The assessment of joint endocrine effects of multi-component mixtures of food contaminants and additives

Area of research interest: Chemical hazards in food and feed
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Background

Current approaches to risk assessment of mixtures of compounds have limitations and do not address the complexity of mixtures of chemicals that may occur in the diet, many of which are present at very low levels.

A considerable body of work exists on mixtures of chemicals with similar modes of action. This has shown that knowledge of the effects of individual chemicals can be used to predict mixture effects accurately using the concept of dose addition. However, there are few data for mixtures of chemicals with diverse modes of action, especially when individual components are present at low levels, that is around the 'no observed effect levels' (NOELs). This project will address this gap, focusing on endocrine disrupting effects (using *in vitro* tests for estrogenicity and anti-androgenicity) and will aim to develop methods of predicting the effects of mixtures of chemicals with known properties.

Specifically, the project aims to address the following points:

- Are multi-component mixtures of food constituents able to act together to produce significant combination effects when present at levels that may be achieved through dietary intake?
- Is mechanistic information useful to anticipate mixture effects of chemicals found in food and to group the chemicals into those acting by similar and dissimilar action?

It is also anticipated that outcomes from this project will provide guidance on approaches to assessing potential health effects from mixtures of food contaminants and additives.

Research Approach

Chemicals to be studied in this project will be selected from groups of known food chemicals. Individually, they will have documented occurrence in food items and available data on their levels in humans.

Initially, this project will investigate dose-response relationships for relevant individual chemicals. These chemicals will then be tested in a series of mixtures.

Three *in vitro* assays will be used in this project: The ER-CALUX assay, which detects chemicals that act on the estrogen receptor; the MCF-7 cell proliferation assay, which detects chemicals that stimulate cell division; and the PALM assay, which detects chemicals that act on the androgen receptor.

Mixtures will be made up of the chemicals which are active in each respective assay and predictions made as to what joint response will be achieved. Subsequently, the mixtures will be tested experimentally and the observed responses compared to the predictions.

The mixtures will also be tested in combination with chemicals that do not induce positive effects in the assays to investigate whether they modify the joint effect of the mixture.

Finally, mixtures (with and without modifiers) constructed to reflect levels of chemicals predicted to be found in human tissue will be tested. This will help to determine whether effects of combinations of chemicals are likely to be significant when present at the levels humans are exposed to from foodstuffs.

Given the complexity of the proposed mixture studies this work will be carried out *in vitro* using a 'fixed mixture ratio design' approach (Altenburger et al, Environ. Toxicol. Chem. 2000, 19, 2341-2347).

Results

Fifty different mixtures containing up to 31 individual chemicals were tested in vitro for endocrine disrupting effects. When mixtures of ten or more active chemicals (i.e. those chemicals that exhibited activity in the in vitro test system when tested alone) were evaluated, the combined effect could usually be predicted using the concept of Concentration Addition. The mixtures were also assessed using an alternative model known as, Independent Action, (whereby the effect, or response, for the mixture is obtained by applying stochastic principles). Using the concept of Independent Action resulted in an underestimation of the observed effects.

When mixtures were tested in combination with chemicals that do not induce positive effects in the in vitro assays, most did not affect the response to the mixtures of active compounds they were tested against, however three chemicals, PCB126, benzo(a)pyrene and cadmium chloride did induce negative estrogenic effects.

Concentration Addition was reasonably predictive of the combined effects of the active chemicals when testing mixtures constructed to reflect levels of chemicals predicted to be found in human tissue. The project team noted that a small number of the individual chemicals appeared to account for the majority of the combined effects observed.

The results of this study support the use of the Concentration Addition approach to predict the effects of mixtures, when the chemicals in the mixture have similar structures and/or act via similar mechanisms. However, the authors note that in vitro observations cannot necessarily be readily extrapolated to an in vivo situation.

The outcomes from this project provide support for the methodology we currently use when conducting risk assessments of mixtures of chemicals acting on the same receptor.

Published Papers

1. Evans, R.M., Rahte, S. & Kortenkamp, A. (2010) Inability to confirm estrogenicity of the heterocyclic amine PhIP in two in vitro assays. *Toxicol. in vitro*, 24(6),1757-1763.

Research report

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