatory measures, individual chemicals and classes of chemicals are still entering the environment in significant quantities and are accumulating in wildlife and humans (eg PFAS, pharmaceuticals and pesticide residues). In addition, risk assessments are conducted on a limited number of species – usually algae, *Daphnia* (a crustacean) and fish – reflecting a minute subset of the diverse life in the natural environment. With some exceptions (eg the risk assessments required for the offshore processing of petroleum<sup>44</sup>), marine and estuarine invertebrate species are poorly represented in these risk assessments.

41 Escher BI *et al.* 2022 Mixture risk assessment of complex real-life mixtures-the PANORAMIX project. *Int. J. Environ. Res. Public Health* 19, 12990.

42 Environment Agency. 2022 Evaluation of the potential approaches to risk assessment of unintentional chemical mixtures for future UK REACH assessments. See [https://assets.publishing.service.gov.uk/media/62ddfd1d3bf7f06eb72b6f6/Evaluation\\_of\\_the\\_potential\\_approaches\\_to\\_risk\\_assessment\\_of\\_unintentional\\_mixtures\\_for\\_future\\_UK\\_REACH\\_assessments\\_-\\_report.pdf](https://assets.publishing.service.gov.uk/media/62ddfd1d3bf7f06eb72b6f6/Evaluation_of_the_potential_approaches_to_risk_assessment_of_unintentional_mixtures_for_future_UK_REACH_assessments_-_report.pdf) (accessed 29 November 2024).

43 The British Toxicology Society. 2021 Assessment and Management of Chemical Mixtures. See <https://www.thebts.org/wp-content/uploads/2021/08/Assessment-Management-Chemical-Mixtures-v1-Jun-2021.pdf> (accessed 30 January 2025).

44 Centre for Environment, Fisheries and Aquacultural Science. Offshore Chemical Notification Scheme (OCNS). See <https://www.cefas.co.uk/data-and-publications/ocns/> (accessed 30 January 2025).

## BOX 2

## Chemicals regulation in the UK

In the UK, chemicals are primarily regulated through UK REACH, which came into effect following the UK's exit from the EU in 2021. The key principles of EU REACH were retained in UK REACH, but the two now operate independently. UK REACH has a broad remit; however, some substances are exempt from certain aspects of REACH because they fall under other, more specific legislation (eg plant protection products and biocides, medicines). Indeed, beyond REACH there are a range of other regulatory frameworks concerning chemical safety in the UK, such as the Classification, Labelling and Packaging Regulation, the Biocidal Products Regulation, Plant Protection Products Regulation and the Persistent Organic Pollutants Regulation.

While UK REACH applies to Great Britain, EU REACH continues to apply in Northern Ireland under the Northern Ireland Protocol. Within this regulatory framework, separate agencies hold responsibility for different aspects of chemicals monitoring and management in the UK. In addition, chemicals have both devolved and reserved aspects.

For example, Scotland and Wales have some policy responsibilities for chemicals within their jurisdiction, related to environmental protection and health and safety. In terms of operationalising UK REACH, several different agencies, governments and departments are involved. This includes the Health and Safety Executive, the Environment Agency, Defra and the Scottish and Welsh governments.

Other relevant regulators for separate legislation include the Medicines and Healthcare products Regulatory Agency (responsible for regulating pharmaceuticals), the Office for Product Safety and Standards (responsible for regulating most consumer products, including cosmetics) and the Veterinary Medicines Directorate (responsible for regulating animal medicines). While agencies collaborate and regulatory frameworks operate in conjunction with each other, this fragmentation nonetheless adds complexity. Appropriately addressing mixture risk will therefore require a high degree of coordination to consider the mixture effects of chemicals which are managed under different jurisdictions.

Freshwater species data are therefore often used to estimate the sensitivity of marine and estuarine species. Under UK REACH, an additional assessment factor of 10 is added to address these uncertainties in extrapolating from freshwater data to other aquatic environments. This means that the level determined to be 'safe' based on freshwater species is then divided by 10 as a precaution<sup>45</sup>. Such a blunt approach cannot account for the distinct physiological adaptations and sensitivities to environmental stressors or differences in chemical speciation in marine and freshwaters and may lead to an underestimation of risks posed by individual chemicals for marine and estuarine species.

Even among species directly tested, assessments may not adequately protect against risk from chemicals as toxicity is assessed under controlled laboratory conditions. These conditions do not capture dynamics of ecosystems such as food web interactions, avoidance of contaminated areas, chronic exposure across a prolonged period and/or interactions with other environmental stressors. Tests are only run for a limited number of endpoints (ie measurable, observable effects). Complex behavioural effects that could impact fitness in natural environments are not routinely assessed in regulatory testing<sup>46</sup>.

Indeed, many environmental chemicals have complex effects on animal behaviour. Examples include affecting time spent searching for food, thereby reducing growth rates<sup>47</sup>, or interfering with the detection of pheromones, impacting reproduction<sup>48</sup>. Where behavioural endpoints are used in toxicity tests, they often constitute basic behavioural observations which do not reflect this complexity (eg immobilisation).

Thus, while UK REACH provides a structured approach to regulating individual chemicals, current risk assessments often fail to account for the real-world complexity of ecosystems and the combined effects of multiple substances. Assessments are typically limited to a small number of species and controlled laboratory conditions, which do not reflect the diversity or interactions found in nature. Advancing risk assessment methodologies to consider mixture effects is one critical step in ensuring that chemical regulations keep pace with the realities of environmental exposure – and reevaluating how the risk of individual chemicals are assessed may be another.

45 Department for Business, Energy & Industrial Strategy. 2020 The United Kingdom Risk-Based Approach Programme. See [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/941162/RBA\\_Guidance\\_December\\_2020\\_v3.1.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/941162/RBA_Guidance_December_2020_v3.1.pdf) (accessed 30 January 2025).

46 Saaristo M *et al.* 2018 Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proc R Soc B* 285, 20181297.

47 Saaristo M *et al.* 2018 Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proc R Soc B* 285, 20181297.

48 Jiang NJ *et al.* 2023 Ozone exposure disrupts insect sexual communication. *Nature Communications* 14, 1186.





# Chapter two

## The effects of chemical mixtures on aquatic ecosystems

**Left**

Bradwell Waterside Essex.  
© iStock.com / Aerial Essex.

# The effects of chemical mixtures on aquatic ecosystems

There is an established body of evidence demonstrating that mixtures of environmental chemicals can exert adverse effects on aquatic ecosystems, even when present at individually ‘safe’ levels<sup>49, 50</sup>. However, uncertainties remain regarding the mechanisms and biological pathways behind this. Predicting how chemicals interact and their effects in real-world environments, as opposed to under laboratory conditions, remains constrained by inadequate scientific understanding.

## What do laboratory-based studies tell us about the effects of mixtures?

The evidence regarding mixture effects is largely derived from laboratory studies. However, such tests are limited in their ability to capture real-world influences on toxicity, such as fluctuations in environmental factors, changing exposure rates and distribution dynamics across inter-linked environments. These studies are also usually conducted on short timescales using unrealistically concentrations of just two to three chemicals and on a limited number of species within standardised laboratory conditions.

They are also overwhelmingly based on freshwater environments and species (see Box 3) and have only been extended to a very small number of the estimated 350,000 chemicals in use worldwide<sup>51</sup>. Therefore, the applicability of these tests to real-world environments where many species are simultaneously exposed to a high number of chemicals, at low concentrations and for a long time period, is very limited. A subset of chemicals has been studied in detail, with some chemical types more extensively studied than others (eg pesticides). However, for most chemicals, hazard information is very limited, further compounding the challenge<sup>52</sup>.

With these limitations in mind, some general insights have emerged. Laboratory studies, conducted under controlled conditions with simple mixtures, have indicated that it is usually a small number of chemicals within a mixture that drive most of its toxicity<sup>53</sup>. Even in more complex mixtures (ie with many chemical components), the most toxic chemicals appear to contribute at least 20 – 25% of the calculated mixture effect<sup>54, 55</sup>. However, in such studies, any conclusions about which chemicals drive this toxicity are limited to those chemicals which are monitored.

49 Council of the European Union. 2009 Council conclusions on combination effects of chemicals 2988th Environment Council meeting Brussels. See [https://www.consilium.europa.eu/uedocs/cms\\_data/docs/pressdata/en/envir/112043.pdf](https://www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/envir/112043.pdf) (accessed 30 January 2025).

50 Environment Agency. 2022 Evaluation of the potential approaches to risk assessment of unintentional chemical mixtures for future UK REACH assessments. See [https://assets.publishing.service.gov.uk/media/62fddfd1d3bf7f06eb72b6f6/Evaluation\\_of\\_the\\_potential\\_approaches\\_to\\_risk\\_assessment\\_of\\_unintentional\\_mixtures\\_for\\_future\\_UK\\_REACH\\_assessments\\_-\\_report.pdf](https://assets.publishing.service.gov.uk/media/62fddfd1d3bf7f06eb72b6f6/Evaluation_of_the_potential_approaches_to_risk_assessment_of_unintentional_mixtures_for_future_UK_REACH_assessments_-_report.pdf) (accessed 29 November 2024).

51 Naidu R *et al.* 2021 Chemical pollution: A growing peril and potential catastrophic risk to humanity. *Environment International*, 156, p.106616.

52 European Environment Agency. 2020 The European environment – state and outlook 2020: knowledge for transition to a sustainable Europe. See <https://www.eea.europa.eu/soer/2020> (accessed 6 December 2024).

53 Drakvik E *et al.* 2020 Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ. Int.* 134, 105267.

54 Spurgeon, D *et al.* 2022 Proportional contributions to organic chemical mixture effects in groundwater and surface water. *Water Research* 220, 118641.

55 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

For example, commissioned analysis for the Royal Society by the UK Centre for Ecology and Hydrology suggests that in UK waters, mixture risk is largely driven by metals and polycyclic aromatic hydrocarbons (PAHs)<sup>56</sup>. However, potentially important emerging substance groups such as PFAS, pharmaceuticals, plasticisers and newer pesticides are not yet captured in this analysis<sup>57</sup>.

Laboratory-based testing has also suggested that ‘additivity’ is the most common type of mixture interaction, where the combined effect of chemicals can be estimated by adding the toxicity of each constituent chemical (Figure 1). Some estimates suggest that between 75 – 90% of chemicals interact in additive ways, meaning additivity is likely to be the most important mixture pattern from a regulatory perspective<sup>58</sup>. When chemicals behave additively in mixtures, even at concentrations deemed ‘safe’ for the individual

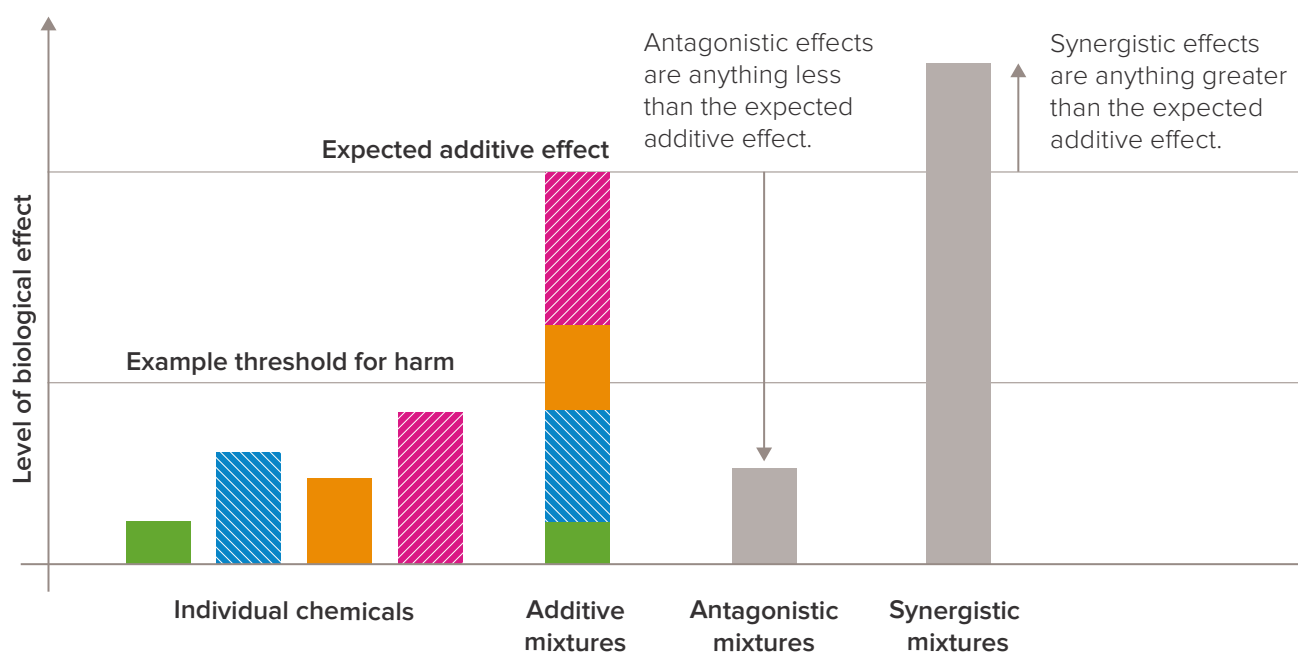
chemical constituents, the combined effect of co-occurring chemicals in situ can still cause harm to aquatic organisms<sup>59,60</sup>. This is because the chemicals target the same biological pathway, or mode of action, meaning their combined effects can result in harmful effects. The evidence for harmful effects due to additivity is strong across a range of chemicals and effects<sup>61</sup>. For example, combined exposure has demonstrable effects on reproduction<sup>62</sup>, impaired growth<sup>63</sup> and loss of photosynthetic function<sup>64</sup>.

In a smaller number of cases (among those chemicals examined so far), chemicals in mixtures may act in ‘antagonistic’ or ‘synergistic’ ways (see Figure 1). Antagonistic mixtures, where the combined toxicity is less than would be expected from the additive effects of constituent chemicals, are commonly found among metal-metal mixtures<sup>65</sup>. Due to their reduced toxicity in combination, they may be considered of lower priority concern to regulators.

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- 56 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).
- 57 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).
- 58 Cedergreen N. 2014 Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. *PLoS one* 9, e96580.
- 59 EU Scientific Committee on Health and Environmental Risks, EU Scientific Committee on Emerging and Newly Identified Health Risks, EU Scientific Committee on Consumer Safety. 2012 Opinion on the Toxicity and Assessment of Chemical Mixture. See [https://ec.europa.eu/health/scientific\\_committees/environmental\\_risks/docs/scher\\_o\\_155.pdf](https://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf) (accessed 10 December 2024).
- 60 Carvalho, RN *et al.* 2014 Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they? *Toxicological Sciences* 141, 218-233.
- 61 Kortenkamp A, Backhaus T, Faust M. 2009 State of the Art Report on Mixture Toxicity. See [https://www.pan-europe.info/old/Campaigns/pesticides/documents/cum\\_syn\\_effects/Kortenkamp%20state%20of%20the%20art%20mixture%20toxicity.pdf](https://www.pan-europe.info/old/Campaigns/pesticides/documents/cum_syn_effects/Kortenkamp%20state%20of%20the%20art%20mixture%20toxicity.pdf) (accessed 10 December 2024).
- 62 Silva P *et al.* 2012 Testing the effects of ethinylestradiol and of an environmentally relevant mixture of xenoestrogens as found in the Douro River (Portugal) on the maturation of fish gonads – A stereological study using the zebrafish (*Danio rerio*) as model. *Aquatic toxicology* 124, 1-10.
- 63 Enserink EL, Maas-Diepeveen JL & Van Leeuwen CJ. 1991 Combined effects of metals; an ecotoxicological evaluation. *Water Research* 25, 679-687.
- 64 Carvalho, RN *et al.* 2014 Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they? *Toxicological Sciences* 141, 218-233.
- 65 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

FIGURE 1

Graphic illustration of the different ways in which chemical pollutants interact as mixture components



Synergism, where toxic effects are greater than additive, is more concerning to regulators due to this potential for amplified effects (see Figure 1). Laboratory-based analyses (on less than a few hundred chemicals) suggest synergism occurs in between 5 – 10% of cases<sup>66, 67</sup>, yet there is variability in frequency across chemical types. For example, one meta-analysis suggested that in mixtures with more than two antifouling agents, synergism occurs in up to 61% of cases<sup>68</sup>.

Comprehensive toxicity data are lacking for most of the 350,000 chemicals registered for use, so the true frequency of synergistic interactions in environmental mixtures remains uncertain. Chemical pollutants can also interact synergistically with other environmental stressors (eg climate stressors), however this is beyond the scope of this report.

66 Belden JB, Gilliom RJ, Lydy MJ. 2007 How well can we predict the toxicity of pesticide mixtures to aquatic life? *Integrated Environmental Assessment and Management* 3, 364-372.

67 Martin O *et al.* 2021 Ten years of research on synergisms and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies. *Environment International* 146, 106206.

68 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

## BOX 3

## Unique pollution-related challenges across riverine, estuarine and coastal environments

**Rivers**

Freshwater ecosystems such as rivers provide numerous critical ecosystem services, including water for drinking and irrigation. Riverine water quality is threatened by multiple pollution sources such as sewage, agricultural runoff and industrial discharges. With respect to the effects of chemical pollution, riverine species are more studied than estuarine and coastal species<sup>69</sup>. This may be for a range of reasons. Firstly, freshwater environments are more routinely monitored for their chemical conditions by regulatory agencies to meet a range of statutory reporting requirements. Secondly, freshwater environments and species are easier to study under laboratory conditions. Thirdly, pollutant concentrations are assumed to be higher in riverine environments as they are less affected by dilution effects than marine waters<sup>70</sup>.

**Estuaries**

Estuaries are critical habitats for water filtration, flood protection, nutrient cycling and more. As transitional zones between riverine and marine environments, estuaries have complex and unique environmental conditions. Given their transitional nature, monitoring estuarine environments can provide insights into the flow rates and quantities of chemical pollutants travelling from freshwater to marine environments. Estuaries can also be hotspots for pollution accumulation, as they are the primary receivers of sediment delivered to the coast by rivers<sup>71</sup>.

Organisms that inhabit estuarine environments are uniquely adapted to cope with fluctuating salinity amidst complex mixing of fresh and salt water, stratification and tidal action<sup>72</sup>. Despite this importance and the uniqueness of these habitats, there are comparatively fewer studies into the effects of pollutants on estuarine species and ecosystems, though an established evidence base does exist<sup>73</sup>.

**Coastal marine environment**

Coastal marine environments provide substantial economic value via fisheries, shipping, tourism and recreation and are home to a diverse range of marine habitats. Compared to freshwater environments, they are more dynamic which can result in more varied and unpredictable distribution patterns of pollutants. As well as being the ultimate sink for pollutants passing through river and estuarine systems, coastal marine environments have their own pollution challenges such as coastal runoff, shipping-related contaminants (eg antifoulants) and impacts from offshore energy industries. Despite having a greater diversity of species<sup>74</sup>, the effects of chemical pollutants on marine species are generally not as well studied compared to freshwater species. Due to the difficulty and expense of hosting marine species in the lab, pollutant effect studies are more often conducted in situ. This may have benefits in terms of understanding the effects of chemicals under environmentally realistic conditions and disadvantages in terms of repeatability and understanding the mechanisms of toxicity.

69 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

70 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

71 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

72 Robins PE *et al.* 2016 A review of climate change impacts on UK estuaries. *Estuar Coast Shelf Sci* 169, 119-135.

73 Matthiessen, P. and Law, R.J., 2002 Contaminants and their effects on estuarine and coastal organisms in the United Kingdom in the late twentieth century. *Environmental pollution* 120, 739-757.

74 ECETOC (European Centre for Ecotoxicology and Toxicology Of Chemicals). 2008 Workshop on the Probabilistic Approaches for Marine Hazard Assessment, Workshop Report No. 15. See [https://www.ecetoc.org/wp-content/uploads/2021/10/ECETOC\\_WR\\_15\\_Probabilistic\\_approaches\\_for\\_marine\\_hazard\\_assessment.pdf](https://www.ecetoc.org/wp-content/uploads/2021/10/ECETOC_WR_15_Probabilistic_approaches_for_marine_hazard_assessment.pdf) (accessed 30 January 2025).

In general terms, synergism results from interactions between chemicals that affect toxicity, including uptake, internal transportation, metabolism, binding at the target site and excretion<sup>75</sup>. Predicting synergistic effects remains challenging due to a lack of mechanistic information regarding the toxicity of specific chemicals involved, their chemical breakdown products and concentrations and the biological system(s) being studied<sup>76</sup>. Despite this, there are several chemicals that are well-documented as acting synergistically, including chemicals that affect metabolic degradation and transformation<sup>77</sup>. For example, organophosphates (a common component of pesticides, flame retardants and plasticisers) are known to act synergistically with many other compounds, including other organophosphates, different pesticide classes and wider contaminants such as heavy metals<sup>78</sup>. Similarly, pyrethroid insecticides, used for agricultural crop protection and mosquito control, are known to act synergistically with azole fungicides and organophosphates. Such compounds may warrant stricter controls due to the risk of amplified effects on ecosystems.

### What do field-based studies tell us about the effects of mixtures?

While there is some degree of understanding regarding the effects of manufactured mixtures under laboratory conditions, the real-world impacts of unintentional mixtures have proved more difficult to disentangle. There are many examples where point source emissions of chemical mixtures (eg from the end of a pipe or a specific site) are linked to adverse effects on aquatic species or ecosystems<sup>79, 80, 81</sup>. However, establishing cause-effect relationships or even associations between non-point source emissions and their biological effects is extremely difficult. Significant data gaps such as lack of toxicity data, complicate progress towards identifying these causal mechanisms. Strategic research efforts and the adoption of innovative approaches will enable science to continue to make incremental but meaningful progress towards establishing probable causality in chemical mixture effects (see Chapter three for discussion of some of these approaches). It is likely significant uncertainties will remain well into the medium term.

75 Cedergreen N. 2014 Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. *PLoS one* 9, e96580.

76 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology.

77 Cedergreen N. 2014 Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. *PLoS one* 9, e96580.

78 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).

79 Rainbow, PS *et al.* 2011. Have the bioavailabilities of trace metals to a suite of biomonitors changed over three decades in SW England estuaries historically affected by mining? *Science of the Total Environment* 409, 1589-1602.

80 Haase, P *et al.* 2023 The recovery of European freshwater biodiversity has come to a halt. *Nature* 620, 582-589.

81 Byrne, P *et al.* 2013 Changes in macroinvertebrate community structure provide evidence of neutral mine drainage impacts. *Environmental Science: Processes & Impacts* 15, 393-404.

Mixture models predict that in the long-term, species richness will decrease as the potential ecological damage of mixtures increases. Yet the presence of other environmental stressors (eg thermal and salinity stress, habitat disturbance or loss) complicates the establishment of cause-effect relationships and their mechanisms<sup>82</sup>. As a result of this complexity, field-based studies on contaminant mixtures and their effects are relatively rare compared to laboratory-based studies. Nonetheless, while constituting a smaller evidence base, field-based studies have indeed found links between mixture exposure and adverse effects on aquatic ecosystems. For example, mixture exposure has been linked to declines in species abundance, diversity and growth<sup>83, 84, 85</sup>, as well as wide-ranging genetic, metabolic, immunological and physiological effects<sup>86, 87, 88</sup>.

Few field-based studies have attempted to validate the predicted effects of laboratory research into chemical mixtures. More field-based studies are still required to provide a better approximation of the real-world effects of chronic exposure to a range of chemicals in the environment. There is also a need to better understand mixtures in estuarine and marine environments, which are historically less studied than freshwater environments due to assumed greater dilution and fewer regulatory requirements for monitoring. See Box 3 for further detail on the unique pollution challenges across these aquatic environments.

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- 82 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).
- 83 Echeveste P, Galbán-Malagón C, Dachs J, Berrojalbiz N, Agustí S. 2016 Toxicity of natural mixtures of organic pollutants in temperate and polar marine phytoplankton. *Science of the Total Environment* 571, 34-41.
- 84 Bighiu MA, Höss S, Traunspurger W, Kahlert M, Goedkoop W. 2020 Limited effects of pesticides on stream macroinvertebrates, biofilm nematodes and algae in intensive agricultural landscapes in Sweden. *Water Research* 174, 115640.
- 85 Ginebreda A *et al.* 2014 Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: Compound prioritization, mixture characterization and relationships with biological descriptors. *Science of the Total Environment* 468, 715-723.
- 86 Galloway TS, Millward N, Browne MA, Depledge MH. 2002 Rapid assessment of organophosphorous/carbamate exposure in the bivalve mollusc *Mytilus edulis* using combined esterase activities as biomarkers. *Aquatic Toxicology* 61, 169-180.
- 87 Lima I, Moreira SM, Rendón-Von Osten J, Soares A, Guilhermino L. 2007 Biochemical responses of the marine mussel *Mytilus galloprovincialis* to petrochemical environmental contamination along the North-western coast of Portugal. *Chemosphere* 66, 1230-1242.
- 88 Xu LL, Ji CL, Zhao, JM, Wu HF. 2016 Metabolic responses to metal pollution in shrimp *Crangon affinis* from the sites along the Laizhou Bay in the Bohai Sea. *Marine Pollution Bulletin* 113, 536-541.



# Chapter three

## Innovative approaches to chemicals management

**Left**

Eastbourne beach.

© iStock.com / George Standen.

# Innovative approaches to chemicals management

As has been identified, current approaches to chemical risk assessment have considerable shortcomings. The sheer number of chemicals in production are now overwhelming our ability to assess their safety using traditional approaches<sup>89</sup>. This is true for individual chemicals and even more so when the additional complexities of mixture risk are considered. Therefore, it is important to leverage the latest science to help to deal with this inherent complexity and develop a more protective and efficient regulatory system.

This section presents several of these innovative approaches that reflect a changing landscape of chemical risk assessment, sometimes referred to as ‘next generation risk assessment’<sup>90</sup>. Many of these approaches hold significant potential for producing faster and more cost-effective toxicological assessment compared with traditional approaches to testing and monitoring. This is because they partly minimise the need for lab-based animal testing<sup>91</sup>. These techniques can add value to both prospective risk assessment (ie pre-market registration of new chemicals) and retrospective risk assessment (ie environmental impact assessment).

Regarding mixtures specifically, ‘next generation’ approaches have the potential to address several key challenges with respect to their regulation and management in aquatic ecosystems. For example, they can advance understanding of:

- the composition and potential biological hazard of chemical mixtures;
- the mechanisms driving mixture toxicity and why some species are more sensitive to effects of mixtures than others; and
- the uses, pathways and environmental fate of chemicals (essential for predicting the composition of mixtures in the environment).

Each approach offers a different set of insights with respect to these challenges and therefore will provide the most benefit when used in combination. Discussion in this section is not comprehensive and these approaches should be considered in conjunction with other advancing techniques (eg high-resolution mass spectrometry<sup>92</sup>).

89 US Environmental Protection Agency. 2014 Next Generation Risk Assessment: Incorporation of Recent Advances in Molecular, Computational and Systems Biology (Final Report). See <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=286690> (accessed 31 January 2025).

90 US Environmental Protection Agency. 2014 Next Generation Risk Assessment: Incorporation Of Recent Advances In Molecular, Computational and Systems Biology (Final Report). See <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=286690> (accessed 31 January 2025).

91 US Environmental Protection Agency. 2014 Next Generation Risk Assessment: Incorporation Of Recent Advances In Molecular, Computational and Systems Biology (Final Report). See <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=286690> (accessed 31 January 2025).

92 Benigni P *et al.* 2015 Targeted high-resolution ion mobility separation coupled to ultrahigh-resolution mass spectrometry of endocrine disruptors in complex mixtures. *Analytical Chemistry* 87, 4321–4325.

It is also critical to recognise that while these innovative approaches hold great promise, major gaps and deficiencies in our current understanding of environmental chemicals will persist for some time to come. For example, there is much greater scientific understanding of legacy pollutants in freshwater systems compared to emerging contaminants in marine environments and the application of new technologies will not immediately rectify this. These approaches must therefore be deployed alongside a strategy for research prioritisation, which addresses critical data gaps (see Recommendation 4) and which also provides a rational basis for managing chemical mixtures now and in the coming years.

### New approach methodologies

New approach methodologies (NAMs) are non-animal<sup>93</sup> methodologies and technologies that can inform chemical hazard assessment. NAMs encompass a wide range of technologies and approaches designed to reduce, refine or replace traditional animal testing in chemical safety assessment and biomedical research<sup>94, 95</sup>. Along with the ethical advantages associated with a shift away from animal-based toxicity testing, NAMs also have the potential to increase efficiencies as vertebrate animal testing can be prohibitively slow and costly<sup>96</sup>. Examples of NAMs include high throughput screening, ‘omics’ technologies and ‘in silico’ computer modelling. This section discusses the broad contributions of NAMs as a group of approaches and the sections that follow examine two types of NAMs in detail – machine learning and ‘omics’ approaches.

Firstly, NAMs present opportunities to advance understanding of mixture risk via their ability to accelerate the generation of toxicity data for the individual components that make up the mixture. As established, our understanding of mixture effects is based on studies of a small subset of the estimated 350,000 chemicals in use worldwide. Addressing gaps in toxicity data for individual chemicals is therefore critical for developing a more comprehensive understanding of mixture risk.

93 NAMs are predominantly non-animal methods, but some approaches can include non-mammalian model species such as *Drosophila* (fruit flies) and *C. elegans* (roundworms).

94 Van Der Zalm A *et al.* 2022 A framework for establishing scientific confidence in new approach methodologies. *Archives of Toxicology* 96, 2865–2879.

95 Sewell F *et al.* 2024 New approach methodologies (NAMs): identifying and overcoming hurdles to accelerated adoption. *Toxicology Research* 13, tfae044.

96 Brescia S, Alexander-White C, Li H, Cayley A. 2023 Risk assessment in the 21st century: where are we heading? *Toxicology Research* 12, 1–11.

NAMs can generate chemical toxicity data for individual chemicals in a more resource-efficient manner, enabling an evaluation of a larger number of chemicals in a shorter timeframe<sup>97,98</sup>. For example, the use of high throughput screening enables the simultaneous testing of thousands of chemicals, dramatically increasing the speed of data generation<sup>99</sup>. Similarly, the use of computational models such as artificial intelligence (AI) and machine learning allow the rapid analysis of large datasets and modelling of chemical effects, enabling faster prioritisation of problematic chemicals and therefore, chemical combinations (further discussion below).

A second critical contribution of NAMs to understanding mixture risk is via the generation of mechanistic insights. Understanding how each chemical may cause harm – such as which biological pathways or molecular targets it affects – helps to inform predictions of how chemicals might interact when combined<sup>100</sup>. Traditional animal studies rarely provide this level of mechanistic detail, often focussing on observable effects (eg organ damage or mortality). By examining cellular and molecular-level responses,

NAMs can generate data to be used in mathematical models to predict mixture effects in a more detailed way<sup>101</sup>. These models can then be validated against real-world data to ensure their environmental relevance.

Finally, NAMs can also be used to directly study the effects of mixtures. Recent studies have used NAMs such as high-throughput analysis and metabolomics to study the toxic combined effects of chemicals in humans and other species. For example, a recent study analysed blood samples from pregnant women, using *in vitro* NAMs to detect toxic effects among the chemicals present in the highest concentrations and frequencies<sup>102</sup>. This data was also used to model mixture toxicity and identify key drivers of toxicity. In freshwater environments, studies have examined the combined effects of pharmaceuticals on water fleas by applying a suite of NAMs to detect sub-lethal physiological and metabolic disturbances<sup>103</sup>. Their findings showed that exposure to pharmaceutical mixtures, even at non-lethal concentrations, led to marked reductions in feeding activity and significant changes in metabolic pathways.

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- 97 National Centre for the Replacement Refinement & Reduction of Animals in Research. 2024 Vision for a modern science-based approach to UK chemicals regulation. See <https://nc3rs.org.uk/uk-chemicals-regulation-vision-january-2024> (accessed 31 January 2025).
- 98 US Food and Drug Association. 2024 New Approach Methods (NAMs). See <https://www.fda.gov/food/toxicology-research/new-approach-methods-nams> (accessed 31 January 2025).
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- 102 Braun G *et al.* 2024 Neurotoxic mixture effects of chemicals extracted from blood of pregnant women. *Science* 386, 301-309.
- 103 Rowan E *et al.* 2025 New approach methodologies: physiological responses of daphnids to pharmaceutical mixtures. *J. Hazard. Mater. Lett.* 6, 100139.

These effects serve as sensitive early warning signs of mixture toxicity that are likely to go undetected by conventional testing methods. Thus, by enabling high-throughput and mechanistic investigations, NAMs can provide a more realistic and comprehensive understanding of environmental and human health risks from combined exposures.

The pace of scientific development in this space has been rapid and there have been some successes already with integrating NAMs into existing chemical regulatory frameworks. For example, the OECD and US Environmental Protection Agency have adopted guidelines allowing for the use of non-animal information sources for skin sensitisation testing<sup>104, 105</sup>. However, uptake within chemical risk assessment in the UK has been limited so far. Barriers to widespread integration appear to include the familiarity and long-term trend data associated with established animal-testing, as well as ambiguity as to what types of testing are acceptable under current legislation such as REACH<sup>106</sup>. Leveraging the full benefits of NAMs for regulatory mixture assessment will require better data sharing and integration across chemical domains and regulatory frameworks (see Recommendation 3).

### Machine learning

Machine learning is a branch of AI that allows computer systems to be trained using data and examples to develop algorithms and build models<sup>107</sup>. These technologies are part of the 'NAMs' suite of techniques and offer opportunities to address some of the regulatory and management challenges posed by chemical mixtures. While the application of computational models to chemical risk assessment is not new, the unique selling point of machine learning models is that they can autonomously identify patterns emerging from input data (ie, they are able to 'learn' without explicit programming of these relationships).

The ability to train machine learning models to predict the effects of mixtures helps to address the practical impossibility of experimentally testing all possible chemical combinations and interactions. Machine learning algorithms can also identify subtle relationships in large, complex datasets that may not be obvious to human analysts<sup>108</sup>. Advanced machine learning techniques may soon be able to identify and predict particularly high-risk chemical combinations, allowing regulators and researchers to focus resources on preventing the environmental co-occurrence of concerning chemical combinations. Some early studies have found that machine learning models outperformed animal testing approaches in terms of identifying toxicity for chemical risk assessments<sup>109</sup>.

104 Casati S, Asturiol D, Browne P, Kleinstreuer N, Régimbald-Krnel M, Therriault P. 2022 Standardisation and international adoption of defined approaches for skin sensitisation. *Frontiers in Toxicology* 4, 943152.

105 US Environmental Protection Agency. 2024 Strategic Vision for Adopting New Approach Methodologies – Replacement Strategies. See <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-new-approach-2> (accessed 31 January 2025).

106 Precision Toxicology. 2023 Report on Socio-Technical Barriers to the Uptake of NAMs. See <https://precisiontox.org/wp-content/uploads/2024/02/D6.1-Report-on-Socio-Technical-Barriers-26Jan.pdf> (accessed 31 January 2025).

107 The Royal Society. 2017 Machine learning: the power and promise of computers that learn by example. See <https://royalsociety.org/-/media/policy/projects/machine-learning/publications/machine-learning-report.pdf> (accessed 31 January 2025).

108 Wittwehr C *et al.* 2020 Artificial Intelligence for chemical risk assessment. *Computational Toxicology* 13, 100114.

109 Miller TH *et al.* 2018 Machine learning for environmental toxicology: a call for integration and innovation. *Environmental Science & Technology* 52, 12953-12955.

However, realising this predictive power largely depends on the quality and comprehensiveness of the initial assumptions and training data<sup>110</sup>. Given existing biases in chemicals data – with some chemicals incredibly well-studied and others lacking toxicity data – machine learning models trained primarily on well-studied chemicals may not accurately predict the effects of less characterised chemicals or their interactions. Further research to prioritise plugging such gaps will be critical to improve the reliability and usefulness of deploying machine learning tools.

Other promising applications for machine learning include its potential to enhance exposure modelling. Exposure modelling uses real-world exposure data (collected via environmental monitoring) to describe and predict the distribution of environmental chemicals over space and time<sup>111</sup>. Machine learning can be used to draw inferences from existing exposure data to prioritise chemicals that are likely to co-occur in the environment<sup>112</sup>.<sup>113</sup> Scientists may then be able to use this knowledge to help design more relevant and targeted laboratory-based experiments. Such applications will advance understanding of the uses, pathways and environmental fate of chemicals, allowing us to predict the likely composition of environmental chemical mixtures, as well as aiding our understanding of the hazards that these mixtures pose.

Despite the opportunities it presents in modernising both prospective risk assessment and environmental monitoring, machine learning is not yet being leveraged for these purposes in the UK. Regulatory acceptance may be a key barrier. Machine learning has often been considered a ‘black box’, lacking transparency and explainability, which can be daunting for regulators who may not understand the decision-making processes of these models<sup>114</sup>. Enhancing the accessibility and interpretability of machine learning models will be essential for fostering their acceptance in regulatory environments.

Skills gaps among regulators, academic researchers and industry alike must be also addressed to enable the adoption of machine learning for chemical risk assessment<sup>115</sup>. Dedicated skills development programmes, in collaboration with industry and academic institutions, will be necessary to ensure toxicologists and ecotoxicologists have the coding, programming and data analysis skills to keep pace with the fast-advancing science (see Recommendation 5). Moreover, leveraging this technology in research will require interdisciplinary collaboration between domain experts (eg biologists, ecologists, toxicologists) and technical experts (eg computer scientists) to frame problems and interpret results in a meaningful way.

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- 110 Schür C, Gasser L, Perez-Cruz F, Schirmer K, Baity-Jesi M. 2023 A benchmark dataset for machine learning in ecotoxicology. *Scientific Data* 10, 718.
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- 112 Drakvik E *et al.* 2020 Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ. Int.* 134, 105267.
- 113 Martinez A, Hornbuckle KC, Jones MP, Westra BD. 2025 Spatial and Temporal Analysis and Machine Learning-Based Prediction of PCB Water Concentrations in U.S. Natural Water Systems. *ACS ES&T Water* 5, 60-69.
- 114 Wassenaar PNH, Minnema J, Vriend J, Peijnenburg WJGM, Pennings JLA, Kienhuis A. 2024 The role of trust in the use of artificial intelligence for chemical risk assessment. *Regulatory Toxicology and Pharmacology* 148, 105589.
- 115 Miller TH *et al.* 2018 Machine learning for environmental toxicology: a call for integration and innovation. *Environmental Science & Technology* 52, 12953-12955.

## BOX 4

## What are biomarkers?

Biomarkers are a well-established and valuable tool for understanding both exposure and the biological effects of chemicals and associated mixtures. A biomarker is a biological characteristic of an organism – whether biochemical, cellular, physiological or behavioural – which provides information regarding exposure to and/or adverse effects of pollution. These can be measured in samples of tissue or body fluid, such as DNA, or by observing the whole organism. Changes in biomarkers can signal exposure to and affects of, one or more chemical pollutants and the effects of chemical pollutants on specific biomarkers are well established<sup>116</sup>. Examples of biomarkers used to assess chemical exposure include enzyme activity<sup>117</sup> and DNA damage<sup>118</sup>.

Typically, biomarkers have been used to detect exposure to a particular class of pollutants (eg heavy metals<sup>119</sup> or pesticides<sup>120</sup>), but it is also possible to measure exposure and ecologically relevant effects relating to suites of different environmental chemicals<sup>121</sup>. This allows the study and analysis of the co-exposure of organisms to different chemical mixtures over time. Biomarkers are therefore particularly valuable for routine environmental monitoring and as early warning indicators of the presence of chemical stressors in the environment<sup>122</sup>. Improvements in analytical techniques will permit rapid, cost-effective biomarker studies to be performed routinely.

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- 116 Depledge MH, Fossi MC. 1994 The role of biomarkers in environmental assessment (2). *Invertebrates. Ecotoxicology* 3, 161-172.
- 117 Gonçalves AM, Rocha CP, Marques JC, Gonçalves FJ. 2021 Enzymes as useful biomarkers to assess the response of freshwater communities to pesticide exposure – A review. *Ecological Indicators* 122, 107303.
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- 121 Lomartire S, Marques JC, Gonçalves AM. 2021 Biomarkers based tools to assess environmental and chemical stressors in aquatic systems. *Ecological Indicators* 122, 107207.
- 122 Lomartire S, Marques JC, Gonçalves AM. 2021 Biomarkers based tools to assess environmental and chemical stressors in aquatic systems. *Ecological Indicators* 122, 107207.

### 'Omics' techniques, such as environmental DNA and RNA

'Omics' techniques are advanced molecular approaches that can give insights into biological responses to chemical exposures. Many of these can also be used as biological markers, or biomarkers (see Box 4). Key omics approaches include genomics (studies genetic code and structure), transcriptomics (analyses RNA transcripts to assess gene expression), proteomics (analyses proteins) and metabolomics (analyses metabolites)<sup>123</sup>. Omics-based technologies can be used for environmental 'surveillance', to understand changes in biological markers for an individual or species which can then act as early warning signals for changes in environmental quality (for example, changes in DNA expression may indicate a more polluted environment)<sup>124</sup>. Omics can also be used to understand variation across species with regard to mechanisms of action and adverse outcome pathways (ie sequences of events within organisms that lead to harmful effects). This can facilitate an understanding of how mixture toxicity and exposure effects may vary between species.

Environmental DNA (eDNA) is an 'omics' biomarker technology that can be used to understand which genes and species are affected by aquatic pollution. eDNA refers to DNA obtained directly from an environmental sample (eg water)<sup>125</sup>. It can be recovered either from fragments of cellular material or from whole organisms (for very small organisms, such as microbes) and their genomes<sup>126</sup>. eDNA can be used to determine which organisms are present in each ecosystem and can be used as a proxy to detect the presence of pollution and its effects on individuals, populations and the wider ecosystem<sup>127</sup>. It allows for large datasets to be generated at low cost compared to more conventional forms of environmental monitoring. eDNA can also be used to detect the presence of certain genes of interest (eg those related to antibiotic resistance).

By combining eDNA data with chemical monitoring data, researchers can establish stronger correlations between chemical mixtures and their resultant ecosystem level effects<sup>128, 129, 130</sup>. eDNA analysis can reveal the entire range of species present in a sample (or at least those which have available genomes or barcodes within reference databases).

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- 123 Zhang X, Xia P, Wang P, Yang J, Baird DJ. 2018 Omics Advances in Ecotoxicology. *Environmental Science & Technology* 52, 3842-3851.
- 124 Rishan ST, Kline RJ, Rahman MS. 2024 Exploitation of environmental DNA (eDNA) for ecotoxicological research: A critical review on eDNA metabarcoding in assessing marine pollution. *Chemosphere* 351, 141238.
- 125 Thomsen PF, Willerslev E. 2015 Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* 183, 4-18.
- 126 The Royal Society. 2025 Environmental DNA: A Policy Explainer. See <https://royalsociety.org/-/media/policy/projects/environmental-dna/environmental-dna-policy-briefing.pdf> (accessed 19 May 2025).
- 127 For example, in monitoring the impact of oil and gas drilling – see Frontalini, *et al.*, 2020. Benthic foraminiferal metabarcoding and morphology-based assessment around three offshore gas platforms: congruence and complementarity. *Environment International* 144, 106049.
- 128 Inostroza PA *et al.* 2025 Multi-compartment impact of micropollutants and particularly antibiotics on bacterial communities using environmental DNA at river basin-level. *Environmental Pollution* 366, 125487.
- 129 Huo M *et al.* 2024 Co-selection mechanism for bacterial resistance to major chemical pollutants in the environment. *Sci. Total Environ.* 912, 169223.
- 130 Eckert EM *et al.* 2023 Increased similarity of aquatic bacterial communities of different origin after antibiotic disturbance. *Environmental Pollution* 316, 120568.

eDNA sampling and analysis therefore allows for a comprehensive assessment of biodiversity changes across taxonomic groups simultaneously, providing a holistic view of how chemical mixtures impact entire ecological communities<sup>131</sup>. Therefore, through repeated eDNA sampling, any changes in species composition over time and as a response to polluted conditions, can be detected. As a result, eDNA approaches can help to overcome the limitations of traditional chemical risk assessments which look at a limited range of test species and do not consider impacts on the wider ecosystem.

Environmental RNA (eRNA) analysis can also be used in a complementary way to understand more detailed changes in gene expression associated with polluted conditions<sup>132, 133</sup>. Whereas eDNA represents the fragmented genomes of cellular organisms present in the environment, eRNA represents only the expressed parts of those genomes (the transcriptome). As gene expression is influenced by external factors, eRNA analysis can allow more detailed inferences on the response of different individual organisms to environmental stressors<sup>134</sup> – giving insights into the biological pathways that are affected by pollutants and non-lethal effects.

These techniques are perhaps most valuable when used in combination. For example, integrating eDNA and eRNA, traditional ecological surveys and other ‘omics’ approaches can yield comprehensive insights into both the biological effects of pollutants within organisms and how pollution affects species, ecosystem health and ecosystem function more widely<sup>135</sup>.

While eDNA has gained traction in various environmental monitoring applications, its use in chemical management is still limited. Over time, projects such as the Darwin Tree of Life<sup>136</sup> will vastly increase the availability of genomic data for UK environmental species and thus the accuracy, potential and value of these methods. For more information on eDNA and its applications, including in pollution monitoring, see the Royal Society’s *Environmental DNA: A policy explainer* report<sup>137</sup>.

131 The Royal Society. 2025 Environmental DNA: A Policy Explainer. See <https://royalsociety.org/-/media/policy/projects/environmental-dna/environmental-dna-policy-briefing.pdf> (accessed 19 May 2025).

132 Littlefair JE, Rennie MD, Cristescu ME. 2022 Environmental nucleic acids: A field-based comparison for monitoring freshwater habitats using eDNA and eRNA. *Molecular Ecology Resources* 22, 2928-2940.

133 Zhang Y *et al.* 2024 Evaluating eDNA and eRNA Metabarcoding for Aquatic Biodiversity Assessment: from Bacteria to Vertebrates. *Environmental Science and Ecotechnology* 21, 100441.

134 Yates MC, Derry AM, Cristescu ME. 2021 Environmental RNA: A Revolution in Ecological Resolution? *Trends in Ecology and Evolution* 36, 601-609.

135 For example, see Giroux MS, Reichman JR, Langknecht T, Burgess RM, Ho KT. 2023 Using eRNA/eDNA metabarcoding to detect community-level impacts of nanoplastic exposure to benthic estuarine ecosystems. *Environmental Pollution* 338, 122650.

136 Darwin Tree of Life. See <https://www.darwintreeoflife.org/> (accessed 4 March 2025).

137 The Royal Society. 2025 Environmental DNA: A Policy Explainer. See <https://royalsociety.org/-/media/policy/projects/environmental-dna/environmental-dna-policy-briefing.pdf> (accessed 19 May 2025).



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**Left**

River flowing through British countryside.

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138 Spurgeon D, Roberts S, Schultz C. 2024 Rapid evidence review – the biological effects of chemical mixtures in aquatic environments. Wallingford, UK: UK Centre for Ecology and Hydrology. See <https://royalsociety.org/-/media/policy/projects/environmental-chemical-mixtures/ukceh-review.pdf> (accessed 6 October 2025).









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