Science for Environment Policy
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SPECIAL ISSUE

Combination Effects of Chemicals

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Towards better assessment of the 'cocktail effect'

Outside the laboratory, living organisms are never subject to single stressors at set doses. In the real world, they face an intricate array of physical, chemical and biological environmental stressors that vary in space and time. The problem of assessing these complex risks for human health and the environment is a great challenge for scientists and regulators alike. For example, even if Maximum Permissible Concentrations (MPCs) for individual contaminants are not exceeded in water, in combination they can still be potentially hazardous to wildlife.

This thematic issue reports on scientific research which can help us overcome some of the challenges associated with assessing the combination effects of chemicals.

The EU's NoMiracle project has adopted a new biology-based approach to assessing combination effects, which considers the interaction of mixtures with biological processes. This receptor-oriented approach puts the exposed individual, population or ecosystem at the heart of assessment; the physiology and behaviour of the receptor are important drivers of cumulative risks.

This new biology-based approach shows great promise and is described in the article 'Biology as important as chemistry in assessing toxic mixtures'. A practical example of how it has been applied is presented in: 'Protecting surface waters from combined effects of chemical contaminants'.

In the US, the Environmental Protection Agency (EPA) are developing the Community-Focussed Exposure and Risk Screening Tool (C-FERST) in order to help safeguard public health from the effects of chemical mixtures. This assessment tool will assess human exposure at several levels and its development is outlined in: 'New US tool to assess cumulative health risk of multiple chemicals'.

Registration, Evaluation and Assessment of Chemicals (REACH) and the Water Framework Directive (WFD) are both large pieces of EU legislation which regulate chemicals. However, they primarily use approaches that assess the toxicity of single chemicals. The review 'Integrating chemical mixture assessments into REACH and the WFD' takes inspiration from the US and Denmark, suggesting steps could be made to limit the mixtures to be assessed based on the 'PEC/PNEC' ratio, e.g. for compounds with ratios larger than 0.1. It recommends using Concentration Addition as a default assessment method of mixtures within the WFD and REACH. This descriptive method is based on concentrations and properties of individual chemicals within the mixtures.

Two practical examples of tools which help predict and manage combination effects are outlined in this issue. 'New tool accurately predicts toxic effects of chemical mixtures' describes how chemical mixture effects can be predicted using a new tool for assessment based on the 'Dynamic Energy Budget' theory. The article 'New maps show "hotspots" of risk to wildlife from chemical mixtures' explains how researchers have developed ecological risk maps which combine wildlife vulnerability maps with soil hazard maps.

Rather than testing a large battery of species, testing resources should be directed towards better mechanistic understanding of mixture/multiple stressor effects in order to develop a mechanism-based framework for interpreting mixture effects. The most important step in future testing would be to change the test schemes to acquire data on how effects change over time to enable better predictions of mixture effects in a dynamic world.

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Biology as important as chemistry in assessing toxic mixtures

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Theme(s): Biodiversity, Chemicals, Risk assessment

Researchers have developed the first biology-based model to predict the sub-lethal effects of chemical mixtures on organisms. Sub-lethal effects do not cause death but can damage processes such as growth and reproduction. The model provided accurate predictions of the sub-lethal impacts of a chemical mixture on water fleas.

"The results raised questions about the use of traditional measurements of toxicity, which would not have yielded useful information about the impacts on survival, growth and reproduction."

Currently the toxicity of chemical mixtures tends to be analysed using descriptive methods based on concentrations and properties of individual chemicals within the mixtures, for example, the methods of 'Concentration Addition' and 'Independent Addition'. Although these are useful as a first step in assessing toxicity, they do not consider the interaction of mixtures with biological processes or explain why the effects of mixtures can change over time.

The research was supported by the EU-funded NoMiracle[1] project. Compared with traditional methods, it adopted a more biology-based approach that considered the interaction of mixtures with several biological processes, such as feeding, maintenance and reproduction. This is known as an 'ecotoxicodynamic' approach. The researchers predicted the impacts of a simple mixture of two polycyclic aromatic hydrocarbons (PAHS), pyrene and fluoranthene, on the growth, reproduction and survival of water fleas (Daphnia magna).

To test the model, the results were compared to experimental results from a 21-day partial life-cycle test. The test comprised measurements of the impacts of the PAH mixture with varying proportions of the two chemical compounds within the mixture. The model provided an accurate description of the impacts of the PAH mixture on growth and reproduction. It indicated that fluroanthene is slightly more toxic than pyrene within the mixture, and was consistent with the assumption that these compounds have the same mechanism of action.

The modelled data were very similar to the real data for growth and reproduction for all treatment. For survival, the general pattern was well described, but for comprising roughly equal levels of pyrene and fluroanthene, there was a notable mismatch between model and data. This could be due to a specific interaction between the two PAHs when mixed at these concentrations.

The results demonstrated the feasibility of using a biology-based approach to assess the patterns of toxicity for different types of impact over time. It also raised questions about the use of traditional measurements of toxicity, which would not have yielded useful information about the impacts on survival, growth and reproduction. Since it is the first of its kind, more data need to be analysed to increase confidence in the method.

Source: Jager, T., Vandenbrouck, T., Baas, J. *et al*. (2010). A biology-based approach for mixture toxicity of multiple endpoints over the life cycle. *Ecotoxicology*. 19:351-361.

1. NoMiracle was supported by the European Commission under the Sixth Framework Programme , under the theme 'Global Change and Ecosystems'. See: http://nomiracle.jrc.ec.europa.eu/default.aspx

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Protecting surface waters from combined effects of chemical contaminants

Surface water is considered to be of good ecological quality if the Maximum Permissible Concentrations (MPCs) of contaminants in the water are not exceeded. However, new research suggests that even when each individual contaminant does not exceed its MPC, water quality may be compromised by the combined effects of contaminants.

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Theme(s): Biodiversity, Chemicals, Water, Risk assessment

"Waters containing cadmium in concentrations close to its Maximum Permissible Concentration (MPC), together with other metals found in typical concentrations, would cause water fleas to die within 30 hours, even though none of the MPCs had been exceeded."

MPCs are concentrations of contaminants in water, above which the risk of adverse effects is considered unacceptable. They are typically chosen as the environmental quality standard for evaluating surface waters. However, MPCs are established for individual compounds and do not account for combined effects of chemical mixtures.

Supported by the EU project NoMiracle[1], the researchers compared the MPCs of 23 pollutants (nine PAHs, seven metals and seven pesticides) found in Dutch surface waters by studying their individual and combined effect on water fleas (Daphnia magna, a commonly used species in toxicity research) exposed to the waters.

Models used to predict the effect of chemical mixtures on the survival of water fleas use the No Effect Concentration (NEC) instead of the more frequently used No Observed Effect Concentration (NOEC). Below the NEC there is no effect on survival even after lengthy exposure. The researchers compared the NECs for the survival of water fleas with the MPCs and the actual measured concentrations in water samples.

Overall, the study suggests that exceeded MPCs for some individual contaminants, such as some metals and pesticides, do not necessarily cause water fleas to die. However, due to the combined effects of chemicals, some mixtures of contaminants may lead to death even if individual MPCs are not exceeded.

Where there is a safe (i.e. large) margin between the NECs and MPCs, as was the case with the PAHs, the study suggests the MPCs provide adequate protection and slight exceedence of the MPCs would not lead to extinction of the water fleas population.

However, the differences between the NECs and MPCs for the metals and pesticides varied widely. For some, the difference was very small and even a slight exceedence of the MPC can result in the death of the water flea population. For example, the researchers calculated that waters containing the metal cadmium in concentrations close to its MPC, together with other metals found in typical concentrations, would cause water fleas to die within 30 hours after the start of the exposure, even though none of the MPCs had been exceeded.

As water fleas are not considered to be especially sensitive to the toxic effect of chemicals, the researchers suggest that if water flea populations can become extinct, other species might not be adequately protected by MPCs.

Source: Baas, J., Kooijman, B. (2010). Chemical contamination and the ecological quality of surface water. *Environmental Pollution*. 158:1603–1607.

1. NoMiracle was supported by the European Commission under the Sixth Framework Programme, under the theme 'Global Change and Ecosystems'. See: http://nomiracle.jrc.ec.europa.eu/default.aspx

New US tool to assess cumulative health risk of multiple chemicals

Assessing the cumulative human health risk caused by multiple toxic substances is a major challenge. New research has reported on developments in the US, where the Environmental Protection Agency (EPA) is creating a tool that will provide maps and other information to depict exposure data and risks at both a national and a local level.

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"So far the programme has reviewed and evaluated existing assessment tools by applying them to case studies. The review indicated that existing tools are difficult to use and do not answer all community questions."

Identifying and prioritising environmental issues within a community requires clear assessment tools that are based on scientific evidence. These tools include exposure models, sampling methods, databases and geographical information systems (GIS). The US EPA is developing models and tools to conduct exposure assessments of multiple stresses to the environment, with the aim of reducing risks and improving health.

The study outlined the research programme behind this initiative. So far the programme has reviewed and evaluated existing assessment tools by applying them to case studies. Alongside discussions with stakeholder groups, which include scientists and regional EPA officers, the review indicated that existing tools are difficult to use and do not answer all community questions. More specifically it suggested that:

- Tools need to be developed collaboratively with end-users to become more user-friendly
- Tools need to quantify cumulative risks and the impact of reduction activities
- More data are needed on both community-level and individual-level exposure and their links to health effects
- Additional research is needed to consider the effects of non-chemical factors that affect risk, such as noise and individual stress

The programme has started to develop the Community-Focussed Exposure and Risk Screening Tool (C-FERST). This will assess exposure at several levels, for example, sources (e.g. airports, traffic), individual toxic substances (e.g. radon, benzene) and health effects (e.g. childhood asthma, lung cancer). It will contain general information about the selected environmental stressor or toxic mixture, such as factsheets and weblinks, more specific information about the population affected, through maps, for example, as well as more details on sources, concentrations, exposures, risks, health effects and actions that can be taken to reduce risk.

Research to underpin the development of C-FERST has started. This includes evaluating existing risk assessment approaches for environmental issues, such as benzene, radon, tobacco smoke and ultraviolet radiation. The aim is to collaborate with community assessments and the National Children's Study to evaluate the tools, and initial efforts have been made to develop models to assess individual exposure to multiple pollutants focussing on asthma and diet.

Eventually C-FERST will identify communities at risk to multiple toxic chemicals, assess the health impacts of these chemical mixtures and evaluate reduction strategies.

Source: Zartarian, V.G. & Schultz, B.D. (2009). The EPA's human exposure research program for assessing cumulative risk in communities. *Journal of Exposure Science and Environmental Epidemiology.* Doi: 10.1038/jes.2009.20.

Integrating chemical mixture assessments into REACH and the WFD

New research has supported a more thorough integration of toxic mixture assessments into two major pieces of EU legislation: REACH and the Water Framework Directive. It recommended constructing a database of harmful chemicals in the environment which, among other uses, could assess mixture toxicity using a 'Concentration Addition' method.

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"Knowledge of exactly how chemicals act is rare and it is difficult, if not impossible, to group chemicals in the environment according to their similarity or dissimilarity."

The effect of chemicals on ecosystems and human health is mainly due to exposures to mixtures rather than to individual chemicals. However, two large pieces of EU legislation aimed at regulating chemicals – the Registration, Evaluation and Assessment of Chemicals (REACH)[1] and the Water Framework Directive (WFD)[2]–primarily use approaches that assess the toxicity of single chemicals. Only specific types of mixtures, such as oil compounds, are covered by REACH.

The study reviewed existing research on risk assessments of chemical mixtures. Currently there are two main models: Concentration Addition (CA) which estimates mixture effects of chemicals that act in a similar way, and Independent Action (IA), which estimates mixture effects of chemicals that act differently to each other.

However, knowledge of exactly how chemicals act is rare and it is difficult, if not impossible, to group chemicals in the environment according to their similarity or dissimilarity. Several pieces of research have argued for the use of CA as it is the most conservative method and therefore the most protective.

The study evaluated the practicality of the models by investigating their use in existing legislation. The relevant environment and health agencies in the US use both CA and IA. Before using either approach, the US Agency for Toxic Substances and Disease Registry (ATSDR) reduces the number of mixtures to be assessed using so-called hazard quotients. In principle these are similar to the PEC/PNEC ratio used in EU legislation where PEC is the background concentration of the mixture in the environment (Predicted Environmental Concentration) and PNEC is the concentration level of the mixture above which it causes harm (Predicted No-Effect Concentration). The researchers suggested that assessment could be limited to mixtures containing chemicals with individual ratios of PEC/PNEC > 0.1.

The Danish regulation on wastewater and air pollution uses a CA method. For mixtures with dissimilar chemicals, the air pollution regulation considers the risk of the chemical that contributes most to the toxic effect, whereas the wastewater regulation recommends that all chemical mixtures are predicted with CA. The study pointed out that initially REACH included the use of CA to assess preparations but it was removed from the final version, perhaps because assessment was considered too complex at the time.

The study recommended the use of CA as a default assessment method of mixtures within the WFD and REACH. Taking inspiration from the US and Denmark, steps could be made to limit the mixtures to be assessed based on the PEC/PNEC ratio.

Syberg, K., Jensen, T.S., Cedergreen, N. & Rank, J. (2009). On the Use of Mixture Toxicity Assessment in REACH and the Water Framework Directive: A Review. *Human and Ecological Risk Assessment: An International Journal.* 15(6): 1257-1272.

1 See http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm
2 See http://ec.europa.eu/environment/water/water-framework/index_en.html

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New tool accurately predicts toxic effects of chemical mixtures

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Themes: Biodiversity, Chemicals, Water, Risk assessment

A new tool that predicts the effects of complex mixtures in water has shown promising results. It correctly predicted the impacts of toxic mixtures on the model species Daphnia magna, or water fleas, in over 90 per cent of cases.

"In the samples where there was complete mortality the model could identify the contaminant, group of contaminants or conditions that was the cause of death."

The most common approaches for predicting the toxicity of chemical mixtures are 'Concentration Addition' and 'Independent Action'. Both these methods focus on specific effects of the mixtures (e.g. on growth) and the results do not apply to other organisms, endpoints, points in time or chemicals.

The research was supported by the EU-funded NoMiracle[1] project. It compared predicted survival with observed survival of water fleas in water samples taken from the Delfland region of the Netherlands, an area with a high concentration of greenhouses. At the time of the research there was no sewage treatment in this region so it is contaminated with heavy metals, pesticides, nutrients and minerals.

The concentrations of over 90 chemical contaminants were measured and, by analysing the chemical composition, the study predicted whether the NECs were exceeded and what the effect on water flea survival was after one week of exposure. 37 datasets were analysed from 17 locations. In 17 of these datasets all water fleas died, but in the other 20 all water fleas survived. Other datasets were also examined which showed partial effects, i.e. some water fleas died, but large differences in mortality levels made it difficult to include these in the main analysis.

The researchers predicted the survival of water fleas using no effect concentrations (NEC) of detected chemical compounds in the water samples. NECs are the concentrations below which there are no effects on the water fleas. For the first time, NECs were also derived for mixtures. They distinguished the following groups on the basis of having similar NECs: poly-aromatic hydrocarbons (PAHs), organophosphorus insecticides, inhibiting acetyl-choline esterases and metals. All other compounds were assumed to have their own individual NECs. The study also considered the impacts of the acidity of water and the level of oxygen.

The concentrations of over 90 chemical contaminants were measured and, by analysing the chemical composition, the study predicted whether the NECs were exceeded and the effect on water flea survival after one week of exposure.

In 19 out of the 20 cases, where all water fleas survived, the model correctly predicted survival. In 15 out of the 17 cases, where all water fleas died, the model correctly predicted death. In the samples where there was complete mortality the model could identify the contaminant, group of contaminants or conditions that was the cause of death, by checking which of the NECs were exceeded. These were mainly high pH, individual pesticides and low oxygen levels.

There was only one case where the observed mortality was caused by a mixture. This was from a mixture of organophosphorus pesticides. However, the researchers suggested that if partial or sub-lethal effects were evaluated then the role of mixtures could be more apparent.

Source: Baas, J., Willems, J., Jager, T. *et al*. (2009). Prediction of Daphnid Survival after *in Situ* Exposure to Complex Mixtures. *Environmental Science & Technology*. 43(15): 6064-6069.

1. NoMiracle was supported by the European Commission under the Sixth Framework Programme , under the theme 'Global Change and Ecosystems'. See: http://nomiracle.jrc.ec.europa.eu/default.aspx

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New maps show "hotspots" of risk to wildlife from chemical mixtures

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Ecological risk maps showing 'hotspots' of risk to wildlife from single or combined soil pollutants have been developed. These maps can be used to improve risk analysis and stakeholder communications.

"The risk assessment of harmful substances in the environment has traditionally been carried out on individual pollutants. In reality, wildlife species are exposed to a mixture of toxic compounds."

Ecological risk maps are made by using and combining information on vulnerability of wildlife species, wildlife habitats, individual soil pollutants and modes of action for mixture effects of the pollutants.

The risk assessment of harmful substances in the environment has traditionally been carried out on individual pollutants. In reality, wildlife species are exposed to a mixture of toxic compounds and more realistic assessments should take account of these combined effects.

As part of the EU-funded NoMiracle[1] project, new methods have been developed to assess the potential risks to wildlife from the combined effect of toxic compounds in the soil.

A new method, vulnerability analysis, uses trait-based ecological risk assessment. This is based on analysing the vulnerability of wildlife species to soil pollutants, by assessing the role of various traits of the species on the effects of pollutants, for example, food preferences, life cycle and behavioural characteristics. This process is based on three factors: 1) exposure of the species to the chemicals; 2) sensitivity of the species to the chemicals; and 3) the potential for populations to recover from exposure.

The results of the vulnerability analysis can be used to produce wildlife vulnerability maps and, when overlaid with maps of soil pollution, ecological risk maps for single or combined soil contaminants. Using Denmark as a case study, the researchers mapped the vulnerability of different habitats to the effects of the metals copper, zinc, cadmium and nickel and the insecticide chlorpyrifos.

As a first step, a suitable habitat map is combined with a vulnerability analysis of the different wildlife species found in each habitat. This process results in five habitat vulnerability maps for wildlife: one for each of the four metals and one for chlorpyrifos.

Next, soil hazard maps are constructed for each of the pollutants, based on soil characteristics and the estimated concentrations of the contaminants (relative to their Maximum Permissible Concentrations) found in Danish soils.

Combining vulnerability maps with soil hazard maps produced ecological risk maps for each of the pollutants. These relative risk maps identify 'hotspots' where wildlife is most at risk from individual pollutants in Denmark.

Finally, different cumulative ecological risk maps are developed by combining maps of individual pollutants according to the various ways in which the pollutants can have a combined effect on wildlife. These maps reveal where wildlife is most at risk (i.e. the 'hotspots') from the combined effects of these pollutants in Denmark.

Source: Lahr, J., Münier, B., De Lange, H.J. *et al.* (2010). Wildlife vulnerability and risk maps for combined pollutants. *Science of the Total Environment.* Doi:10.1016/j.scitotenv.2009.11.018 (8 pages)

1. NoMiracle was supported by the European Commission under the Sixth Framework Programme , under the theme 'Global Change and Ecosystems'. See: http://nomiracle.jrc.ec.europa.eu/default.aspx

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A selection of articles on Combination Effects of Chemicals from the *Science for Environment Policy* news alert.

How toxic are unregulated wastewater pollutants? (07/05/09)
Spanish and Dutch researchers have evaluated the environmental impact of chemical pollutants in wastewater in Spain. The results suggest that the most problematic pollutants may be derived from newer pharmaceutical and personal care products, such as everyday painkillers and soaps, not yet regulated.

Hormonal cocktail feminises male fish (12/03/09)
Rivers that contain treated wastewater can cause feminisation and de-masculinisation of male fish. A recent study of UK rivers suggests that a more complex chemical mixture is responsible for these changes than previously thought. The study even suggests a possible link between exposure to these chemicals and rising fertility problems in humans.

New risks identified for aquatic wildlife from plastic compounds (04/12/08)
New evidence suggests that the adverse effects on aquatic ecosystems of chemical compounds used in the manufacture of plastics are greater than previously thought. The study reviewed data on five substances with known endocrine-disrupting effects on wildlife in rivers and waterways.

The true toxicity of combined pollutants in freshwater (25/09/08)
Aquatic organisms are exposed to many stressors, including a variety of pollutants from human activities. New research suggests that commonly used models in toxicological studies can fail to adequately predict the range of effects of complex mixtures of chemicals in aquatic environments.

Chemicals may increase risk of breast cancer (15/05/08)
Breast cancer has increased dramatically in Europe over the past 20 years, with a doubling of the number of cases in some countries over this time period. A recent report presented to the European Parliament suggests that by reducing our exposure to certain chemicals, the number of women who develop breast cancer could also be reduced.

Nanoparticles affect pollutant toxicity (06/03/08)
Nanoscience and nanotechnology are relatively new, but already nanoparticles made from C60 (Buckminster fullerenes) are finding potential applications in consumer products ranging from car lubricants to cosmetics and medicines. New research suggests that nanoparticles, when released into water systems, may interact with other common pollutants in aquatic environments with important consequences for their toxicity to plant and animal life.

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