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Invited Perspective: How Relevant Are Mode-of-Action Considerations for the Assessment and Prediction of Mixture Effects?

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Distinguishing chemical mixtures in terms of the similarity vs. dissimilarity of their components' mechanisms or modes of action (MOAs) is perceived as a key issue in mixture risk assessment. However, the well-designed study by Van der Ven et al.[1] of a mixture of chemicals producing craniofacial malformations in zebrafish embryos calls the relevance of such distinctions into question.

The theoretical importance of separating similar and dissimilar actions derives from the assumptions that underpin dose addition and independent action, the two concepts typically applied to predicting effects of a mixture. Dose addition, a model developed by Loewe and Muischneck[2], views chemicals that produce the same effects (i.e., similar action) as dilutions of each other. Independent action was conceived by Bliss[3] to address irreversible events, such as mortality, where probabilistic principles apply. With simultaneous exposures to multiple chemicals, these principles are only valid when the constituent chemicals interact with different molecular targets by different mechanisms (i.e., dissimilar action).

These distinctions may seem overly theoretical, but they have important implications when it comes to deciding which chemicals to include in a mixture risk assessment. Under the U.S. Food Quality Protection Act of 1996[4], cumulative risk assessment considers simultaneous exposures to multiple pesticides that act through a common mechanism of toxicity. Possible mixture risks from pesticides with dissimilar mechanisms are not considered. As a result, pesticide mixture risk assessment in the United States uses tightly defined "common mechanism groups." Because of the strict criteria for similarity, these groupings are rather small (4–5 substances).

Separating similar from dissimilar action is not straightforward. First, the mechanistic information needed for such decisions is often not available. It is also unclear how the terms "mode of action" or "mechanism" should be applied to build common assessment groups. For example, as shown in rodents, phthalates[5], as well as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[6] are capable of reducing sperm numbers after exposure during gestation (similar action?), but through different pathways and mechanisms (dissimilar action?). Advocates of grouping compounds by common mechanism based on strict similarity of action would deny the possibility of mixture effects and therefore regard the grouping of phthalates and TCDD in common assessment groups as inappropriate. Yet there is clear evidence that mixture effects between phthalates and TCDD occur in rats.[5] Is there a way to resolve such difficulties?

Van der Ven et al.[1] assessed the accuracy of dose addition with a mixture of eight chemicals that produce malformations by diverse mechanisms. By carefully distinguishing major MOAs and placing them in pathway networks, the authors identified informative gene markers and tested their expression. In this way, they were able to ascertain that the principles of similar action did not apply to their chosen combination of algicides, antioxidants, plant growth regulators, flavoring agents, and fungicides. Yet, they demonstrated convincingly that the observed combined effects were predicted well by dose addition and even showed that malformations occurred when all components were combined at low doses, around no observed adverse effect levels.

Van der Ven et al.[1] concluded that dose addition does not depend on the MOA of the components in the mixture. Work with chemical mixtures in fish led van Leeuwen[7] to a comparable insight in 1995. He wrote that "chemicals with different modes of. . .action can often almost behave according to concentration–addition." Dose addition has also performed well in approximating the effects of chemicals that disrupt male sexual development by different mechanisms[8], whereas assuming independent action has often led to underestimations of effects (reviewed by Kortenkamp[9]). Thus, the applicability domain of dose addition is clearly larger than is suggested by ideas about strict similarity of action and narrowly defined assessment groups.

Are dissimilarity of action and independent action therefore theoretically relevant but of limited practical applicability? The available evidence suggests this is the case. With strictly dissimilar mixtures of around 15 chemicals in luminescent bacteria and algae, Backhaus et al.[10] and Faust et al.[11] showed the superior performance of independent action. However, further reference cases for independent action are difficult to find. The only example of independent action applying to multicomponent mixtures in higher organisms is in fish exposed to different hormonally active chemicals.[12]

The lack of further reference cases for independent action suggests that the theoretical principles of strict dissimilarity are in reality often confounded by the convergence of multiple effector chains on common downstream pathways that are better described by dose addition. There are biological limits to the number of strictly dissimilar MOAs, and with rising numbers of mixture components, combined effects approaching similar action are increasingly likely.

The work by Van der Ven et al.[1] supports the idea of the default application of dose addition, even to mixtures viewed as acting dissimilarly, as advocated by the European Food Safety Authority.[13] Regulators need broader criteria for building common assessment groups based on common adverse outcomes, regardless of perceived mechanisms. Application of such principles will help to develop badly needed international harmonized approaches to mixture risk assessment. Debates about similar or dissimilar action, isolated from adverse outcome pathway networks, will likely lead to a dead end.

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