chapter thirteen

Toxicology of mixtures in the light of food safety

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13.1 Introduction

This chapter deals with health effects resulting from the combined actions of food components. These actions include synergism leading to increases in toxicity (of nonnutritive components in particular), as well as interactions between nonnutritive components and nutrients resulting in deficiencies.

In the medical field, there are many examples of both increases and decreases in toxicity after taking combinations of drugs. An interesting example is the case of the anticoagulant dicoumarol and certain other drugs. To maintain the desired prolongation of the prothrombin time, the dose of dicoumarol is critical. Drugs such as aspirin, phenylbutazone, and sulfonamides that displace dicoumarol from its binding sites on plasma proteins, enhance the effect of the anticoagulant. The administration of such drugs during treatment with anticoagulants has resulted in serious cases of bleeding and even fatalities. The anticoagulant effect of dicoumarol may also be reduced if it is administered in combination with drugs that induce the enzyme, mediating the metabolic elimination of dicoumarol. The enzyme involved is mixed-function oxidase (MFO). Examples of MFO inducers are the barbiturates. Simultaneous treatment with barbiturates requires a higher dose of the anticoagulant. On the other hand, cessation of barbiturate administration has been reported to result in an unexpected enhancement of the anticoagulant effect.

As far as nutrition is concerned, combined action of food components has only occasionally been reported. The more frequent occurrence of observable effects of combined actions of drugs is largely due to the combined use of a relatively small number of drugs. Further, drugs are prescribed at effective dose levels, and usually there is medical surveillance. In food, on the contrary, large numbers of components are present at dose levels intended or expected to be far below the effect level. In the case of food additives, the acceptable daily intake is often a hundredth part of the no-observed-adverse-effect level (NOAEL) in experimental animals. Acute toxic effects of combinations of food components are rare, but if they were to occur, they would be easily noticed. The main cause for concern is the not easily recognizable unspecific and chronic effects, such as growth retardation in children and poor state of health in adults. In addition, deficiencies may develop, resulting from interactions between non-nutritive components and nutrients.

Prevention of adverse combined actions is also not easy. Toxicological risk evaluation of food components, such as additives and pesticide residues, is based on the results of toxicity tests with single substances. Each food chemical, however, is a component of a complex mixture of many substances with the chance of interactions and toxic combined actions. It is impossible to test all combinations for potentiation, addition or antagonism. In specific cases, however, prediction of the possible interactions can be made on the basis of theoretical considerations of the underlying mechanisms. For that purpose, classifying combined actions according to the mechanisms involved is helpful.

13.2 Classification of combined actions

A useful classification of combined biological actions of chemicals distinguishes the type of site of action as well as the interactive potency. First, a distinction is made between combinations of substances with common sites of main action and combinations of substances with different sites of action. A second distinction concerns the occurrence of combinations of interacting substances and combinations of noninteracting substances. This leads to the following definitions:

- *simple similar action:* common site(s) of main action, and no interaction between the components. The action can be additive;
- *independent action:* different sites of action, and no interaction;
- *complex similar action:* common site(s) of action, and interaction;
- *dependent action:* different sites of action, and interaction.

Interactions can result in a higher intensity (potentiation) or a lower intensity (antagonism) of the effects of one of the components. If it is impossible to discriminate between potentiation and addition, the term synergism is used.

The next section deals with examples of the four types of combined action. In the subsequent sections, attention will be paid to interactions of food components with nonfood factors and the consequences of combined action for food additive policy.

13.2.1 Examples of combined action

13.2.1.1 Simple similar action

Induction of effects by combining of substances with a common mode of action complies with the additivity rule as long as the receptors are not saturated.

Poisoning resulting from simple similar actions of food components has not yet been reported. However, it is beyond doubt that such combined actions occur. Biologically active secondary plant metabolites usually occur in food as mixtures of homologs and isomers. A number of these have similar actions. An example is the intoxication following the intake of green potatoes (see Part 2, Chapter 11). The toxic agents are solanine and chaconine. Their combined disturbing actions on biomembranes are assumed to be additive.

Another illustrative example is the combined toxic action of mixtures of polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls (particularly congeners with planar structures), occurring in, for example, mother's milk. The effects of these substances have been shown to be additive. Usually, the toxicity of such mixtures is expressed in terms of the concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), by adding the so-called TCDD toxicity equivalent concentrations of the individual components — concentration addition. Concentration addition is also used in the assessment of tolerances to "simple similar acting" pesticide residues in food products.

Difficulties in obtaining conclusive evidence of additivity (for the effects of food components) have not been encountered in studies on the toxicology of water pollutants. Therefore, it is important to look at the results of aquatic toxicological studies. The concentrations of the components to which the organisms under investigation are exposed can be maintained constant, and the effects of metabolite formation are minimal.

A study on the exposure of fish to a mixture of 50 different relatively stable lipophilic industrial chemicals may serve as an example. The toxicity of these substances is related to the general depressive action on the central nervous system. Their common sites of action are the nerve membranes. If each of the substances was added at 1/50 of its LC_{50}, the lethality of the mixture appeared to be approximately as high as that of one LC_{50}.

However, it should be noted that the induction of neurodepressive effects by combinations of lipophilic substances is only based on additivity for the general unspecific depressive action on the central nervous system. In addition, compounds may induce effects through interactions with specific receptors in the central nervous system. If food components have the same site of action, additivity is also possible for effects induced through interactions with specific receptors.

13.2.1.2 Independent action

Independency here means different sites of action and no interaction. However, different mechanisms can underlie the same effect and this may mean that the effects of some components of a mixture consisting of a large number of substances are similar, and are integrated into an overall effect (effect integration).

Acceptable daily intakes of food additives are estimated by dividing the NOAEL by safety or uncertainty factors. However, at the NOAEL a substance can still give rise to an effect, i.e., an unobserved effect. With regard to the validity of the NOAEL as basis for evaluation, it is important to know whether in the case of independency of action unobserved effects induced by the components of a mixture consisting of a large number of substances can be integrated into observable, or even adverse effects. The experience that lifelong daily intake of thousands of different food components with many independent actions is tolerated without any clear implication to health, provides insufficient evidence that this will always be the case. This problem was addressed in a recent study on the effects of mixtures of eight substances with different modes of action. The mixtures contained sodium metabisulfite, mirex (the insecticide decachlorooctahydro–methenocyclobutapentalene), loperamide (an antidiarrheal phenylpiperidine derivative), metaldehyde, di-*n*-octyltin dichloride, stannous chloride, lysinoalanine, and potassium nitrite. The mixtures were given to rats in the diet during 4 weeks. The dose levels were 0 (control), 0.1 and 0.33 of the NOAEL, the NOAEL and the marginal-observed-adverse-effect level (MOAEL) (see Section 18.3.5). After administration of 0.1 and 0.33 of the NOAEL, no effects were found that could result from the treatment. In the NOAEL group, on the contrary, effects were observed that might be attributed to effect integration: slightly decreased hemoglobin content and slightly increased kidney weight. The animals of the MOAEL group showed effects that were more serious as well as some that were less serious than the effects after administration of the individual components at their MOAELs.

The more serious effects included reduced food intake, impaired general state of health, growth inhibition, and liver damage. These findings provided no conclusive evidence for an increased risk from combined administration of chemicals at their NOAELs.

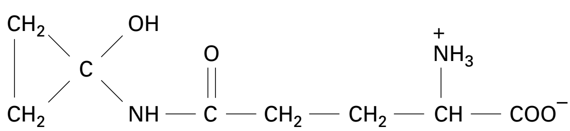
13.2.1.3 *Complex similar action*

Interaction can occur at the level of a common receptor, i.e., the site of main action. This can be competitive as well as noncompetitive by nature. Examples with respect to food are rare. Protection against goitrogens of the thiocarbamate type (e.g., goitrin) by iodine treatment through the diet can be regarded as a case of complex similar action. These goitrogens prevent the incorporation of iodine into tyrosine, the first step in thyroid hormone biosynthesis.

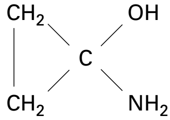
13.2.1.4 *Dependent action*

In the case of dependent action, interactions occur mainly at a pharmacokinetic / toxicokinetic level. This may lead to higher as well as lower intensities / toxicities.

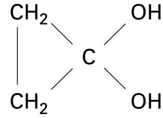
In the medicinal field, there are many examples of enhancement of the effect of one drug by another, resulting from inhibition of the elimination of the latter. In general, however, this type of interaction is expected to be only of minor importance at the relatively low intake levels of nonnutritive food components. An interesting exception is the poisoning following consumption of the (edible) Inky cap mushroom (*Coprinus atramentarius*) in combination with alcoholic beverages. Characteristic symptoms are flushing, hypotension, headache, nausea, and vomiting. The combined action is similar to that of the combination of disulfiram and alcohol, which ends in inhibition of acetaldehyde dehydrogenase. This enzyme is involved in the elimination of acetaldehyde, the primary metabolite of alcohol. The toxic effects are attributed to acetaldehyde accumulation, resulting from inhibition of the dehydrogenase. The mushroom contains the precursor of an inhibitor of acetaldehyde dehydrogenase: the amino acid coprine. In the body, coprine is hydrolyzed under the formation of aminocyclopropanol or cyclopropanone (hydrate). The actual inhibitor of the dehydrogenase is probably the cyclopropaniminium ion. It is believed it reacts with thiol groups of the enzyme.



Coprine



Aminocyclo-
propanol



Cyclopropanone
(hydrate)

The fungicide thiram (tetramethylthiuram disulfide), the methyl analog of disulfiram, is also known to cause alcohol intolerance. Thiram may be present in vegetables as a residue, originating from its agricultural application.

Dependent action can also lead to a decrease in effect of one of the components of a mixture. This is, for example, the case if one component decreases the bioavailability of another.

An illustrative example is the decrease in absorption of metals as a result of the presence of phytic acid (inositol hexakisphosphoric ester) in the diet. Phytic acid occurs in cereal products and legumes. It forms insoluble salts with di- and tervalent metal ions. In that way, the absorption of zinc and calcium is inhibited by phytic acid. This is important if soybean proteins are used instead of animal proteins. The phytic acid content of soybeans is high and their zinc content is low. Consequently, dietary use of soybeans may lead to zinc deficiency. Intake of a balanced diet, i.e., with sufficient calcium and vitamin D, will prevent calcium deficiency.

Decrease in absorption of nutrients has also been reported to result from interaction without similarity of site of action after combined intake of thiamine (vitamin B_1) and the additive sulfite. Thiamine can undergo degradation by sulfite in the intestinal tract. In the case of thiamine deficiency, it is important to pay attention to the presence of antithiamines in food.

Another example of dependent action in relation to effects on the absorption of metals is the effects of interactions between heavy metals on their absorption. In industrial areas, the dietary intake of heavy metals can be high as a consequence of pollution. On the other hand, the absorption of heavy metals is usually low. In the case of lead, the average absorption is estimated at 10% of the intake.

Interaction between nutritional factors and heavy metals, i.e., effects of nutritional factors on the toxicity of heavy metals, have been the object of many studies. Synergism as well as antagonism have been reported. In the majority of cases, no conclusive evidence was obtained for the underlying mechanisms.

The complexity of the interactions is illustrated with the following example. It has been shown that calcium affords protection against the toxic effects of lead and cadmium. Further, calcium deficiency appeared to promote the absorption of both metals. The interaction between calcium on the one hand, and lead and cadmium on the other, is believed to be a competition for binding sites on a carrier protein which is involved in the uptake of the metals from the mucosal wall.

13.3 Toxic interactions after combined intake of food and nonfood chemicals

Toxic interactions need not only take place between food components, but can also involve food components and nonfood chemicals. In this context, interactions between drugs and food components are of particular importance. A well-known example is the inhibition of the metabolic inactivation of the pressor substance tyramine contained in food by antidepressant drugs like iproniazid. Tyramine is present in foods such as cheese, wines and coffee. It is detoxicated by monoamine oxidase (MAO). Iproniazid is an inhibitor of MAO.

Further, in mice a high intake of iron appeared to enhance the porphyrogenic activity of halogenated hydrocarbons such as the environmental pollutants hexachlorobenzene and polychlorinated biphenyls. The mechanism of this interaction has not yet been fully elucidated. In addition, an increased incidence of hepatic tumors has been found. This is believed to result from oxidative DNA damage by hydroxyl radicals formed from hydrogen peroxide by uroporphyrin in the presence of iron.

13.4 How does combined action affect food safety assessment?

The safety factors applied for the calculation of ADIs of food additives and food contaminants should not only account for inter- and intraspecies differences, but also for combined action. This means that the level of addivity should be known. However, to establish this level is very complex, if not impossible.

Potential consequences of combined action for food safety assessment are illustrated by the following arithmetic example. Assume a mixture consists of 10 components. The intake of each component is 20% of the ADI of the component. If the level of additivity is 100%, the total intake is twice the ADI. The safety factor for the mixture is cut by one half.

13.5 Summary

A classification of combined actions of chemicals in mixtures is given, together with examples of each type of combined action. While there have been many cases of acute or subacute poisoning due to (unexpected) combined actions of drugs, examples of combined actions of non-nutritive food components are scarce. Not easily recognizable unspecific effects, such as decreased growth in children and poor state of health in adults, are main causes for concern. The possibility of adverse effects from combined actions is one of the justifications of applying large safety factors in the calculation of ADIs.