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Combined ecotoxicological risk assessment in the frame of European authorization of pesticides

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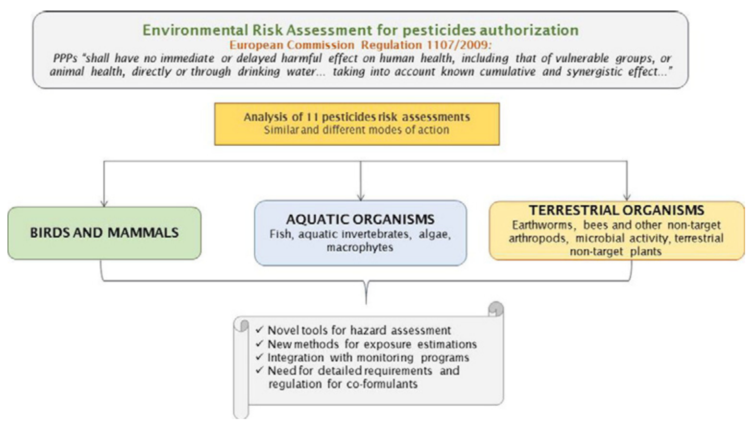
HIGHLIGHTS

- Current EU Reg 1107/2009 explicitly requires pesticides cumulative risk assessment
- We reviewed the standard approaches to assess the effects on non-target organisms
- The Concentration Addition concept is usually adopted as first conservative option
- Inhomogeneous approaches are adopted for different non-target organisms categories
- Clear indications for the assessment of co-formulants are needed

GRAPHICAL ABSTRACT



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ABSTRACT

Organisms are frequently exposed to mixtures of chemical contaminants in the environment, causing a potential "cocktail effect", or combined effect. The joint action of different molecules with similar or different modes of action could result in a potentially unlimited number of additives, synergistic or antagonistic combinations. Since the large number of contaminants makes it impossible to perform ecotoxicity tests for each potential mixture, a robust approach for prospective environmental risk assessment of chemical mixtures is needed.
A number of recent publications by the European Commission and the authorities in charge prove the increasing interest that is spreading in the European community towards the topic of the assessment of chemical mixtures. The current EU regulation for Plant Protection Products authorization (Reg. 1107/2009 EC) explicitly requires the evaluation of the potential combined effects of active substances.
We reviewed current methods and limitations of mixture assessment of pesticides (7 fungicides and 4 herbicides) through the analysis of the approaches adopted to investigate possible risks for different non-target organisms.
The Concentration Addition (CA) approach was the most used approach to predict multiple toxicity to non-target organisms. The guidance for birds and mammals first introduced standard procedures to assess the multiple toxicity based on on CA concept. The recent aquatic EFSA guidance introduced some requirements to evaluate potential mixture toxicity, while the current guidance requirements for terrestrial organisms still lack clear indications on how to conduct the assessment. Moreover, new indications come from the draft guidance for the assessment

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of terrestrial plants and in-soil organisms. However, the approval and implementation of these new guidelines are still at a developmental stage.
Some final considerations are drawn on the future possibilities to improve risk assessment procedures so as to identify harmful effects of pesticides mixtures on non-target organisms.

1. Introduction

Humans and ecosystems are exposed to a very complex mixture of chemicals. In the great majority of risk assessments, only a single chemical is considered (SCHER/SCENIHR/SCCS, 2012) although there is a consensus in the field of toxicology that the routine chemical-by-chemical approach to risk assessment might be too simplistic (Bopp et al., 2016).

Since the assessment of the toxicity of a given chemical is normally done individually, the possible adverse effect of mixtures may remain unknown. The joint action of different molecules with similar or different modes of action could result in a potentially unlimited number of additive, synergistic or antagonistic combinations (Kortenkamp et al., 2009; Beyer et al., 2014). A robust approach to assess the ecotoxicity of chemical mixtures is therefore needed to evaluate the environmental risk in many sectors, since the large number of potential chemical contaminants makes it impossible to perform toxicity tests for each potential mixture (Lydy et al., 2004; Kienzler et al., 2014).

Several pieces of European Union (EU) legislation are in force to regulate single and multiple compounds. The main sectors where more experience has already been gained in assessing mixtures can be found in the area of Plant Protection Products (PPPs) (EC, 2009) and chemicals falling under the Regulation for the Registration, Evaluation, Authorisation and Restriction of Chemicals -REACH (EC, 2006) (Kienzler et al., 2014).

The interest towards Combined Risk Assessment (CRA) has increased over the last years, as is proved by the publications of several reports, scientific opinions and guidances by European and American institutions (USEPA, 2006a; USEPA, 2016; OECD, 2011; EC, 2012; EFSA, 2014a). A recent study (Busch et al., 2016) showed that chemicals occurrence in European freshwaters seems to be highly variable in composition and relative abundances and that the largest group of compounds found in European rivers consist of pesticides. Bopp et al., 2016, concluded that chemicals need to be further addressed not only in single substance risk assessment and that mixtures should be considered also across chemical classes and legislative sectors.

Although the general concepts of mixture risk assessment are comparable for humans and the environment, which both make use of the tiered approach, there are substantial differences between human and Ecological Risk Assessment (ERA). In the first place, different types of environmental exposure assessments are needed when dealing with risk assessment of different non-target organisms, making it more complex to design a tiered framework for combined exposure assessments. Secondly, while human toxicologists have traditionally refuted the possibility of mixture effects in the low dose range, ecotoxicologists have acknowledged the possibility of combination effects when each chemical is present at its Predicted No-Effect Concentration (Péry et al., 2013). In the case of pesticides, the assessment for terrestrial mammals and birds has some similarities to the human health assessment, whereas the framework to assess the risk for aquatic and terrestrial organisms is completely different (EFSA, 2014b).

Predictive approaches for mixture toxicity are valuable tools in the European product risk assessment and they are considered to be essential for ethical and economic reasons in order to reduce the use of laboratory animals in the light of the Directive on the protection of animals used for scientific purposes (EC, 2010).

The present work aims to provide the regulatory state-of–art in dealing with pesticides made of two active substances in the frame of the ERA presented for formulated products, with special attention to risk assessment procedures set for the EU. In particular, EC, 2009 requires in article 29 that "*interaction between the active substance, safeners, synergists and co-formulants shall be taken into account*" in the evaluation and authorisation. This article explicitly refers to marketed PPP, which are usually technical mixtures containing one or more active substances, plus several co-formulants. Mixtures with more than two active substances are out of the scope of this work.

Therefore, in the first part of the review, we present the two main predictive methods used to estimate mixture toxicity in the regulatory context. In the second part, we analyse 11 risk dossiers presented for the registration of pesticide formulations in the EU in order to evaluate the level of compliance with the in force European legislations of the registration reports of PPPs. The evaluation was carried out for three different non-target organisms groups (birds and mammals, aquatic and terrestrial organisms). In the following discussion, we highlight the limitations found in the revised reports. Finally, we advance some proposal for conducing CRA in light of the recent literature in this field.

1.1. Predictive methods used for the assessment of mixture toxicity

The choice of model in a specific situation depends on the knowledge about the chemical's mode of action. Two mathematical models, Concentration Addition (CA) and Independent Action (IA), have frequently been proposed for assessing mixtures as an alternative to testing all mixtures.

The underlying principle of the CA approach is that different compounds in a mixture have a similar mode of action and it is generally assumed that the compounds have the same target site in the organism (Loewe, 1927). CA implies that individual components of the mixture contribute to mixture toxicity in proportion to their individual concentration and potency (Kortenkamp et al., 2009).

The concept of CA is mathematically expressed by the equation:

$$\mathrm{ECx_{mix}} = \left(\sum\nolimits_{i=1}^{n} \frac{pi}{ECxi}\right)^{-1} \quad (1)$$

Where ECx_{mix} is the effect of the mixture of n compounds provoking x % effect, p_i is the relative fraction of the compound i in the mixture (note: Σ pi must be 1) and ECx_i is the concentration of component i provoking x % effect.

The concept of IA was first applied to biological data by Bliss (1939) and can be mathematically explained by the equation

$$\mathrm{EC_{mix}} = 1-\prod\nolimits_{i=1}^{n}(1-ECi) \quad (2)$$

Where $ECmix$ is the effect of the mixture of n compounds and Ec_i is the effect of substance i when applied singly.

Conceptually, the IA model is a statistical approach to predict the likelihood that one of multiple possible events will occur (Altenburger et al., 2003). IA has been suggested for the prediction of the joint effects of dissimilarly acting components acting in a strictly independent manner (SCHER/SCENIHR/SCCS, 2012).

For a realistic and accurate risk assessment, the consideration of potential interactions between chemicals in a mixture is important (Bopp et al., 2015). Antagonism occurs if the chemicals interact to produce an effect larger than predicted by the CA, while synergism occurs if chemicals interact to produce a smaller effect than predicted (Greco et al. 1995). Cases of interactions have been described in the literature, but they are