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Commentary

Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives

Aude Kienzler, Stephanie K. Bopp*, Sander van der Linden, Elisabet Berggren, Andrew Worth

European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Systems Toxicology Unit and EURL ECVAM, Via Enrico Fermi 2749, 21027, Ispra, VA, Italy

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ABSTRACT

This paper reviews regulatory requirements and recent case studies to illustrate how the risk assessment (RA) of chemical mixtures is conducted, considering both the effects on human health and on the environment. A broad range of chemicals, regulations and RA methodologies are covered, in order to identify mixtures of concern, gaps in the regulatory framework, data needs, and further work to be carried out. Also the current and potential future use of novel tools (Adverse Outcome Pathways, *in silico* tools, toxicokinetic modelling, etc.) in the RA of combined effects were reviewed.

The assumptions made in the RA, predictive model specifications and the choice of toxic reference values can greatly influence the assessment outcome, and should therefore be specifically justified. Novel tools could support mixture RA mainly by providing a better understanding of the underlying mechanisms of combined effects. Nevertheless, their use is currently limited because of a lack of guidance, data, and expertise. More guidance is needed to facilitate their application. As far as the authors are aware, no prospective RA concerning chemicals related to various regulatory sectors has been performed to date, even though numerous chemicals are registered under several regulatory frameworks.

1. Introduction

The number of chemicals and combinations thereof to which humans and the environment are continuously exposed is potentially enormous, ever changing in concentration and identity and to a large extent unknown. This makes it neither realistic nor useful to test every possible combination. However, current human risk assessment (HRA) and environmental risk assessment (ERA) of chemicals mainly focuses on exposure to individual chemicals, mostly considering only a single source.

In 2012, the European Commission published a communication on the combined effects of chemicals (EC, 2012), expressing concerns about the current limitations of assessing compounds individually and proposing a path forward to ensure that risks associated with chemical mixtures are properly understood and assessed. It states that EU laws set strict limits for the amounts of particular chemicals allowed in food, water, air and manufactured products, but that the potential risks of these chemicals in combination are rarely examined.

The hazard and/or risk assessment (RA) requirements for (components of) products on the European market are laid down in specific EU legislations primarily depending on the intended use of the product. These products, e.g. biocides, pesticides, food or feed additives, pharmaceuticals, can consist of an individual compound or of mixtures of several compounds. As the composition of these products is generally known, and the relevant compounds are relatively well assessed individually, the RA is performed prospectively, based on the properties of the individual constituents. Where appropriate, tests can also be carried out on the formulated products. However, when several formulated products are used in combination, i.e. for the application of plant protection products (PPPs) in the field or for the use of personal care products at home, the combined resulting risk is generally not assessed. Similarly, the prospective RA often considers only one route of exposure, e.g. linked to occupational exposure to pesticide, and does not consider potential additional sources of exposure such as the intake via food consumption.

In addition to the regulations that cover the intentional mixtures that are present in specific products, several others focus on

* Corresponding author.
E-mail address: Stephanie.BOPP@ec.europa.eu (S.K. Bopp).

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Abbreviations

ADI	acceptable daily intake
AOP	adverse outcome pathway
ARfD	acute reference dose
AS	active substance
BPR	biocidal product regulation
CA	concentration addition
DEB	dynamic energy budget modelling
EQS	environmental quality standard
ERA	environmental risk assessment
ILSI-HESI	Health and Environmental Sciences Institute
HI	hazard index
HRA	human risk assessment
IA	independent action
IPCS	International Program on Chemical Safety
MCR	maximum cumulative ratio
MCS	multi constituent substances
MoA	mode of action
MRLs	maximum residue levels
PBPK	physiologically based pharmacokinetic modelling
PPP	plant protection products
PPPR	plant protection product regulation
QSAR	quantitative structure activity relationship
TEFs	toxic equivalency factor
TTC	threshold of toxicological concern
UVCBs	substances of unknown or variable composition, complex reaction products or biological materials
WFD	water framework directive

the exposure to unintentional mixtures. These can be mixtures that are unintentionally formed during the production process or mixtures that are found in the environmental matrix after being emitted (also defined as coincidental mixture, Table 1). Examples of regulations that address these types of mixtures are the Water Framework Directive (WFD), Marine Water Strategy, or Air and Soil related regulation.

Because of their varying composition in space and time, due to both the environmental fate of chemicals and the constant entry of new pollutants, exposure to coincidental mixtures in the environment is never assessed prospectively, and retrospective RAs are scarce, even if this is the most common situation. In special cases, if more information on use would be available, some unintentional mixtures might be assessed prospectively, e.g. PPPs tank mixtures (mixtures of individually assessed formulation that are mixed by the user), or mixtures of chemicals found in the environment after being emitted at the same place and the same time or sequentially (e.g. production plants of specific substances). These cases are currently not covered under the legislation. However several different guidance documents on how to deal with mixtures have been published recently, each focusing on a specific group of compounds or type of assessment. Examples are the guidance on aquatic RA under REACH (Bunke et al., 2013), the assessment of mixture effects of biocides (ECHA, 2014), and for pesticides, how to assess exposure scenarios for RA both using MRL or actual exposures based on monitoring data (EFSA, 2012).

Although methodologies for assessing the combination effects of chemicals are being developed and applied by scientists and regulators in specific circumstances, so far there is no systematic, consistent, comprehensive and integrated approach across different pieces of legislation. As a step forward, a widely accepted framework for the RA of combined exposure to multiple chemicals was developed in a WHO/IPCS workshop (Meek et al., 2011). This framework describes a general approach for RA of combined exposure to multiple chemicals that could be adapted to the needs of specific users. However, its use is often hampered by large data gaps on exposure as well as hazard information.

This review presents an overview of the current regulatory requirements for chemical mixture assessment, with emphasis on the extent to which they address the assessment of intentional and unintentional mixtures. Recent case studies, specifically focusing on mixture RA beyond the current regulatory requirements are summarized to illustrate what lessons can be learned in terms of methodology being used and existing regulatory and data gaps. The lessons learned from the case studies are supplemented by the results from an expert survey. This survey, which was carried out to explore the current use of different approaches for assessing human and environmental health risks from combined effects and the added value of several novel tools that could provide some of the missing information currently hampering the toxicological assessment of mixtures.

2. Mixture terminology and assessment concepts

2.1. Mixture assessment terminology

While the term mixture might seem a clear term at first sight, many similar - but different terms - are used in parallel, to indicate

Table 1
Types of mixtures, characterisation and related regulation.

Type of mixture	Definition	Characterisation	Assessment	Example of related regulation
Intentional	Formulated products marketed as such	Usually of known or well-known composition	Usually prospective based on the properties of the constituents supplemented, where appropriate, by tests carried out on the entire products	Plant Protection Products, Biocides, Pharmaceuticals, Food additives …
Unintentional	Usually from one source; generated by discharge during production, transport, use or disposal of goods	The composition can either be known (effluent) or unknown	If composition unknown, whole-mixture approach.	Water Framework Directive or waste-related regulation.
Coincidental	Originating from various sources	Composition unknown, varying in space and time	Usually not required	a) water/soil/air-related regulation; b) exposure of workers in the workplace, for which a risk assessment is required for all hazardous chemicals, including in combination; c) exposure of humans to multiple chemicals from food and drinking water.