Part 2

Adverse effects of food and nutrition

chapter eight

Introduction to adverse effects of food and nutrition

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8.1 Introduction

Part 2 consists of eight chapters dealing with the induction of toxic effects by food components and food products, and the mechanisms underlying these adverse effects. Disorders related to food, like obesity, will not be dealt with, as the central theme of this book is food safety, treated from a toxicological point of view.

8.2 Two major problems in food safety assessment

Food toxicologists are confronted with two major problems:

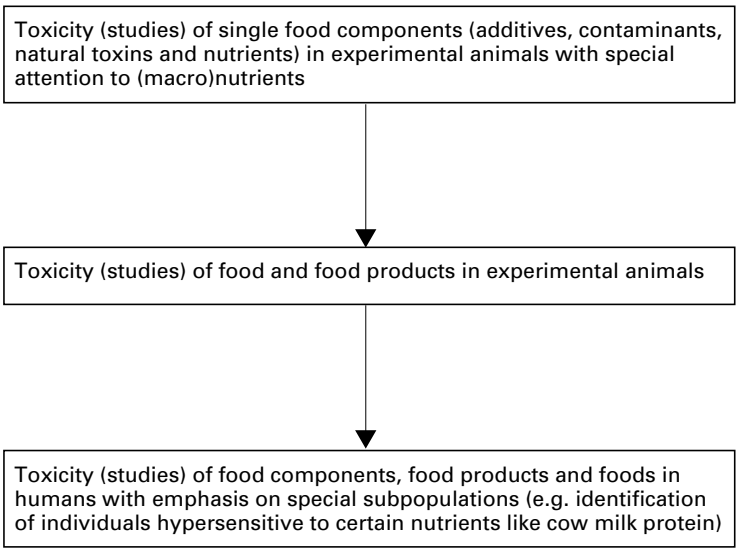
(a) food and food products are complex chemical mixtures of variable composition;
(b) the existing procedures for extrapolation of animal toxicity data to man are incompatible with Recommended Dietary Allowances (RDAs) (see Chapter 12, Section 12.1; and Chapter 17, Section 17.2.1) for many essential nutrients and also with the

normal use of many common foods and food products. Current guidelines for toxicity testing of chemicals are inappropriate for (macro) food compounds, food products, and foods. Specific approaches for the safety evaluation of foods and food chemicals are to be pursued.

Aspects of these two problems will be discussed in several chapters of Part 2, and will return in certain sections of Part 3 dealing with risk assessment.

8.3 *Toxicity (testing) of food chemicals and foods*

In order to outline the way in which adverse effects of foods and food chemicals are currently measured and the underlying mechanisms are examined, the toxicity (testing) of food chemicals, food products, and foods will be discussed along the following lines:



In essence, two lines are combined using this approach: one from single substances to complex mixtures, and the other from studies in experimental animals to studies in humans. The combination of both lines ends in the assessment of toxicological risks due to foods and food products (complex mixtures) in humans, including high-risk groups. The titles of the various chapters reflect this approach.

This chapter begins with a brief description of a number of general toxicological principles (Section 8.4) followed by a discussion of the above-mentioned major problems facing food toxicologists (Sections 8.5 and 8.6). Further, a short survey is given of the toxicology of the various categories of food components (Section 8.7). Next, the topics discussed in the other seven chapters of Part 2 are touched upon (Section 8.8). Finally, the characteristics and practical aspects of toxicity testing of food chemicals and (complex) food products are described (Section 8.9).

8.4 *Toxicity of (food) chemicals*

Toxicity (or hazard) is the potential of a chemical to induce an adverse effect in a living organism e.g., man. Each chemical, and thus also each food component, whether it is an

essential amino acid, a trace element, vitamin, contaminant or additive, has its own specific toxicity. Whether a food component is of natural origin or is man-made is irrelevant for its health hazard.

Generally, information on the toxicity (hazard) of food chemicals is obtained from studies in experimental animals, *in vitro* studies, studies in volunteers, or epidemiological studies. The main goals of these studies are to determine (a) the type of adverse effects, (b) dose–effect relationships including the no-observed-adverse-effect levels, and (c) the mechanisms underlying the adverse effects.

The induction of biological effects or toxic effects largely depends on the disposition of the substances concerned. The interaction of a substance with a living organism can be divided into a kinetic phase and a dynamic phase. The kinetic phase comprises absorption, distribution, metabolism, and excretion. It concerns the fate of a substance in the body: along which routes does the substance enter the body, and in what way is it distributed, metabolized, and excreted? For example, the pathway along which a substance is absorbed may largely affect the type and intensity of its effects. An inhaled substance reaches the blood circulation through the lungs, while a food component passes the liver, which is the main organ involved in biotransformation. This means that the body has a number of defense mechanisms at various levels of the kinetic phase, metabolism, and excretion. These mechanisms are aimed at detoxication of the substances that enter the body. However, the systems involved in detoxication may be saturated with certain chemicals. But also, they may convert the parent substance into a toxic reactive intermediate (bioactivation).

8.5 *Food, a complex mixture of variable composition*

Part 1 has shown that the chemical composition of food can be extremely complex and variable. Food products are estimated to consist of several hundred thousands of different chemicals. Usually, the toxicity of such a complex mixture does not simply depend on the toxicities of the individual components. Interactions may occur that lead to synergism or antagonism. Moreover, the rule of additivity may apply to the induction of effects (summation of effects). This means that combined actions may occur. It is impracticable to test every single substance for toxicity. Even if the toxicity data and Acceptable Daily Intakes (ADIs) (see Chapter 17, Sections 17.3.2 and 17.3.3) of all food components were available, there would still be the problem of many possible interactions and combined actions.

Addition of new chemicals to food should meet the requirements that guarantee safety. The only adequate way to deal with the safety problems of food chemicals already in use is the development and implementation of a priority-setting system based on the amount ingested, the number of consumers, potential toxic effects of food components, or combinations of groups of food components, and possible interactions between components.

8.6 *Problems in toxicity testing and extrapolation of animal data to man*

For most food additives and for many contaminants, the amount allowed for human consumption is at most 1% of the highest dose shown to cause no adverse effect in an appropriate animal study.

Obviously, it is impossible to give animals 100 times the amount of a macronutrient (e.g., single-cell protein, fat substitute, chemically modified starch) anticipated to be consumed by humans. Therefore, a safety factor of 100 cannot be applied to calculate an ADI. Instead, the safety data base of such food products should be expanded beyond the

traditional requirements or, in other words, the safety factor may be reduced on the basis of additional information. This information may be obtained from studies on absorption, distribution, metabolism, and excretion in humans and non-human primates, from long-term studies in appropriate primates, from studies in humans on the possible effects on vitamin and mineral state, and from very specific toxicity tests, e.g., for immunotoxicity and neurotoxicity. In brief, the safety evaluation of macronutrients requires more fundamental information on their effects on physiology and their toxicology. Such information forces toxicologists to abandon their strict safety procedures and to seek integration of their approach with that of nutritionists. This does not ask for more rules but rather requires a case-by-case approach on the basis of carefully discussed, well-reasoned safety evaluation procedures for (macro)nutrients, food products, and foods.

8.7 Categories of food components

Generally, food components are classified into four groups: nutrients, non-nutritive naturally occurring components, including antinutritives and natural toxins, and man-made contaminants and additives. For many food chemicals which are necessary for life, the margin between RDA and minimum toxic dose is often much smaller than a factor 100 or even 2, for example for fat. This is quite understandable, since nutrients play an essential role in the maintenance of homeostases. A slight overintake of nutrients may lead to exceeding the limits within which the homeostases should be kept.

As will be discussed in Section 8.8, there are large gaps in our knowledge of the toxic potential of the majority of natural food components and the consequences of their intake for human health. It is clear that this group of food components should have a high priority with regard to further toxicological research.

The requirements for testing the toxicity of man-made contaminants and their toxicological evaluation are similar to those for additives. ADIs are assessed and standards are set.

Before a chemical is admitted as a food additive, extensive toxicological research is required. The results are often the basis for assessing the recommended limit values, such as ADIs, usually applying a safety factor of 100. The levels of additives in food are usually much lower than the ADIs. Therefore, food additives are relatively safe.

Chapters 9 through 12 deal with the toxicology of the various groups of food components in general and the mechanisms underlying their toxic effects in particular. Consumers are increasingly confronted with food products and food components produced with modern technological methods.

Chapter 13 concerns the toxicology of mixtures of the chemical substances that make up our food. It looks at the different types of possible interactions between substances (antagonistic or synergistic) and of independent combined actions of substances (presence or absence of additivity or no additivity).

Not enough is known yet about the prevalence of food allergies and intolerances. Estimates vary widely and are unreliable. It is not easy to diagnose a food allergy and to identify the food component provoking the allergic reaction. The same holds for food intolerance. In Chapter 14, the different types of food allergy and food intolerance and the associated problems are discussed.

The final chapter of Part 2 is an introduction to the use of epidemiological methods in general, and to the application of epidemiology in studying associations between diet and adverse effects of food (components) in particular. The basic principles of epidemiology will be covered at an introductory level. Topics include types of study design, dietary exposure, disease outcome, causality, validity, bias, interpretation and integration of epidemiological data with animal data. Special attention will be paid to the possibilities and

limitations of diet assessment methods and the use of biomarkers in studying diet–disease associations.

8.8 Rank order of hazards from food components

In general, in developed countries food safety is adequate. However, it should be noted that the information on the (chronic) toxicity of natural food components is insufficient. Further, a number of important health problems such as cardiovascular disorders, diabetes, osteoporosis, obesity, allergy, and cancer are believed to be related to nutrition. Nutritional interventions could drastically reduce the incidence of these diseases.

There is a consensus of opinion among experts (nutritionists and food toxicologists) that food hazards should decrease in the following order of importance:

8.8.1 Wrong dietary habits

These are believed to be main causes for the nutrition-related disorders mentioned above. A more balanced diet means changes in dietary habits: energy according to need, and less fat, cholesterol, salt, sugar, and alcohol, and more dietary fiber. Nutritionists and toxicologists are well aware of the fact that for nutrients the margin between physiological need and safe dose is often very small. Large safety factors cannot be applied. More basic information on the physiology and toxicology of macro- and micronutrients is required. Such information may be used for recommendations aimed at changing dietary habits (see also Part 1B, and Part 3, Chapter 22).

8.8.2 Microbial contamination

Food can serve as a vehicle or growth medium for pathogenic microorganisms. The incidence of food-borne diseases due to microorganisms is estimated at some hundred thousands of cases per year in the Netherlands and a staggering 20 million or more cases per year in the US. Worldwide, the number of cases of food-borne diseases is astronomical.

A distinction can be made between food-borne infections and microbial food intoxications. The former are caused by the pathogenic microorganisms themselves, the latter by toxins produced by microorganisms in the food. Table 8.1 lists the main microorganisms involved in either food infection or food intoxication. In view of the central theme of this book, food infections will not be dealt with any further. Food intoxications will be discussed in detail in Chapter 11 in which naturally occurring toxins are discussed.

8.8.3 Natural toxins

The number of naturally occurring non-nutritive chemicals in foods is unknown, but is probably larger than 500,000. Only a small portion has been identified chemically and only a few have been submitted to adequate toxicological examination. In contrast, synthetic pesticides, food additives, and industrial contaminants have been subjected to extensive toxicological screening. This has led the public to believe that man-made chemicals are potentially more hazardous to humans than natural chemicals.

Based on their origin, natural toxins associated with foods can be divided into four groups, as listed in Table 8.2. The toxicology of the different classes of natural toxins will be discussed in Chapter 11, using important representatives as examples.

8.8.4 Man-made contaminants

Man-made contaminants are substances unintentionally present in foodstuffs or their raw materials. They may occur as the result of production, processing, preparation, packaging,

Table 8.1 Microorganisms causing food-infections or food intoxications

Microorganism	Pathogenicity
Salmonella	infection
Shigella	infection
Escherichia coli	infection
Yersinia enterocolitica	infection
Campylobacter jejuni	infection
Listeria monocytogenes	infection
Vibrio parahaemolyticus	infection
Aeromonas hydrophila	infection
Staphylococcus aureus	enterotoxin
Clostridium botulinum	botulinum toxins
Clostridium perfringens	enterotoxin
Bacillus cereus	enterotoxin, emetic toxin
Aspergillus flavus	aflatoxins
Penicillium citrinum	citrinin
Aspergillus ochraceus	ochratoxin
Aspergillus versicolor	sterigmatocystin
Penicillium claviforme	patulin
Fusarium graminearum	zearalenone

Table 8.2 Classification of natural toxins according to their origin

Toxins	Organism	Toxic product (examples)
Bacterial toxins	Bacteria	Botulinum toxin
Mycotoxins	Fungi	Aflatoxin
Fycotoxins	Algae	Diarrhetic shellfish poison
Fytotoxins	Plants	Solanin

transport or storage of foods or their raw materials, or as a result of environmental contamination. By definition, contaminants are unintentional, but some are present as a result of intentional applications, e.g., residues of pesticides, additives to feedstuffs, or veterinary drugs.

To protect people against hazards from contaminants, governmental agencies in many countries have developed and implemented legislation in which approval and establishing of ADIs is regulated. Once a pesticide is approved, conditions leading to its safe use are imposed. For example, a safety period between the last treatment of a crop and its harvest is specified. Also, the maximum residue level must be as low as consistent with Good Agricultural Practice and always low enough to avoid exceeding the ADI.

8.8.5 Additives

Food additives are chemicals that are intentionally added to foods or their raw materials to preserve or improve the quality of the product. The increasing demand for food by an ever-increasing world population, as well as by changes in lifestyles in developed societies, has led to the use of additives to preserve foods or to process raw foods into nutritionally adequate ready-to-eat foods. Examples of types of additives are preservatives, antioxidants, colorings and color-preserving substances, flavorings, thickening and emulsifying agents, stabilizers, bleaching agents, moisture repellants, and defoaming agents.

In the Netherlands, for example, the admittance of additives is regulated in the Commodities Act. Regulation under this act has taken the form of a so-called positive list. This list contains all chemicals that have been approved as food additives. It also incorporates detailed specifications concerning identity and purity of the substance, the purpose of using it, and its maximum permitted concentration in foodstuffs or categories of foodstuffs designated by name.

Nearly all major additives have been subjected to a thorough toxicological evaluation on the basis of which an ADI is established. Because of this rigorous toxicological evaluation and the application of large safety factors (generally 100 or more) in calculating ADIs for food additives, this category of food components ranks at the bottom of the list of foodborne hazards, far behind nutrients, microbial toxins, food infections, natural toxins, pesticides, and environmental contaminants.

8.9 Identification of health hazards due to food chemicals and foods

The results of proper toxicological and epidemiological studies are the only scientific basis for assessing the level of exposure to a specific (food) chemical that is low enough to avoid unacceptable health risks. Toxicological data are generally obtained from various types of animal experiments, *in vitro* studies, and studies in humans. Studies in experimental animals have become the main source of toxicological data, although ideally the data should be obtained from humans because the ultimate goal is to assess the health risk from chemicals to humans. *In vitro* studies using organ and cell cultures of animal and human origin are increasingly used to study the mechanisms underlying the adverse effects.

Epidemiological studies are one type of studies in humans. The possibilities of epidemiological studies to detect and quantify adverse effects of food components and foodstuffs are discussed in Chapter 15.

The next sections introduce animal experiments, *in vitro* studies and studies in human volunteers, focusing on aspects of particular interest in testing food components, food products, and foodstuffs.

8.9.1 Animal experiments

Guidelines drawn up by the Joint FAO/WHO Expert Committee on Food Additives and the Scientific Committee for Food of the European Union provide a general outline for the toxicity testing of food components. The guidelines of the Organization for Economic Cooperation and Development (OECD) for toxicity testing of chemicals give more details of the design of studies, the way in which studies should be carried out, and the parameters to be used. However, it is evident that the end points specified in the OECD guidelines are not always appropriate for providing relevant toxicity data on a food component, particularly in the case of macroingredients such as bulk sweeteners, fat substitutes, modified starches, and novel food products. Some experienced food toxicologists believe that the traditional (guideline) approach to toxicity testing has not only impeded the development of toxicology as a science, but has priced itself out of the market as far as food chemicals are concerned. Others are somewhat more cautious with their criticism but feel that an effort should be made to relate toxicological findings more to the human situation. For instance, more attention should be paid to parameters characteristic of the cardiovascular system, the immune system, and the central nervous system.

The relevance of the major end points specified in the OECD guidelines for the hazard assessment of food chemicals is critically analyzed in the following paragraphs.

Acute toxicity. A potential food component rules itself out if it is acutely toxic to a considerable extent. Therefore, determination of LD_{50} (acute dose that is lethal to half of the exposed animals) should not be required as a major end point for a food component. Only range-finding studies (e.g., a one-week multiple dose feeding study in rats) would be necessary to ensure that the ingredient proposed for use in food has a low acute toxicity.

Subacute/subchronic repeated dose studies. These are important for examining the safety of food components. The substance is added to the feed or drinking water to imitate exposure to humans. Special attention should be paid to the composition of the diet, if the substance under investigation is a macronutrient, because in that case it usually has to be incorporated into the diet at levels as high as 20 to 60% at the expense of a comparable nutrient. Examples are alkaline treated proteins, protein concentrates from bacteria or yeasts, and chemically modified potato or maize starch. Aspects to be checked are, for example, vitamin and mineral content and their bioavailability to avoid nutrient deficiencies, which could strongly influence the results of the toxicity studies and, thus, lead to erroneous conclusions. The problems associated with toxicity testing of macronutrients, food products, and new foods have already been touched upon (Section 8.6) and will be discussed in more detail in Chapter 12.

Allergy. Testing for allergic sensitization is highly relevant. However, the commonly used animal models only detect substances that are active on the skin and/or after inhalation. Substances which are highly active in such tests are unsuitable as food components. Some food additives may cause intolerance reactions in certain individuals with symptoms similar to genuine allergic reactions. Therefore, there is a need for studying these end points in the testing of food ingredients. Currently, however, there is no animal model or *in vitro* test system available that unequivocally reveals intolerance. Testing in volunteers should be considered (see also Chapter 15).

Reproductive toxicity. Reproduction toxicity tests of food components are necessary. They should include male and female fertility and reproduction, multi-generation, and teratogenicity tests.

Long-term studies. For food components, long-term studies may not always be necessary. In the guidelines of the Scientific Committee for Food of the European Union, a decision point approach is recommended. For example, if the food ingredient is a simple ester that on hydrolysis yields products identical to substances of the normal metabolism, no testing beyond a subchronic study is needed. Similarly, chronic toxicity and carcinogenicity tests may be unnecessary for peptides, proteins, carbohydrates, and fats which by chemical analytical and metabolism studies can be shown to consist of well-known sequences of amino acids, mono- and disaccharides, and fatty acids. Nevertheless, if such substances are to be used in large amounts or will have a widespread use, long-term studies may be warranted.

Mutagenicity tests. The testing of mutagenicity as an end point is a subject of discussion concerning its relevance to food components. The present state may be summarized as follows. The significance of mutagenicity per se as an end point for food components is not clear and no regulatory agency seems willing to use positive results in mutagenicity tests alone as grounds for non-admittance of a food component. In addition, the faith in mutagenicity tests as pre-screens for carcinogenicity is declining. A positive response does not need to be proof of carcinogenicity. However, mutagenicity or genotoxicity is considered a very important end point in evaluating carcinogenicity data from animal tests. If a substance is found to be genotoxic, especially when tested *in vivo*, positive results of carcinogenicity tests make admittance as a food component very difficult if not impossible. On the other hand, quite a few non-genotoxic carcinogens are widely used as food additives, for example, butylated hydroxyanisole as an antioxidant, cyclamate, saccharin, and lactitol as artificial sweeteners, and propionic acid as a preservative. For these non-

genotoxic carcinogens ADIs have been calculated in a way similar to that used for other non-genotoxic, non-carcinogenic substances (see also Chapters 19 and 21).

8.9.2 In vitro *studies*

Isolated cells, tissues, and organs are increasingly used in toxicological research. Major advantages of these *in vitro* systems are:

- toxic effects can be studied independent of other compartments in the body;
- the systems are often very sensitive, and effects can be measured or calculated directly;
- *in vitro* systems are excellent tools for screening substances for organ-directed toxicity;
- molecular studies are easier than *in vivo* studies;
- phenomena and mechanisms can be studied in human cells which allows direct comparison of effects on human cells with effects on animal cells, which possibly makes extrapolation of toxicity data from animal to man more meaningful.

On the other hand, each model system has its limitations. The major disadvantage of *in vitro* systems is that there is no integration of cells, tissues, or organs as in an intact and functioning whole animal or human physiological system, and hence, no elimination by excretion whether or not in combination with biotransformation.

Of special interest to food toxicologists are *in vitro* systems using pieces of intestine and intestinal epithelial cells, for instance to examine the mechanism of absorption of substances, and interactions at the absorption level between xenobiotics, or between micronutrients and other food components. Such *in vitro* intestinal systems are successfully used to study the mode of action of so-called antinutritive factors such as lectins.

8.9.3 *Studies in volunteers*

From an ethical point of view, studies in volunteers can only be carried out if careful evaluation of all available data leads to the conclusion (preferably drawn by an independent ethical committee) that no unacceptable risk is being run. The end points should be short-term and indicative of reversible disturbances of physiology rather than of cell, tissue, or organ damage. Of particular relevance are absorption, distribution, metabolism, and excretion studies. Such studies in man would certainly contribute to more confident interspecies extrapolation (see also Chapter 18).

8.10 *Summary*

This first chapter of Part 2 dealing with adverse effects of food, introduces the characteristics of the toxicology and toxicity testing of food chemicals, food products, and foods. Food is a complex mixture of chemicals, the toxicity of which also depends on possible interactions between components. The current safety evaluation procedures of food additives and contaminants are incompatible with the RDAs of many essential nutrients and also with the normal use of many common foods. Hazards posed by food decrease in the following order: wrong dietary habits (too much food, too fat, too salt, too few fresh vegetables and fruits), food infection, natural toxins including microbial toxins, man-made contaminants, and finally additives (considered among the safest food components). Major aspects of the toxicology of the various categories of food chemicals (additives, contaminants, natural toxins, and nutrients) are briefly discussed. Finally, a brief description is

given of the methods for studying the toxicity of food chemicals and foods (studies in animals, *in vitro* studies and studies in volunteers) focusing on experiments in animals as the main source of toxicological data.

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