

Food Safety

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Abstract Food can never be entirely safe. Food safety is threatened by numerous pathogens that cause a variety of foodborne diseases, algal toxins that cause mostly acute disease, and fungal toxins that may be acutely toxic but may also have chronic sequelae, such as teratogenic, immunotoxic, nephrotoxic, and estrogenic effects. Perhaps more worrisome, the industrial activities of the last century and more have resulted in massive increases in our exposure to toxic metals such as lead, cadmium, mercury, and arsenic, which now are present in the entire food chain and exhibit various toxicities. Industrial processes also released chemicals that, although banned a long time ago, persist in the environment and contaminate our food. These include organochlorine compounds, such as 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (dichlorodiphenyl dichloroethene) (DDT), other pesticides, dioxins, and dioxin-like compounds. DDT and its breakdown product dichlorophenyl dichloroethylene affect the developing male and female reproductive organs. In addition, there is increasing evidence that they exhibit neurodevelopmental toxicities in human infants and children. They share this characteristic with the dioxins and dioxin-like compounds. Other food contaminants can arise from the treatment of animals with veterinary drugs or the spraying of food crops, which may leave residues. Among the pesticides applied to food crops,

the organophosphates have been the focus of much regulatory attention because there is growing evidence that they, too, affect the developing brain. Numerous chemical contaminants are formed during the processing and cooking of foods. Many of them are known or suspected carcinogens. Other food contaminants leach from the packaging or storage containers. Examples that have garnered increasing attention in recent years are phthalates, which have been shown to induce malformations in the male reproductive system in laboratory animals, and bisphenol A, which negatively affects the development of the central nervous system and the male reproductive organs. Genetically modified foods present new challenges to regulatory agencies around the world because consumer fears that the possible health risks of these foods have not been allayed. An emerging threat to food safety possibly comes from the increasing use of nanomaterials, which are already used in packaging materials, even though their toxicity remains largely unexplored. Numerous scientific groups have underscored the importance of addressing this issue and developing the necessary tools for doing so. Governmental agencies such as the US Food and Drug Administration and other agencies in the USA and their counterparts in other nations have the increasingly difficult task of monitoring the food supply for these chemicals and determining the human health risks associated with exposure to these substances. The approach taken until recently focused on one chemical at a time and one exposure route (oral, inhalational, dermal) at a time. It is increasingly recognized, however, that many of the numerous chemicals we are exposed to everyday are ubiquitous, resulting in exposure from food, water, air, dust, and soil. In addition, many of these chemicals act on the same target tissue by similar mechanisms. “Mixture toxicology” is a rapidly growing science that addresses the complex interactions

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between chemicals and investigates the effects of cumulative exposure to such “common mechanism groups” of chemicals. It is to be hoped that this results in a deeper understanding of the risks we face from multiple concurrent exposures and makes our food supply safer.

Keywords Infection · Food allergies · Food additives · Toxicology · Diarrhea · Food safety

Abbreviations

ACh	Acetyl choline
AChE	Acetyl cholinesterase
ADI	Acceptable daily intake
AGD	Anogenital distance
AR	Androgen receptor
ATSDR	Agency for Toxic Substances and Disease Registry
BBP	Benzyl butyl phthalate
BSE	Bovine spongiform encephalopathy
bw	Body weight
CDC	Centers for Disease Control and Prevention
CONTAM	Panel on Contaminants in the Food Chain (EU)
DAP	Dialkyl phosphate
DBP	Di(<i>n</i> -butyl) phthalate
DDT	1,1,1-Trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane (dichlorodiphenyl dichloroethene)
DEHP	Di-(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
EFSA	European Food Safety Authority
EU	European Union
DON	Deoxynivalenol (a mycotoxin)
FB1	Fumonisin B1
FSIS	Food Safety Inspection Service
GM	Genetically modified
IARC	International Agency for Research on Cancer
JECFA	Joint (WHO/FAO) Expert Committee for Food Additives and Contaminants
MRL	Maximum residue limit
NHANES	National Health and Nutrition Examination Survey
NOAEL	No observed adverse effect level
NRC	National Research Council
OP	Organophosphate
OTA	Ochratoxin A
OVA	Ovalbumin
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	Polychlorinated dibenzofuran
PMTDI	Provisional maximum tolerable daily intake
PTWI	Provisional tolerable weekly intake
Rfd	Reference dose (set by the USEPA)
SCF	Scientific Committee for Food

TCDD	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin
TDI	Tolerable daily intake
TWI	Tolerable weekly intake
vCJD	Variant Creutzfeldt-Jakob disease
USEPA	US Environmental Protection Agency
USFDA	US Food and Drug Administration
USDA	US Department of Agriculture
ZEA	Zearalenone (a mycotoxin)

Introduction

There can never be an absolute guarantee that our food is safe. It is simply impossible to test every single item for every imaginable toxin, contaminant, adulterant, or food-borne pathogen, not to mention that this would make our food prohibitively expensive. Every country has an agency that oversees food safety, defined as a “reasonable certainty of no harm,” and regulates what additives are allowed in food and what levels of unavoidable contaminants are acceptable. In the USA, the Food and Drug Agency (USFDA) is responsible for the safety of all foods except meat, poultry, and egg products, which are regulated by the Food Safety Inspection Service (FSIS) of the US Department of Agriculture (USDA). In addition, the Environmental Protection Agency (USEPA) regulates drinking water from public systems and pesticides. In order to determine acceptable levels of contaminants and toxins, the responsible agencies regularly monitor the food supply, and if their own research or scientific discoveries indicate a new hazard or higher risk than previously recognized from a known hazard, they conduct risk assessments. Risk is a function of exposure and hazard or toxicity. Therefore, risk assessment consists of hazard identification and characterization, exposure assessments, and subsequent risk characterization.

The assessment of exposure to food toxicants or contaminants requires data on the dietary intake of food items or groups that are known or are most likely to contain the chemical of interest. There are three basic approaches to determining dietary intake: (1) total diet study, (2) survey of individual households or individuals, using prospective food records or dietary recall, and (3) duplicate diet studies. Data on dietary intake then need to be combined with databases (e.g., from governmental monitoring programs) on the concentration of the contaminant of interest in foods. One of the challenges facing risk assessors is that food consumption databases were generally compiled by nutritionists, who were interested in assessing nutrient intake. Such databases do not necessarily contain detailed data on the food groups most likely to contain the additive or contaminant of interest. Therefore, these databases need to be adjusted, or new surveys need to be conducted.

The approaches currently in use for combining dietary intake and contaminant concentration data in order to arrive at a dietary exposure estimate are either deterministic or probabilistic. In the deterministic approach, dietary exposure is calculated by multiplying a fixed value for consumption of a food (usually the mean population value) with a fixed value for the chemical concentration in that food (usually the mean concentration or the maximum level permitted). Then, the intake from all foods is summed in order to arrive at a point estimate. This is a relatively simple, straightforward approach, yielding results that can be easily understood, but it has the major drawback of not providing insight into the range of possible exposures and the proportion of the population that remains at risk. Semiprobabilistic or simple distribution models use a fixed value for the concentration of the chemical of interest in food but employ simple distributions of food intake. In many cases, this still yields data only on the upper-bound estimate of exposure. The probabilistic approach takes into account the variability in food consumption and consumer body weight (bw) as well as the variability in contaminant concentrations by using data on the total distributions of consumption as well as contaminant/toxin content of foods. This is particularly important for many substances, such as veterinary drug residues in meat or pesticide residues on fruits and vegetables, which cannot be detected in a majority of samples. By representing each uncertain variable as a distribution function rather than a single value, this method can be used to determine the likelihood with which a certain exposure level will occur. Which model is most appropriate appears to critically depend on the distribution of the data on occurrence in food, e.g., undetectable levels in many foods and low levels in much of the remainder or low levels in many foods and very high, but variable, concentrations in a significant number of samples.

In some cases, measuring contaminant levels in food may not be feasible (e.g., because of laboratory contamination with the chemical to be measured, as in the case of phthalates). In other cases, dietary exposure is not the only or not even the major route of exposure, and measurements in other media (air, dust, soil, water) may be difficult. For the purposes of total exposure measurements, it is therefore desirable to conduct biomonitoring studies. The most commonly used biomarkers of exposure are the concentrations of the parent compound or its metabolites in urine or, more rarely, in plasma or serum. In order to extrapolate to the level of exposure that results in these biomarker concentrations, it is necessary to have information on the extent of absorption of the parent compound, its metabolism, and the relative abundance of the resulting metabolites and, for urinary measurements, their fractional excretion. Knowledge on the toxicity of the metabolites and their

relationship to possible human health risks is also highly desirable.

Once dietary exposure data are available, the next step is to determine whether this level of exposure constitutes a human health risk. For many food toxicants and contaminants, data on their toxicities are only available from studies in laboratory animals, most commonly rodents. For regulatory purposes, governments distinguish between genotoxic substances and nongenotoxic substances (including those that are carcinogens by nongenotoxic mechanisms). For nongenotoxic substances, the most sensitive toxicity end point is established from the available data, and the no observed adverse effect level (NOAEL), i.e., the dose at which no detrimental effects are seen in laboratory animals, is determined. It needs to be taken into account that there are interspecies differences and that humans may exhibit substantial differences in their sensitivity to certain insults due to differences in metabolic pathways and other factors. Therefore, in extrapolating from toxicities observed in laboratory animals to health risks in humans, uncertainty factors are applied, most commonly a factor of 10 for interspecies differences and a factor of up to 10 (depending on the extent and quality of the available human data) for human variability. According to the definition provided by the Joint (World Health Organization (WHO)/Food and Agriculture Organization (FAO)) Expert Committee for Food Additives and Contaminants (JECFA), the resulting tolerable daily intake (TDI) values “provide an estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable risk (standard human = 60 kg).” These intake values are referred to as acceptable daily intake (ADI) by the USFDA, whereas the USEPA uses the term reference dose (Rfd). Even though regulatory agencies (or the expert committees or panels advising them) generally rely on the same data, they frequently reach quite different conclusions concerning the level of human exposure they deem acceptable. These differences arise when the experts judge differently on the quality of the existing studies and on their relevance to humans.

The TDI or ADI is then used to determine the maximum allowable levels of a particular chemical in a specific food, depending on the extent to which this food contributes to the overall intake of that chemical. These are called maximum limits for some chemicals and maximum residue limits (MRLs) for substances such as pesticide and veterinary drug and hormone residues, the latter being referred to as tolerances rather than MRLs in the US.

For genotoxic carcinogens, there is no dose without adverse effects, and regulatory agencies apply the “acceptable risk concept.” The approach is to determine the additional cancer risk from lifetime exposure to low doses

of the chemical. What level of excess cancer risk is deemed acceptable is the result of social convention, but frequently this is a value of one additional case per million. In the case of genotoxic carcinogens, the aim is to keep the exposure level as low as technologically achievable.

Regulatory agencies around the world most commonly take a chemical-by-chemical approach to risk assessment. However, it is increasingly acknowledged that we are exposed to hundreds of chemicals on a regular basis and that many of these chemicals may share a common mode of action and affect the same target organ(s) or tissue(s). The new approaches required for the risk assessment of mixtures and the data that have emerged from the fairly new, but rapidly expanding, field of mixture toxicology will be discussed at the end of this paper.

Foodborne diseases

Bacterial, parasitic, and viral foodborne diseases

According to the Centers for Disease Control and Prevention (CDC), foodborne diseases arising from a known pathogen are responsible for an estimated 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths each year in the USA (www.cdc.gov/ncidod/dbmd/diseaseinfo/foodborneinfections_t.htm). The Foodborne Diseases Active Surveillance Network, a collaborative effort of the CDC, USDA, and USFDA along with selected state health departments, conducts active surveillance for seven bacteria and two parasites that cause foodborne diseases in a defined population of almost 46 million Americans (~15% of the US population). Their data indicate that the 2008 incidence (in cases per 100,000 people) of laboratory-confirmed infections was 12.68 for *Campylobacter*, 16.2 for *Salmonella*, 6.59 for *Shigella*, 2.25 for *Cryptosporidium*, 1.12 for *Escherichia coli* O157, and below one for the other pathogens included in the surveillance.

The major bacterial pathogens involved in foodborne diseases include over 2,300 types of *Salmonella*, over 30 types of *Shigella*, *Campylobacter jejuni*, and strain O157:H7 as well as several other strains of *E. coli*. In addition, *Listeria monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus*, *Vibrio*, and *Yersinia* as well as certain parasites like *Cryptosporidium*, *Cyclospora*, and *Giardia* can cause foodborne disease. See Table 1 for the transmission routes and the symptoms these pathogens cause. In addition to bacteria and parasites, foodborne viruses are implicated in an increasing number of disease outbreaks. They can be divided into viruses that cause gastroenteritis and enterically transmitted hepatitis viruses (e.g., hepatitis A virus). Examples of viruses that cause gastrointestinal symptoms are norovirus and rotavirus. The former is

thought to be the single most common cause of gastroenteritis in people of all age groups [1].

One of the objectives of the US Healthy People 2010 initiative is to reduce infections caused by foodborne pathogens and another is to reduce outbreaks of infections caused by key foodborne pathogens. It is well recognized that prevention constitutes the most important measure for reducing foodborne infections. For this purpose, The USFDA regularly conducts food field exams, inspections, and sample collection for further analysis. Note that monitoring of the food supply for viruses is currently impossible because of the lack of a simple validated method. Standard methods for assessing viral inactivation are also unavailable since viruses frequently cannot be propagated in cell cultures and no suitable animal models exist. In addition, the USFDA publishes guidance on how to prevent microbial contamination of foods and is involved in the training and education on hygiene measures of growers and food handlers in the entire food chain since it has been recognized that improper storage (e.g., at inappropriate holding temperatures), improper preparation (inadequate cooking), poor personal hygiene among food handlers, and contaminated equipment are major contributors to outbreaks of foodborne diseases. Since the vast majority of food is prepared at home, the education of consumers on improving the way they store and cook food is another task.

Another aspect of prevention is the targeting of educational messages to persons at higher than average risk of foodborne illness from particular pathogens, specifically those with primary immune defects or secondary immunodeficiency (for example, human immunodeficiency virus, chemotherapy, or organ transplantation) and pregnant women [2,3]. Pregnant women have an impaired ability to clear intracellular pathogens due to the immunosuppressive effects of pregnancy, which evolved to maintain the fetus. Depending on the particular immune phenotype, a consumer may be more susceptible to certain bacterial foodborne illnesses, as with *L. monocytogenes* in pregnancy, and to chronic colonization or symptomatic disease with intestinal parasites that are also frequently waterborne, such as *Cryptosporidium* or *Giardia lamblia* [4]. *L. monocytogenes* is an intracellular bacterium that can have devastating effects on a fetus and infect other immune-compromised individuals, including the elderly.

If an outbreak of foodborne disease occurs, the CDC is responsible for investigating the outbreak and identifying its cause. Once it identifies possible foods, the food product implicated determines which regulatory agency has primary jurisdiction. This agency is then notified and subsequently attempts to trace the outbreak back to a specific source and to remove this source from the market as quickly as possible.

Zoonoses

Zoonosis is defined as an infectious disease that can be transmitted from other animals to humans. The infectious agents can be parasites, fungi, bacteria, viruses, and, as most recently discovered, prions (or prions). It is thought that zoonoses usually result from direct contact with infected animals, but zoonotic pathogens include *E. coli* 0157:H7, *Campylobacter*, *Salmonella*, and *Caliciviridae* (subdivided into two genera, *Norovirus* and *Sapovirus*), which can also cause foodborne disease via fecal–oral contamination. The zoonosis that has garnered by far the most attention in recent years is the emergence of a new transmissible spongiform encephalopathy in humans, namely variant Creutzfeldt-Jakob disease (vCJD), which is thought to have been caused by the consumption of meat from cows infected with bovine spongiform encephalopathy (BSE) [5]. This disease is called a prion disease because there is strong evidence that it is caused by an incorrectly folded isoform of prion proteins that converts native prion proteins into replicates of the infectious prion isoform. This triggers a chain reaction that results in the conversion of more and more native prions into infectious prion isoform replicates. These then form aggregates that disrupt cell function and cause cell death. Native prion proteins are normal constituents of cell membranes in vertebrates and are found at particularly high concentrations in nervous tissue, which is the tissue affected by BSE and vCJD. The first case of BSE was diagnosed in the UK in 1986, although cases are thought to have occurred as early as in the 1970s, and several cases were retrospectively diagnosed in 1985. Since then, more than 184,500 cases have been reported in the UK alone, the peak incidence occurring in 1992 (close to 36,700 cases) [5]. Once it was recognized that meat and bone meal used in concentrated cattle feed was the most likely source of infectious material, the use of ruminant protein in ruminant feed was banned in 1988. This reduced the number of new infections but was not entirely effective in terminating the epidemic, most likely because cross-contamination of feed occurred in feed mills. Further reductions in new infections were only achieved through a ban on feeding of all mammalian protein to all farm animal species in 1996 [5].

There are several other transmissible spongiform encephalopathies in various animal species, such as scrapie in sheep and transmissible mink encephalopathy and chronic wasting disease in deer and elk, but none of these forms has ever been reported to be transmitted from animals to humans [5,6]. It was not until 1995 that the first case of vCJD was diagnosed in the UK, and a comparison of the biochemical characteristics of the prion isoform in vCJD patients with that of the BSE-associated isoform revealed them to be the same, suggesting that BSE was transmissible

from cows to humans [7]. This is thought to have occurred via the consumption of beef carrying the infective agent. Like classic CJD, this degenerative neurological disorder is incurable and invariably fatal, usually within a few months to a year. Altogether, there have been about 200 cases of vCJD worldwide, 162 of them in the UK [8]. Although it has been claimed that the human risk from BSE was recognized in the UK from the beginning, one wonders why tissues likely to contain the highest concentrations of the transmissible agent (brain, spinal cord, tonsil) were not banned for human food use until 1989. Spleen and thymus were added to the list in 1994. After the first cases of vCJD were recognized as probably linked to BSE, the UK government restricted the use of cattle for human food to animals under the age of 30 months since BSE is rare in animals that are less than 30 months old. The sale of beef on the bone was banned in 1997 [5]. Cases of BSE also occurred in other European countries, but with a much lower incidence [8]. In 1996, the European Union (EU) banned the import of cattle and beef from the UK. Once the epidemic was declining in the UK, the ban was eased in 1999 to allow export of boneless beef products from animals 6–30 months of age. The complete lifting of the ban did not come until 2006. All EU countries maintain an active surveillance system for the monitoring of BSE in cattle [5].

Many of the steps taken by the USFDA in order to protect US consumers from BSE mirror those taken by the UK government, although they generally came considerably later. In 1997, the agency banned the use of most mammalian proteins in ruminant feed and started routine testing of cows for BSE. After the emergence of the first case of BSE in the US, the USFDA elaborated an emergency response plan. In 2004, it prohibited specified risk materials (brain and spinal cord from cattle >30 months of age and other materials likely to contain high levels of infectious agents) from use in the human food supply, and in 2008 it published a new feed rule banning the use of specified risk materials from all animal feed. Until now, there have been only a few isolated cases of BSE in the US and Canada; no human cases of vCJD in association with the consumption of domestic US beef have been reported, and the risks of BSE in cattle and of vCJD in humans are considered very low [9,10].

Toxins—shellfish and fish poisons

A variety of toxins that are produced mainly by dinoflagellates but also some other algae are taken up by mussels, oysters, crabs, and other aquatic species and thereby enter the human food chain. The frequency as well as the geographic distribution of harmful algal blooms has been increasing worldwide, suggesting that more people

Table 1 Common pathogens in foodborne illness (*Bad Bug Book*, www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodbornePathogensNaturalToxins/BadBugBook/default.htm)

Pathogen	Occurrence	Main transmission routes	Symptoms	Possible complications or chronic sequelae
<i>Campylobacter jejuni</i>	Intestines of animals and birds, raw milk, untreated water, sewage sludge	Contaminated water, raw milk, raw or undercooked meat or poultry, or shellfish	Headache, fever, and muscle pain 2–5 days after ingestion; later severe abdominal pain, nausea, and diarrhea (sometimes bloody). May last 7–10 days	May lead to reactive arthritis and neurological disorders in 2–10% of patients
<i>Listeria monocytogenes</i>	Intestines of humans and animals, soil, silage	Raw milk, cheeses (particularly soft-ripened ones), ice cream, raw meats and poultry, raw and smoked fish	Listeriosis may begin with influenza-like symptoms; gastrointestinal symptoms (nausea, vomiting, and diarrhea) may occur—usually after at least 12 h—and may remain the only symptom. In serious cases, it can manifest as septicemia, meningitis, or meningoencephalitis, encephalitis, and intrauterine or cervical infections	Intrauterine and cervical infections in pregnant women may result in spontaneous abortion or stillbirth
<i>Salmonella</i>	Intestines and feces of animals; <i>Salmonella enteritidis</i> in eggs	Raw or undercooked food of animal origin, mainly meat, poultry, eggs, and milk	Fever, headache, nausea, vomiting, abdominal pain, and diarrhea appearing 9–72 h after infection lasting 1–7 days	In ~2% of culture-proven cases, reactive arthritis may occur after ~3 weeks; also Reiter's syndrome
<i>Shigella</i>	Human intestinal tract	Person-to-person by fecal–oral route or fecal contamination of food handled by workers with poor personal hygiene	Diarrhea and dysentery (diarrhea with blood and mucus in the stools), vomiting, abdominal cramps, rectal pain and fever, appearing 12–50 h after infection and lasting a few days to 2 weeks	Reiter's syndrome, reactive arthritis, and hemolytic uremic syndrome. Some strains are associated with fatality rates of 10–15%
<i>Escherichia coli</i> O157:H7	Intestines of some mammals, raw milk, unchlorinated water	Contaminated water, raw milk, raw undercooked ground meat products, fruits and vegetables, unpasteurized apple juice	Diarrhea (can be bloody), abdominal cramps, nausea, malaise, possibly fever, and vomiting within 3–8 days after infection. Recovery usually within 8–10 days. <i>E. coli</i> O157:H7 produces toxins (verotoxins or Shiga-like toxins)	Especially young children can develop hemolytic–uremic syndrome, which can cause acute renal failure and has a case fatality rate of 3–5%
<i>Vibrio vulnificus</i>	Shellfish and finfish; also found in all US coastal waters	Raw oysters, clams, crabs	Gastroenteritis within 16 h after ingestion	May cause primary septicemia in individuals with certain underlying chronic diseases (this form is associated with a 50% mortality)
<i>Yersinia enterocolitica</i>	Soil, water, often isolated from pigs, cats, dogs, birds, and beavers	Meats, oysters, fish, raw milk	Fever and abdominal pain, frequently also gastroenteritis with diarrhea and/or vomiting within 24–48 h after infection; may mimic appendicitis	Can cause “primary septicemia” in individuals with underlying chronic disease (e.g., diabetes, cirrhosis, AIDS, leukemia). This form is associated with 50% mortality

<i>Staphylococcus aureus</i>	Human and other animal skin, hair, in nasal passage and throat	Food handler to food (meat, meat products, poultry, egg products, salads, bakery products, milk, and dairy products)	Symptoms are caused by the enterotoxins that some strains produce and occur within 1–6 h after ingestion. They include severe nausea, abdominal cramps, vomiting, and diarrhea; in more severe cases, headache, muscle cramping, changed blood pressure and heart rate. Recovery within 2–3 days	Associated with reactive arthritis. Can be mistaken for appendicitis, resulting in unnecessary appendectomies
<i>Clostridium botulinum</i>	Soil, water, plants, and intestines of animals and fish	Improperly canned foods, garlic in oil, tightly wrapped food	Symptoms are caused by the toxin released by the bacterium and are that of an intoxication: marked fatigue, weakness, and vertigo, followed by blurred vision, dry mouth, and difficulty in speaking and swallowing, possibly vomiting, diarrhea, constipation, or abdominal swelling. These symptoms usually occur within 12–36 h (range 4 h to 8 days). They can progress to weakness in the neck and arms, paralysis of the respiratory muscles, and death if not treated with antitoxin	

will be exposed to these toxins. According to the main symptoms they cause, these poisonings are grouped into paralytic (PSP), diarrhetic (DSP), neurotoxic (NSP), and amnesic shellfish poisoning (ASP). In addition, there are ciguatera fish poisoning and azaspiracid shellfish poisoning and yessotoxin and palytoxin poisonings. See Table 2 for a summary of the toxins involved, their sources, mechanisms, or action, and the acute symptoms they cause. Many of the dinoflagellate toxins are neurotoxins that interact with voltage-gated sodium and or calcium channels in different ways and cause either increases or decreases in the flux of these ions, thereby resulting in different sets of symptoms. PSP and NSP toxins as well as ciguatoxins, azaspiracids, yessotoxins, and palytoxin all belong to this group.

PSP The major PSP toxins belong to the saxitoxin group, of which at least 29 congeners are known and which are produced mainly by members of the *Alexandrium*, *Gymnodinium*, and *Pyridinium* genera of dinoflagellates. Symptoms after ingestion of PSP toxins develop rapidly (within 0.5–2 h) and include tingling sensation of the lips, mouth, and tongue, gastrointestinal problems, numbness of the extremities, difficulties with muscle coordination, respiratory distress, and paralysis. Severe cases can proceed to respiratory arrest and cardiovascular shock, the fatality rate being approximately 20% [11,12]. The lethal dose in humans is between 1 and 4 mg. The European Food Safety Authority (EFSA) set an acute Rfd (ARfd) of 0.5 µg/kg bw. Both the USFDA and the EFSA have a maximum limit of 80 µg/100 g (or 800 µg/1 kg) of PSP toxins in saxitoxin equivalents in shellfish tissue. Ingestion of a somewhat large 400-g portion of shellfish containing the maximum allowable level of saxitoxin equivalents would result in an acute exposure of 320-µg toxins or 5.3 µg/kg bw in a 60-kg adult. Since this intake is tenfold higher than the ARfd, it has been suggested that more appropriate limits (e.g., a more than tenfold reduction of the current limits) should be considered for saxitoxin equivalents in shellfish [13].

NSP *Karenia brevis* and several other dinoflagellates (see also Table 2) produce hemolytic and neurotoxic substances, the latter being designated as brevetoxins. There are a total of ten known brevetoxins, subdivided into type 1 and type 2 based on the structure of their backbones [14]. Brevetoxins and some of their molluskan metabolites cause the typical symptoms of NSP, which are milder than those of PSP and include nausea, tingling, and numbness of the lips, mouth, and face, paresthesia, loss of motor control, and severe muscular pain [11,14]. The pathogenic dose for humans is between 42 and 72 mouse units. Note that many of the shellfish poison levels are still measured in mouse units, which are defined as the amount of shellfish poison required to kill a 20-g mouse within 15 min after

Table 2 Shellfish poisoning toxins: sources, vectors, and symptoms adapted from Wang et al. [11]

Type of poisoning	Toxin	Sources of toxin	Primary vector	Mechanism	Symptoms
PSP	Saxitoxins, gonyautoxins	<i>Alexandrium</i> spp., <i>Gymnodinium</i> spp., <i>Pyridinium</i> spp.	Shellfish	Voltage-gated sodium channel 1	Tingling of perioral area, gastrointestinal problems, numbness of extremities, disturbed muscle coordination, respiratory distress, paralysis; 20% mortality
NSP	Brevetoxins	<i>Karenia brevis</i> , <i>Chattonella marina</i> , <i>Chattonella antiqua</i> , <i>Fibrocapsa japonica</i> , <i>Heterosigma akashiwo</i>	Shellfish	Neurotoxin acting via voltage-gated sodium channel 5	Nausea, numbness of perioral area, paresthesia, disturbed motor control, severe muscular pain
Ciguatera fish poisoning	Ciguatoxins, maitotoxins	<i>Gambierdiscus toxicus</i> , <i>G. belizeanus</i> , <i>G. yasumotoi</i>	Coral reef fish	Voltage-gated sodium channel 5, voltage-gated calcium channel	>175 gastrointestinal, neurological, cardiovascular, and general symptoms; can be fatal
AZP	Azaspiracids	<i>Protoperidinium crassipes</i>	Shellfish	Voltage-gated calcium channel	Nausea, vomiting, severe diarrhea, stomach cramps
Palytoxin	Palytoxins	<i>Palythoa toxica</i> , <i>Ostreopsis siamensis</i>	Shellfish	Sodium–potassium ATPase	Fever, ataxia, drowsiness, often fatal
Yessotoxin poisoning	Yessotoxins	<i>Protoceratium reticulatum</i> , <i>Lingulodinium polyedrum</i> , <i>Gonyaulax spinifera</i>	Shellfish	Possibly voltage-gated calcium/sodium channel	
DSP	Okadaic acids	<i>Dinophysis</i> spp.	Shellfish	Inhibition of phosphatases and of protein synthesis	
ASP	Domoic acids	<i>Pseudo-nitzschia</i> spp.	Shellfish	Activation of the kainate glutamate receptor	Vomiting, diarrhea, abdominal cramps, severe headache, loss of short-term memory, can be fatal

intraperitoneal injection. Brevetoxin levels in shellfish tissue have been set at 20 mouse units/100-g shellfish or, more recently, 80 µg/100-g shellfish in brevetoxin equivalents [14].

ASP The only known outbreak of ASP occurred in Canada in 1987 [15]. The toxins responsible for ASP symptoms were identified as domoic acid and its ten isomers, which are the only shellfish toxins not produced by dinoflagellates but by diatoms of the genus *Pseudo-nitzschia*. These toxins accumulate in a wide variety of shellfish species, including crabs, mussels, razor clams, scallops, and cockles, and much lower levels have also been detected in anchovies and mackerel. Symptoms after ingestion of domoic-acid-contaminated shellfish include gastrointestinal symptoms such as vomiting, abdominal cramps, and diarrhea and neurological symptoms such as debilitating headache and loss of short-term memory (seen in only 25% of patients but responsible for the name of the poisons). Three of the 107 patients that fulfilled the clinical definition of the illness died. Rough exposure estimates suggest that 1 mg domoic acid per kilogram bw is sufficient to induce

gastrointestinal illness, whereas neurological symptoms may require ~4.5 mg/kg bw [15].

Ciguatera fish poisoning This poisoning is caused by the consumption of contaminated coral reef fishes, such as barracuda, grouper, and snapper. The dinoflagellate *Gambierdiscus toxicus* produces maitotoxins, which are bio-transformed into ciguatoxins by herbivorous fishes and invertebrates. Ingestion of these toxins causes >170 gastrointestinal, neurological, cardiovascular, and general symptoms, with neurological symptoms predominating in the Pacific Ocean, whereas mostly gastrointestinal disturbances are seen in the Caribbean. Ciguatoxins are highly toxic, with as little as 0.1 µg being sufficient to cause illness in humans [11,12].

AZP The first report of an AZP incident came from the Netherlands and was associated with Irish mussels, but the group of toxins causing it has since been found to constitute a more widespread problem in Europe [11]. These toxins are produced by *Protoperidinium crassipes* and are derivatives of azaspiracid, of which at least 11 have been identified.

Ingestion of contaminated shellfish results in symptoms similar to those seen with DSP, i.e., nausea, vomiting, severe diarrhea, and stomach cramps. However, in mice, the effects of AZP differ markedly from those seen with DSP in that they include severe neurological symptoms, such as respiratory distress, spasms, and paralysis of the limbs.

DSP DSP is caused by okadaic acid and some of its congeners, of which at least seven have been identified and which are called dinophysistoxins [11]. The major producers of this group of toxins are various *Dinophysis* species. Acute symptoms after ingestion of contaminated shellfish include diarrhea, nausea, vomiting, and abdominal pain. No fatalities have been reported to date. Okadaic acid inhibits protein synthesis and also is an inhibitor of phosphatases. In addition, it increases DNA methylation, which is an important mechanism of gene regulation. It is thought that this ability to interfere with gene regulation is involved in the potent tumor-promoting effects that okadaic acid exerts in laboratory animals [16]. The CONTAM panel set a new lower ARfd of 0.3 µg okadaic acid equivalents per kilogram bw [17]. As in the case of saxitoxins, a large portion (400 g) of shellfish contaminated with the maximum of 160 µg okadaic acid equivalents per kilogram shellfish would exceed the ARfd by a factor of 3. Hence, a reduction in the maximum level was deemed desirable [17].

Other shellfish poisonings Several algae toxins were originally classified as DSP because they frequently co-occur with DSP toxins and are sometimes produced by the same species of algae. However, they were subsequently discovered to not (or only weakly) cause diarrhea or inhibit phosphatases. These include the pectenotoxins, which are produced mainly by several *Dinophysis* species and are hepatotoxic, and the yessotoxins, which are synthesized by *Protoceratium reticulatum* and *Lingulodinium polyedrum* and mainly target the heart, at least in mice [11].

Note that some of the so-called shellfish poisons are not restricted to shellfish but may also occur in other commonly consumed fish species, though at much lower levels. In addition, it is only during certain times of the year that they accumulate in shellfish to levels that cause acute toxicity, but they can be present at lower levels throughout much of the remainder of the year [11,12]. For example, *K. brevis* counts of 1,000 cells per liter of seawater are considered background levels at the Florida coast, and Florida shellfish beds are closed only when counts are equal to 5,000 per liter seawater or higher [14]. Very little is known about the chronic toxicity of low levels of exposure to these toxins. This is particularly worrisome given that some of them are already suspected of having carcinogenic or hepatotoxic effects.

Scombroid fish poisoning is a very common cause of adverse reactions to fish that is not due to zooplankton

toxins but is actually histamine poisoning due to bacterial action, with contribution from other biogenic amines [18]. Numerous case series document emergency department visits (sometimes considered acute allergic reactions) [19] that on investigation were found to be from ingestion of spoiled fish. Symptoms usually start within 15 min to 2 h and can include flushing, hypotension, palpitations, loss of consciousness, headache, skin rashes, nausea, diarrhea, vomiting, and shortness of breath or wheezing. Dark-fleshed fish of the Scombridae family, especially, contain higher levels of free histidine that is decarboxylated to histamine by bacteria. This can occur after just a few hours at ambient temperatures and has even been reported in fish that is chilled but not adequately so. Histamine is heat stable, so cooking the fish will not prevent the toxicity. Persons may differ substantially in their sensitivity to the ingested histamine, which may be directly due to their endogenous diamine oxidase activity in the small intestine.

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi that infect a variety of crops, including cereals, nuts, spices, and in some cases fruit.

Aflatoxins Aflatoxins are produced by three species of *Aspergillus*, namely *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, with *A. flavus* synthesizing only B aflatoxins, whereas both B and G aflatoxins occur in the other species [20]. Peanuts and maize are the most frequently contaminated foods, but pistachios and other nuts can also contain very high levels. Hydroxylation of B1 and B2 aflatoxins yields M1 and M2 aflatoxins, respectively. These metabolites are found in milk from animals that consumed aflatoxin-contaminated feed. Aflatoxin intake is very difficult to estimate because the contamination levels in foods are frequently below the limit of detection. Assuming that samples without detectable aflatoxins B1, B2, G1, and G2 contained concentrations of one half the limit of quantitation, mean dietary intake estimates of 0.12 and 0.32 ng/kg bw in adults and children, respectively, were obtained in a recent French total diet study [21]. Aflatoxin M1 intake was estimated at 0.09 ng/kg bw per day in adults and 0.22 in children (see also Table 3). According to an investigation using a dietary questionnaire for assessing food consumption, Swedish adults are exposed to a mean of 0.76 ng/kg bw per day (P95 2.1 ng/kg bw per day) [22]. In this study, a majority of samples were above the detection limit. The rather high intake was driven almost exclusively by extremely high levels of contamination in Brazil nuts and pistachios.

Aflatoxin B1 is highly mutagenic and carcinogenic and is one of the most potent liver carcinogens known.

Table 3 Mean daily dietary mycotoxin intake in nanogram per kilogram body weight per day (P95 where available)^a [20–22,26,27,32]

	Aflatoxin ^b	DON	NIV	HT-2	T2	OTA	Fumonisin	Zearalenone	Patulin
France									
Adults	0.117 (0.345)	281 (571)	88 (157)			2.16 (3.63)	14 (64)	33 (70)	18.0 (56.7)
Children	0.323 (0.888)	451 (929)	163 (300)			4.07 (7.77)	46 (175)	66 (132)	29.6 (106)
France (SCOOP)									
Adult		461 (1,667)	58 (199)	30 (98)	45 (156)	2.31	219		
Children		725 (2,430)	94 (307)	44 (143)	67 (207)	3.39	355		
Norway (SCOOP)									
Adult females		300 (530)	50 (93)	26 (59)	30 (57)				
Adult males		343 (628)	57 (110)	30 (69)	34 (67)				
Sweden (SCOOP)									
18–74		78 (155)	6 (13)						
Sweden									
Adults	0.76 (2.1)			39 (69)	1.2 (1.9)				
Children (7–14)				72 (130)	1.4 (2.6)				
Finland (SCOOP)									
Female adult		142	17	6	6	1.71			
Male adult		176	25	12	11	0.53			
Children (1.5–4.5)		483	64	18	14	1.42			
Germany						1.09	129		

Omitted values are either not available or are based on analysis of a very limited number of sampled food groups

^a P95; 95th percentile

^b The aflatoxin levels in food were all below the limit of quantitation. This estimate is based on the assumption that nondetectable levels are equal to one half the limit of quantitation

Aflatoxin M1 is approximately tenfold less potent. The JECFA did not set a TDI for aflatoxin B1 because even very low levels of exposure increase the risk of liver cancer. Instead, it was calculated that intake of as little as 1 ng/kg bw per day would result in one extra cancer case in 10⁵ individuals. Subjects with hepatitis B infection are at increased risk of hepatocellular carcinoma from aflatoxin exposure. It is recommended to keep the contamination levels as low as possible through good manufacturing and storing practices. If contamination is present, there are a variety of chemical or physical means for reducing the aflatoxin content [20,23].

Ochratoxins Ochratoxins are a group of structurally related secondary metabolites that are produced mainly by *Penicillium verrucosum* and *Aspergillus ochraceus*, occasionally also by isolates of *Aspergillus niger* [20]. The main and most toxic mycotoxin in this group is ochratoxin A (OTA), which is found in cereals, oil seeds, coffee beans, pulses, wine, and poultry meat. In the dietary exposure assessment performed by SCOOP, a scientific cooperation of EU states and Norway, the main dietary source was cereal grains in most European countries, but coffee and wine made the major contribution to exposure in Greece and Italy, respectively [24,25]. Dietary intake was estimated to be ~1–3 ng/kg bw per day (see also Table 3), but this is thought to underestimate actual intake because not all sources were taken into account in determining exposure. In a French study on dietary mycotoxin exposure, bread was the major source of OTA, accounting for one third of total intake [21]. Much of the remainder of the intake came from other flour-containing food types. A duplicate diet study of 123 Dutch participants indicated a mean OTA intake of 1.2 ng/kg bw per day [26], whereas in a UK duplicate diet study, where each participant collected duplicates for 30 days and one intake value was determined for the entire month, a mean dietary exposure of 0.94 ng/kg bw was calculated [27].

The absorption of OTA occurs in the upper gastrointestinal tract and ranges between 40% and 66% in various animal species [28]. Essentially, all OTA in blood is bound to proteins. It is distributed mainly to the kidney and also to liver, muscle, and fat. It has been shown to cross the placenta in different animal species and has been detected in human breast milk [29,30]. There are substantial interspecies differences in serum half-lives, ranging from 1 day in mice to 21 days in monkeys and ~35 days in humans. Excretion occurs via bile and urine, and there are indications of enterohepatic circulation.

OTA has been shown to be nephrotoxic in almost all animals species investigated to date. In humans, OTA exposure is thought to be associated with Balkan endemic nephropathy, but a causal connection has not been proven so far [31]. At much higher doses than those needed to

induce progressive nephropathy in animals, OTA has hepatotoxic, teratogenic, and immunotoxic effects. In addition, it has been reported to cause kidney tumors in mice and rats, with male animals being more sensitive than females. The International Agency for Research on Cancer (IARC) classified it as a probable carcinogen to humans (group 2B) [31]. The JECFA set a provisional maximum TDI of 0.014 µg/kg bw (see also Table 4) [23].

Fusarium toxins A variety of *Fusarium* species which can infect cereal crops in the field synthesize toxins. Toxin production occurs mainly before harvesting but can take place postharvest, if the crop is not handled properly. There are three major groups of *Fusarium* toxins, the trichothecenes, fumonisins, and zearalenone (ZEA).

Trichothecenes are structurally related sesquiterpenoids produced by several fungi and are subdivided into four categories depending on their functional groups. Major representatives of the type B trichothecenes are deoxynivalenol, nivalenol, and 3-acetyldeoxynivalenol, while T-2 toxin and HT-2 toxin are type A trichothecenes.

Estimates of mean dietary intake of deoxynivalenol (DON) in various European countries ranged between 78 and 725 ng/kg bw per day [32–34]. The major sources were cereals and cereal products, particularly corn [35]. The JECFA, the Scientific Committee for Food (SCF) for the EU, and the Nordic Working Group have all conducted risk assessments and established TDIs for some of the trichothecenes (see also Table 4): some of these values are provisional or temporary because *Fusarium* species are capable of producing several trichothecenes, and these may share a common mechanism of toxicity, making it desirable to take cumulative effects into account [31]. In the case of

DON, the SCF concluded that the limited data available did not support the establishment of a group TDI, and they set a final TDI of 1 µg/kg bw. At the high end of DON intake, mean dietary exposures far exceeded the TDI in some European countries [34].

DON is rapidly and extensively absorbed in swine, followed by wide but transient tissue distribution and rapid excretion, the elimination half-life being only 3.9 h. Studies in rodents also indicate that DON does not accumulate in the body. Ruminants and poultry show very limited absorption and little susceptibility to the toxicity of this compound [36]. In various animal species, a major effect of subchronic/chronic exposure to DON is decreased weight gain and anorexia due to feed aversion. This is also thought to be responsible for the fetal toxicity and teratogenicity, which are generally observed only at levels that induce maternal toxicity. In several animal species, DON is associated with immunotoxicity, including impaired delayed type hypersensitivity responses, antigen-specific antibody production, and host resistance and altered cytokine production. In rodents, but not in swine, serum total IgA (and sometimes IgG) levels are markedly elevated, resulting in IgA immune complexes that are deposited in the kidneys, resulting in a glomerulonephritis that resembles human IgA nephropathy [36]. Whether DON exerts similar effects in humans remains to be investigated.

T-2 and HT-2 are produced mainly by *Fusarium sporotrichioides* and to a lesser extent by *Fusarium poae*, *Fusarium equiseti*, and *Fusarium acuminatum*, affecting mostly corn, wheat, and oats. In a recent European survey, dietary intake of T-2 and HT-2 was found to exceed the European group TDI of 0.06 µg/kg bw per day in a large proportion of the population, with some infants reaching

Table 4 TDI values of mycotoxins in microgram per kilogram body weight per day [23,32,33]

Type of toxin	Specific toxin	SCF/EU	JECFA	Nordic working group	USEPA (Rfd)	Canada
Type B trichothecenes	Deoxynivalenol	1.0	1.0 ^a	1.0 ^b		
	Nivalenol	0.7 ^b		Insufficient data		
Type A trichothecenes	T-2 toxin	0.06 ^b	0.6 ^a	0.2 ^b		
	HT-2					
Zearalenone		0.2 ^b	0.07 ^{a, c}	0.1 ^b		0.1 ^b
Fumonisins	Fumonisin B1	2.0	2.0 ^a			
	Fumonisin B2					
	Fumonisin B3					
Ochratoxins	Ochratoxin A	0.005	0.014 ^c	0.005	0.12	0.0012–0.0057
Patulin		Endorsed the JECFA value	0.4 ^a			

^a Provisional maximum tolerable daily intake

^b Temporary TDI

^c Calculated from a provisional tolerable weekly intake

>500% of the TDI (see Tables 3 and 4) [32]. Note, however, that <20% of the samples tested positive, so the question of how samples below the limit of detection are treated (considered to be zero or equal to one half of the limit of quantitation) has a major influence on the estimate. Wheat and products made from wheat (e.g., bread and pasta) constituted the predominant dietary source of these toxins. T-2 is rapidly metabolized to HT-2, making it difficult to determine which of the two compounds is responsible for the toxic effects. Acute poisoning is associated with nausea, vomiting, abdominal pain, diarrhea (at times bloody) dizziness, and chills and is lethal in a high proportion of cases. The chronic toxicity is a result of the potent ability of T-2 (and HT-2) to inhibit protein synthesis, which is most obvious in the immune system. Decreased antibody production and delayed type hypersensitivity responses as well as changes in leukocyte counts due to selective depletion of leukocyte precursors have been observed. In addition, there is concern that T-2 can have carcinogenic effects [20].

ZEA is another mycotoxin produced by several fungi of the genus *Fusarium* [33]. It occurs mostly in maize (corn) but can contaminate other cereal grains and also soybeans. ZEA usually is present together with other *Fusarium* toxins, such as trichothecenes and fumonisins. Whereas the main sources of ZEA intake in Northern America are corn and wheat products, in Europe they are wheat, rye, and oats. Intake estimates are 20 ng/kg bw per day for Canada, Denmark, and Norway and 30 ng/kg bw per day in the USA (see also Table 3). Small children may have intakes as high as 55 ng/kg bw per day [33]. This is well below the tolerable intake values established by various regulatory agencies (between 0.1 and 0.5 µg/kg bw per day, see also Table 4). After oral administration, ZEA is readily absorbed in rats, rabbits, pigs, and humans. It is then extensively metabolized in the intestine and/or liver, with hydroxylation resulting in the formation of α - and β -ZEA. Intestinal cells also seem to be able to form α - and β -zearealenol. ZEA and its metabolites are conjugated with glucuronic acid, which would facilitate their urinary excretion. There are indications of extensive enterohepatic recirculation and, consequently, elimination via feces. Whether and to what extent these processes occur in humans remain to be elucidated [20]. ZEA has fairly low acute toxicity. In chronic exposure studies, it exhibits hematotoxicity and hepatotoxicity in rats and rabbits, and high doses are associated with hepatocellular and pituitary adenomas [20]. At much lower doses than are required for carcinogenic effects, ZEA shows estrogenic activity, resulting in reproductive toxicities in various animal species, with pigs and sheep being more sensitive than rodents. These include decreased fertility, reduced litter size, altered weight of adrenal, thyroid, and pituitary glands [33]. The IARC considered it as not classifiable as to its human carcinogenicity (group 3).

Fumonisins are produced by certain *Fusarium* species that mainly contaminate maize and maize products but also other cereal grains [20]. Accordingly, the major dietary sources of fumonisins in various European countries were cereals, particularly corn and wheat products. The most toxic compound is fumonisin B1 (FB1), and this is also the fumonisin for which there are the most data. See Table 3 for mean dietary intake levels. FB1 is poorly absorbed and rapidly eliminated; however, there are indications that a small pool of FB1 or its metabolites are retained in the liver and kidneys. It is therefore not surprising that FB1 targets the liver and kidney in almost all species examined today, with mice and rats showing the greatest susceptibility. In addition, FB1 has species-dependent organ-specific toxicities, such as porcine pulmonary edema in pigs and equine leukoencephalomalacia in horses.

In mouse embryo cultures and in LM/Bc mice, FB1 causes neural tube and craniofacial defects, which can be partially prevented by supplementation with folic acid, while ganglioside supplementation is almost completely protective [37]. The mechanism is thought to involve the ability of fumonisins to disrupt sphingolipid biosynthesis. The resulting lack of complex sphingolipids impedes the function of certain membrane proteins, including the folate-binding protein. Further support for the hypothesis that fumonisin exposure is causally involved in neural tube defects comes from a study of Mexican–American women living along the Texas–Mexico border, an area with a high incidence of these birth defects [38]. The results of this investigation indicated that maternal corn tortilla intake (as a surrogate for fumonisin exposure) during the first trimester of pregnancy and a biomarker of fumonisin exposure (the sphinganine-to-sphingosine ratio in postpartum serum) were significantly associated with the occurrence of neural tube defects. Furthermore, there is an elevated incidence of neural tube defects in other areas where high dietary intake of fumonisins is likely (Guatemala, South Africa, and China). Of note, it has been generally assumed that FB1 does not cross the placenta. However, disturbances in fetal sphingolipid profiles and folate receptor expression after maternal FB1 supplementation, as observed in LM/Bc mice [37], suggest that FB1 or its metabolites can be transferred across the placenta.

Patulin Patulin is a secondary metabolite produced by fungi belonging to several genera, including *Penicillium*, *Aspergillus*, and *Byssoschlamys*. Although many fruits, grains, and other foods can be infected, the commodities with the highest concentrations are generally apples and apple juice, and these are also the major sources of dietary intake [39]. Mean intakes for the populations of various EU countries and Norway generally ranged between 1.1 and 9.6 ng/kg bw per day, with children having several-fold higher mean dietary exposures. Available data indicate that

patulin has no reproductive or teratogenic effects and exhibits embryotoxicity only at doses that are toxic to the mother. It is not mutagenic but induces chromosomal damage. A provisional maximum tolerable daily intake of 0.4 µg/kg bw was established by JECFA [40].

Substances entering the food chain from the environment

Heavy metals

Lead (Pb) Since the phasing out of leaded gasoline, major sources of high Pb exposure are Pb paint (still found in an estimated 38 million homes in the USA) and drinking water contaminated from Pb pipes or brass fixtures, which may contain up to 8% of this metal. However, lead also persists in the environment, including soils that food crops are grown on, and food appears to be the major source of Pb exposure. Local Pb contamination of pastures can result in considerable contamination of meat and milk, and fish generally also contain high Pb concentrations. In some recent analyses from Spain, the food groups “fish and shellfish” and “cold meats and sausages” were found to contain the highest Pb concentrations, but substantial amounts were also detected not only in meat, eggs, and dairy products but also in fruits, vegetables, cereals, and tubers and in alcoholic beverages [41,42]. The highest contribution to adult Pb intake came from fish and shellfish, whereas cereals were the major source of Pb for children and adolescents [41]. In Lebanon, bread accounted for up to 28% of total dietary Pb intake. In the Canary Islands, Spain, water contained only 7.3 µg Pb per kilogram but was estimated to constitute ~20% of daily Pb intake at an average consumption of 2 l/day [42]. Mean dietary intake values are summarized in Table 5.

The extent of gastrointestinal Pb absorption depends on nutritional status, with iron deficiency resulting in increased and calcium supplementation in decreased Pb absorption. Age is another factor that influences Pb absorption. Generally, children have a much higher capacity for absorbing orally ingested Pb (up to 75%) than adults (only 10–15%). Once absorbed, the metal is then slowly (over a period of 4–6 weeks) distributed to various tissues, including liver, renal cortex, brain, and bone, the latter constituting the major storage site for Pb. Pb is remobilized from the exchangeable pool at the bone surface, a process that is particularly obvious during conditions associated with increased bone turnover, including pregnancy and lactation. Inorganic lead does not undergo metabolism, and the majority is excreted via urine, while the extent of fecal excretion remains to be established.

The primary target of lead toxicity is the central and peripheral nervous system. In adults, this most commonly

manifests as peripheral neuropathy involving extensor muscles, but not sensory nerves. The developing brain is much more susceptible to the neurotoxic effects of Pb than the adult brain. Numerous studies have shown that Pb exposure in infants and children is associated with learning disabilities, decreased IQ, behavioral problems, and disturbances in fine motor function. Some of these effects are seen below blood Pb concentrations of 10 µl/dl, which the CDC and the American Pediatric Association consider the level of concern. Of note, among the children examined as part of the nationally representative National Health and Nutrition Examination Survey (NHANES) III (1999–2000), the upper bound of the 95% confidence interval of the 95th percentile of blood Pb concentration was 9.90 in the 1–5-year-old age group [43].

Another Pb-associated toxicity is kidney damage, which is not only well established in occupational Pb exposure but is seen at much lower exposures in the general population. In addition, Pb exposure is associated with an increased risk of hypertension, cerebrovascular, and cardiovascular disease. Furthermore, the IARC has reclassified Pb from a possible to a probable human carcinogen, since there is increasing evidence of an association between Pb exposure and overall cancer incidence but particularly stomach, lung, and bladder cancer. WHO set a provisional tolerable weekly intake (PTWI) of 25 µg/kg bw. In contrast, the USEPA and Agency for Toxic Substances and Disease Registry (ATSDR/CDC) agree that there is no threshold for lead below which no harm occurs.

Mercury (Hg) It is estimated that less than 50% of global Hg releases arise from natural sources, with the remainder coming from human activities, such as burning of coal, cement production, mining, and metal processing. Anthropogenic emissions, particularly from Asia, are expected to increase significantly in coming decades. Long-range transport results in the deposition of Hg throughout the hemisphere in which it was emitted. When inorganic Hg reaches aquatic environments, sediment bacteria convert it into methyl mercury (MeHg), which is then bioaccumulated and biomagnified in the aquatic food chain. As a result, fish at the topmost trophic levels contain the greatest concentrations of MeHg, as do sea mammals. More than 90% of total Hg in fish is thought to be present in the form of MeHg. The highest levels of MeHg are found in predatory species such as shark, swordfish, marlin, pike, large tuna, and king mackerel. As a result, humans are exposed to this metal mainly through the consumption of fish and, if applicable, sea mammals. Using fish meal as animal feed can result in substantial levels of MeHg in meat and other animal products. Table 5 summarizes mean dietary intake values for total Hg. The ethyl mercury compound thimerosal, which is used as a preservative in certain childhood

Table 5 Dietary intake of lead (Pb), total mercury (Hg), total arsenic (As), and cadmium (Cd) in microgram per day (from total diet studies unless indicated otherwise)

Country	Age group	Lead	Mercury (total)	Arsenic	Cadmium
US 1982–1984	Infants (6–11 months)		0.49		
	Children (2 years)		1.3		
	Female adults		2.9		
	Male adults		3.9		
US 1986–1991	Female adults		8.2	50.6	
	Male adults		8.6	58.5	
NHEXAS Maryland		8.14			
NHEXAS Arizona (aggregate lead exposure)	Male adults	42			
	Female adult	35			
	Children (<18 years)	27			
Greenland		2.1	121		143
France (duplicate diet from mass catering establishments)		52		109	17
France	Adults	57	8.48	148	10.6
	Children (3–14)	40	5.86	101	7.8
Germany	Adults	47	9.91	37	19.2
	Children (4–6)	26	5.56	20	13.6
	Children (10–12)	34	7.51	27	17.4
UK	Adults	27	3.02	66	12.1
Denmark	Adults	18	3.5	37	16
Catalonia, Spain	Children	23.0	18.63	132.82	13.2
	Adolescents	25.7	16.57	157.82	14.8
	Female adults	23.1	16.71	190.38	12.0
	Male adults	28.4	21.22	223.59	15.7
	Seniors	23.4	16.95	192.12	12.7
Catalonia, Spain	Adults	59.3	9.89	261.01	10.0
Canary Islands, Spain		58.2 ^a			11.2
Beirut, Lebanon		15.5 ^a			9.3
Japan (duplicate diet) 1980	Female adults				37.5
	Male adults				52.0
Japan (duplicate diet) 1995	Female adults				26.2
	Male adults				26.7

^a After subtracting 14.6 µg/day for water, which was not included in the other analyses

vaccines, is another source of exposure. Dental amalgams may also make substantial contributions to total exposure.

MeHg after fish consumption is almost completely absorbed and distributed throughout the body within the following 30–40 h. It accumulates particularly in the liver and kidney, but about 10% is found in the brain. In addition to crossing the blood brain barrier, MeHg can cross the placenta, resulting in similar or higher levels in cord blood compared to maternal blood, whereas the fetal brain exhibits at least fivefold higher concentrations than maternal blood. The intestinal microflora can metabolize MeHg to inorganic Hg, which is either excreted via bile or slowly accumulates in the body but is thought to be present in inert form. Urinary excretion accounts for less than 10% of total

elimination from the body. The half-life in the body is ~50 days. Of note, bacterial demethylation and biliary excretion are not observed in suckling animals, and a similar inability to metabolize MeHg may underlie the failure of human neonates to excrete this compound.

Numerous animal studies have shown that MeHg is a developmental neurotoxicant and also has potent neurotoxic effects in adult animals [44]. Even at exposure levels well below those that affect the central nervous system, MeHg can profoundly affect the immune system. The extent and nature of these immunotoxicities depends on the timing, dose, type of Hg compound (metallic, inorganic, or organic), and the genetic characteristics of the host. For example, depending on the mouse strain, inorganic Hg was

found to reduce thymus weight, yet may either enhance, decrease, or not significantly affect the lymphoproliferative response. In addition, exposure to inorganic Hg results in polyclonal B and T cell activation, specific nucleolar autoantibody production, deposition of immune complexes in the kidney, and glomerulonephritis in susceptible mouse strains. A similar autoimmune syndrome is observed in certain rats. Clinical manifestations subside spontaneously in both species but can be reinduced in mice, whereas rats become resistant due to the development of suppressor CD8 T cells. Like inorganic Hg, organic Hg decreases thymus weight but can augment the lymphoproliferative response. It is also capable of inducing autoantibody production in susceptible animals but does so with much slower kinetics, suggesting that conversion to inorganic Hg is required. Of note, occupationally exposed cohorts exhibit increased levels of several autoantibodies as well as T cell lymphoproliferation. This suggests that the ability of Hg to break tolerance and induce autoimmunity may be of human relevance, at least for genetically susceptible populations.

Several large-scale poisoning incidents involving highly contaminated fish in Japan in the 1950s and 1960s and seed grain that was used for human consumption in Iraq in the 1970s have highlighted that the major target of MeHg toxicity in humans is the brain and that the developing brain is uniquely susceptible to MeHg neurotoxicity. Since then, evidence has emerged that much lower levels of prenatal MeHg exposure can affect human neurodevelopment, resulting in altered motor function as well as memory and learning deficits that may persist at least into adolescence. These effects were particularly obvious in a large prospective birth cohort assembled in the Faroe Islands, where mothers are exposed to high levels of dietary MeHg due to the occasional consumption of pilot whale meat [45]. Of note, this population is also exposed to very high environmental levels of polychlorinated biphenyls (PCBs), which themselves have been shown to affect cognitive development. There are indications that the effects of MeHg can be enhanced at the highest levels of PCB exposure [46]. Note, however, that PCB exposure was adjusted for in the Faroe Island cohort [45]. Some cross-sectional studies also found an association between prenatal MeHg exposure and neurobehavioral outcomes. In contrast, another large longitudinal study from the Seychelles Islands did not yield any indications of such an association. Since the two longitudinal studies have been used by various regulatory agencies in setting tolerable intakes, the interpretation and weighting of the respective results have had a major influence on the resulting values, which are nonetheless all in the range of 0.1 µg/kg bw per day (USEPA) and 0.3 µg/kg bw per day (ATSDR), while the USFDA still lists an ADI of 0.4 µg/kg bw per day based on data from the Japan and Iraq poisoning incidences [47]. The FAO/WHO

reduced its PTWI from originally 3.3 to now 1.6 µg/kg bw (corresponding to 0.23 µg/kg bw per day). Note, however, that a single meal of one of the more highly contaminated species (such as swordfish or marlin) would result in an intake of >2 µg/kg bw, which vastly exceeds the USEPA Rfd and is higher even than the PTWI of the FAO/WHO. The USFDA therefore advises pregnant or nursing women and young children to avoid highly contaminated species and to eat fish at most twice a week.

Cadmium (Cd) This element occurs naturally in ore, usually as Cd oxide, Cd chloride, Cd sulfate, or Cd sulfide and enters the environment from weathering of Cd-containing rocks as well as mining activities. The production and emission of Cd has increased dramatically over the last century due to its use as an anticorrosion agent, stabilizer in polyvinyl chloride (PVC) products, color pigment, and most commonly now in rechargeable nickel–Cd batteries.

A major source of Cd exposure is cigarette smoking, but food constitutes the most important contributor in non-smokers. The Cd content of crops grown for human consumption reflects the Cd concentration of the soil they were grown on, which can be naturally high or can be increased by industrial emissions or the application of contaminated fertilizing agents. It is not surprising, therefore, that cereals, tubers, and pulses were found to make the highest contribution (37–50%, depending on the age group) to Cd exposure in a Spanish population [41], whereas rice was a major determinant of Cd intake in a Japanese duplicate diet study [48]. Note, however, that by far the highest concentrations are found in fish and shellfish [41]. In duplicate diet studies in an industrial and a nonindustrial area in Germany, children (mean age 3.8 years) were found to have a Cd intake of 0.49 and 0.37 µg/kg bw per day, respectively [49]. Adults from the industrial area had a mean intake of 0.37 µg/kg bw per day. Maximum intake reached 120% of the current PTWI of 7 µg/kg bw per week. Other intake data are summarized in Table 5. In addition, data from the NHANES III (1999–2000), which is representative of the US population 6 years and older, indicate that even the 95th percentile of urinary Cd excretion is below 1.5 µg/g creatinine [43]. This is well below the level of 10 µg/g creatinine that was thought to represent a threshold for the occurrence of mild kidney dysfunction manifested as reversible low-molecular mass proteinuria. However, this threshold has recently been revised down to 1 µg/g creatinine (see below) [50] and that value is exceeded at the higher end of exposure in the US population, even in adolescents (12–18 years of age) [43].

Cd is not well absorbed: the inhalation, oral, and dermal routes are associated with absorptions of 25%, 1–10%, and

<1%, respectively. Generally, females absorb a higher proportion than do males. Cd is widely distributed throughout the body, but the highest levels are found in liver and kidney. It is not known to undergo any metabolic conversion and is eliminated very slowly, with 0.007% and 0.009% of total body burden being excreted daily via urine and feces, respectively. Despite its low absorption, this extremely limited elimination results in the life-long accumulation of Cd in the kidney.

The IARC has classified Cd as a human carcinogen (group I), but the evidence from human studies showing an association between Cd exposure and cancer of the lung, urinary bladder, kidney, and prostate is not deemed entirely convincing (even by the IARC itself). There is, however, strong evidence that Cd dose-dependently induces kidney tubular damage and a decrease in glomerular filtration rate that becomes irreversible at higher levels of exposure. The FAO/WHO set a PTWI of 7 µg/kg bw (which would correspond to 60 µg/day or 420 µg/week for a person weighing 60 kg). This is supposed to represent an intake that can be maintained over a lifetime without resulting in Cd concentration in the renal cortex of more than 200 mg/kg, a concentration at which tubular dysfunction occurs. In contrast, the USEPA has an oral Rfd for Cd from food of 5 µg/kg bw per day, i.e., 35 µg/kg bw per week. The European SCF initially endorsed the value set by the FAO/WHO. Recently, however, its successor established a new TWI of 2.5 µg/kg bw [50]. For its risk assessment, it used urinary Cd levels as an indicator of body burden and urinary β2-microglobulin excretion as a marker of tubular effects. A one-compartment model was then employed to determine that an increase of urinary Cd of 4 µg/g creatinine was associated with a 5% increase in the prevalence of elevated β2-microglobulin. To account for interindividual variability in urinary Cd excretion, a Cd-specific adjustment factor of 3.9 was applied, indicating that urinary Cd concentrations should not exceed 1 µg Cd per gram creatinine. Based on the analysis of dietary Cd intake and urinary excretion data from a large nonsmoking cohort, it was then established that dietary intake of 0.36 µg Cd/kg bw per day (or 2.52 µg/kg bw) over 50 years would lead to a urinary Cd concentration not exceeding the critical value of 1 µg/g creatinine determined from the preceding analysis, hence the TWI of 2.5 µg/kg bw.

Arsenic (As) This element occurs naturally in inorganic form (mostly arsenite As₂O₃ and arsenate As₂O₅, with AsIII being 60 times more toxic than AsV), and high levels of inorganic As are found in certain water supplies, particularly in India and Bangladesh, but also in certain areas of the USA and of other parts of the world. The vast majority of As in food is in the organic relatively nontoxic form, with ≤1.5% of As in fish and 10–20% of As in

shellfish being in the inorganic form. As is well absorbed from the small intestine, extensively methylated, and, to a lesser extent, conjugated to glutathione and quite rapidly excreted, mostly in urine. Therefore, the low dietary intakes of mostly organic As reported from around the world (see Table 5) indicate that food per se does not pose a substantial risk in the USA or other industrialized countries. In contrast, inorganic As is acutely toxic, with nausea, vomiting, and abdominal pain constituting the initial symptoms followed by psychosis, toxic cardiomyopathy, seizures, and possibly death. In addition, high sublethal levels of inorganic As in drinking water result in chronic As toxicity characterized by keratosis and hyperpigmentation of the skin, hepatomegaly, various neurological effects, in particular peripheral neuropathy, both restrictive and obstructive lung disease, hematological abnormalities, and increased risk of cardiovascular and cerebrovascular disease and diabetes. There also is a strong association between As exposure and cancer of the skin, lung, and bladder, but so far no studies from the USA have reported an increased risk of these cancers in association with the drinking water levels of As. The WHO has set a PTWI of 15 µg/kg bw per week, corresponding to 2.14 µg/kg bw per day.

Organochlorine compounds

A variety of pesticides belong to the organochlorine group of compounds, including 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (dichlorodiphenyl dichloroethene) (DDT), lindane, and other hexachlorocyclohexanes, cyclodienes such as dieldrin, chlordane, and heptachlor, and hexachlorobenzene. Their production and use in the USA were banned as early as 1965 for hexachlorobenzene, in 1972 for DDT, and in the late 1980s for most other compounds. However, it was only in 2006 that the EPA called for voluntary cancellation of products containing lindane, and the last use date for lindane was not until July 1, 2007. Many organochlorines, in particular the more heavily chlorinated ones, are lipophilic and very resistant to degradation. Both factors contribute not only to their bioaccumulation in all parts of the environment but also to their bioconcentration from one trophic level to the next. In this section, we focus on DDT and its breakdown product dichlorophenyl dichloroethylene (DDE) as examples of the toxicities associated with organochlorine pesticides.

DDT and DDE The major route of human exposure to DDT and its metabolite DDE is thought to be dietary ingestion. DDT is well absorbed (70–80% depending on the matrix in which it is present). It is stored mostly in adipose tissue, and humans retain DDT much longer than any other

species examined to date, including monkeys. The half-lives of DDT and DDE in humans have been estimated to be 7 and 10 years, respectively. Nonetheless, exposure and body burden have been steadily declining in populations from countries where DDT is not used for malaria control.

The majority of the available data indicate that DDT and DDE are not genotoxic, although conflicting results have been obtained. However, both are carcinogenic in animals, and the IARC has classified them as probably (group 2B) human carcinogens. Studies in human cohorts with relatively high exposures provide strong evidence for an association between DDE and testicular germ cell tumors and DDT and liver cancer. The data on other types of cancer are controversial and insufficient to determine whether DDT and its metabolites are associated with increased risk. An association between DDT and/or DDE and type 2 diabetes has been observed in several studies but may be confounded by concurrent exposures to other organochlorines.

Studies in laboratory animals document that gestational exposure particularly to DDE exposure during gestation has antiandrogenic effects. This is consistent with its ability to bind to the androgen receptor (AR) and inhibit androgen binding. In contrast, *o,p'*-DDT, a minor component of technical-grade DDT, and some DDT metabolites exhibit estrogenic activity. The effects of DDE on the male reproductive organs include reduced weight of testes, seminal vesicles, glans penis and cauda epididymis, decreased sperm count and motility, reduced anogenital distance, and nipple retention. In rabbits, a low incidence of cryptorchidism was also observed. In adult animals, DDT exposure can result in reduced testosterone production. Several studies in adult men suggest an association between DDE and/or DDT exposure and semen quality, but the findings are not consistent. In women, there are indications that DDT and DDE exposure is associated with alterations in estrogen and progesterone levels at different phases of the menstrual cycle, and this may represent an increased risk for fetal loss. There are several reports that the levels of DDT/DDE exposure seen during the peak usage of this pesticide in the 1950s and 1960s were associated with an increased risk of preterm birth, being small for gestational age, and having reduced birth weight. More recent data suggest that these effects are no longer seen at current reduced exposure levels.

In recent years, more information has become available on the possible association of DDT or its breakdown product DDE and neurodevelopmental outcomes. In a birth cohort from North Carolina, transplacental exposure to DDE was associated with hyporeflexia in neonates [51] but did not affect later behavioral or cognitive development [52,53]. A similar lack of association was described in

children of a birth cohort from Oswego, New York [54,55]. In contrast, cord blood concentrations of DDE in neonates of mothers who lived near a PCB-contaminated harbor in Massachusetts were negatively associated with some items on the Neonatal Behavioral Assessment Scale relating to attention [56]. Equal or stronger associations were seen with PCB exposure, and there was no attempt to correct for possible confounding (nor for the multiple comparisons).

Both DDT and DDE were included in an investigation of the CHAMACOS birth cohort from the Salinas Valley in California. This cohort mainly included mothers who had lived in Mexico for most of their lives, and whereas DDT was essentially banned in the USA in 1973, DDT use for malaria control continued in Mexico until the year 2000. The mothers' serum concentrations of DDT and DDE (obtained during pregnancy) were not associated with behavioral assessment scores in their neonates [57] but were associated with decreased scores in psychomotor and mental development assessments in their children examined at 6, 12, and 24 months of age [58]. Specifically, DDT was associated with the psychomotor development index at 6 and 12 months, but not 24 months, whereas DDE showed an association only at 6 months. The mental development index was not related to DDT or DDE exposure at 6 months but was inversely correlated with both compounds at 12 and 24 months. Note that DDE and DDT were highly correlated in this cohort, making it difficult to separate their effects. However, the results were independent of possible neurodevelopmental effects of PCBs, Pb, organophosphate (OP) pesticides, or hexachlorobenzene. In another group of Mexican women from an area with endemic malaria, maternal DDE levels during the first trimester of pregnancy, but not those obtained during the second or third trimester, showed a significant inverse association with the psychomotor development index (but not the mental development index) on the Bayley Scales for Infant Development in their infants at 3, 6, and 12 months of age [59]. Too many of the samples contained undetectable levels of DDT to allow analysis of an association between this compound and neurodevelopment.

In two Spanish birth cohorts, gestational DDT exposure, as assessed by DDT concentrations in cord blood, was significantly associated with decreased general cognitive, memory, and verbal scores in the McCarthy Scales of Children's Abilities [60]. This association remained after adjustment for DDE concentrations, whereas the only significant association seen with DDE (decreased memory scores) disappeared after adjustment for DDT exposure.

To summarize, data on associations between prenatal exposure to the parent compound DDT and neurodevelopmental outcomes are limited but show a consistent negative effect on mental and behavioral development that is independent of other exposures implicated in disturbed

early cognitive functioning. While more data exist on the possible effects of gestational exposure to the breakdown product DDE and neurodevelopment, the results are conflicting. Some of these discrepancies may be due to different exposure levels but also to differences in the analytical procedures (cord blood versus maternal serum) and the control for covariates in the statistical analysis.

Dioxins and dioxin-like compounds In addition to DDT and other pesticides, the organochlorines include polychlorinated dibenzofurans (PCDFs) and dibenzodioxins (PCDDs), PCBs and polybrominated biphenyls (PBBs), and polychlorinated naphthalenes. Whereas PCBs and PBBs were purposely produced for use in transformers and capacitors, hydraulic fluid, plasticizers, and fire retardants, PCDD/Fs are byproducts of thermal and industrial processes, now stemming mostly from incineration of municipal and hazardous waste. According to conservative estimates, ~1.3 million tons of PCBs were produced, almost exclusively in the Northern hemisphere. Some of the major PCB congeners were recently estimated to have environmental residence times of 70–100 years. This suggests that, even though the production of PCBs was halted ~30 years ago, exposure will continue for decades, if not centuries.

Potentially, there could be 75 different PCDD and 135 PCDF congeners (isomers with similar halogen substitution patterns), but the actual number present in biotic samples is much lower, with mainly 2,3,7,8-substituted congeners being detected. The most toxic congener is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is often referred to simply as “dioxin.” The term “dioxins” refers to PCDDs in general. Although 209 PCB congeners are possible, only between 50 and 150 congeners are detectable in biotic samples [61]. Whereas PCDDs and PCDFs have rigid planar structures, PCB molecules can be more flexible, depending on the number and positions of the chlorine substituents. The least-flexible, planar PCBs exhibit the greatest resemblance with the dioxins and are often referred to as “dioxin-like” compounds.

Like DDT and DDE, dioxin and dioxin-like compounds accumulate in the environment and are bioconcentrated in higher trophic levels of the food chain. There are indications that ~90% of current human exposure to PCBs, dioxins, and dibenzofurans occurs via dietary intake of contaminated foods. The highest levels of contamination are usually found in foods containing animal fats, such as meat, dairy products, and fish, particularly fish from highly contaminated waters like the Great Lakes or the Baltic Sea. The results of exposure assessment are expressed in toxic equivalent (TEQ), which are derived by determining the potency of PCDDs, PCDFs, and certain PCBs relative to the most toxic compound, TCDD, then multiplying the

result by the concentration of the individual dioxins and dioxin-like compounds in the diet. Dietary exposure and body burden have been declining over the last decades. Relatively current mean intake estimates range from 0.38 pg TEQ per kilogram bw from PCDD/Fs for the US population and from 0.4 to 1.5 pg TEQ per kilogram bw per day in various European countries, with PCBs adding another 0.8–1.5 pg TEQ per kilogram bw per day. The total exposure in Europe therefore amounts to 1.2 to 3 pg TEQ per kilogram bw per day. That indicates that a substantial portion of the population is exposed to levels higher than the TWI of 14 pg TEQ per kilogram bw set by the JECFA and other regulatory agencies. Children generally are exposed to considerably higher amounts of dioxins and dioxin-like compounds. In the USA, children at the age of 2 years had a mean intake of PCDD/Fs of 1.2 pg TEQ per kilogram bw per day, and European studies also indicate that the dietary exposure of children is up to threefold higher than that of adults. Note that it is deemed advisable to include polychlorinated naphthalenes in the TEQ scheme, but sufficient data for comparing its potency to that of TCDD are not yet available [62]. In one of the rare studies examining exposure to polychlorinated naphthalenes, mean dietary intake of this compound was 0.1 ng/kg bw per day in a Spanish population, which represented an 80% decrease compared to results obtained in 2000 [63].

Of particular note, dietary exposure to PCDD/Fs and PCBs starts in utero as evidenced by their detection in amniotic fluid and cord blood. Even though breast milk levels have been declining over the last decades in most industrialized countries, infant exposures from breast feeding still frequently exceed the most commonly used TDI values by two to three orders of magnitude. Such values are based on lifetime intakes and not intended to be applied to the relatively short nursing period. On the other hand, the exposure during nursing has been found to be a determining factor of total body burden well into adolescence. In addition, infants are likely to be considerably more susceptible to the various toxic effects of environmental pollutants, including organochlorine compounds.

Although only limited data are available, it is generally accepted that humans readily and almost completely absorb lower-chlorinated PCB and PCDD/F congeners, while absorption of higher chlorinated congeners is lower but still substantial. Based on animal studies, dermal absorption is estimated to range from 8% to 20%, depending on the degree of chlorination and on methodological factors. PCBs are initially distributed to highly perfused tissues such as liver and muscle but are then redistributed to and stored in adipose tissue and skin. In partial contrast, >95% of the body burden of PCDD/Fs is found in the liver and adipose tissues. Many PCBs and dioxins have long half-lives in humans, with estimates of ≥ 30 years for the more

persistent PCBs, 7 or 8 years for TCDD, >15 years for other PCDDs, and up to 20 years for some PCDFs. The major metabolites of PCBs are methyl sulfones and polychlorobiphenyls (OH-PCBs). Some OH-PCBs are selectively retained, mainly by binding to plasma proteins, such as albumin and the thyroid hormone transport protein, transthyretin (TTR). In vitro, certain OH-PCBs have fourfold higher affinity for TTR than its natural ligand thyroxine, and their ability to interfere with thyroid hormone homeostasis may contribute to the neurodevelopmental effects of PCB exposure.

Health risks of dioxins and dioxin-like compounds

In animals, dioxins and dioxin-like compounds exhibit a broad array of toxicities, ranging from disturbances of multiple hormone systems and toxicities of the liver, the developing immune, nervous, and reproductive systems to carcinogenesis and outright lethality. There are marked interspecies differences in the susceptibility to dioxin lethality and certain other outcomes, whereas some effects are seen at similar body burden in essentially all species examined to date. The immune system, particularly during fetal development, represents one of the most sensitive targets of TCDD, other dioxins, and dioxin-like compounds. Gestational or perinatal exposure results in thymic atrophy at relatively high doses, but even low doses lead to altered structural and functional development of the immune system and permanent suppression in delayed-type hypersensitivity. In adult laboratory animals, including nonhuman primates but also in marine mammals, chronic low-dose exposure to dioxins suppresses both humoral and cell-mediated immune responses and is associated with impaired host resistance to various infectious diseases. Another highly sensitive target is the developing brain. Gestational exposure of rodents and monkeys to PCBs consistently results in negative effects on learning and locomotor activity and function [61].

The IARC has classified TCDD as a human carcinogen (group 1) but considered other PCDDs and PCDFs as not classifiable. In occupationally and otherwise highly exposed cohorts, TCDD and possibly other PCDD/Fs are associated with increased mortality from ischemic heart disease, but this is not an entirely consistent finding. There are also indications that even background levels of TCDD exposure may increase the risk of type 2 diabetes, but such an association was not detected in other studies. Higher levels of paternal exposure to TCDD stemming from an industrial accident in Seveso, Italy, were found to be associated with a decreased male-to-female sex ratio in their children. Similar observations were reported from workers from a Russian pesticide-producing plant exposed to high levels of dioxin. On the other hand, no significant

changes in the sex ratio were found in the children of three other cohorts exposed to high levels of PCBs, PCDFs, and thermal degradation products of these compounds. Unlike in laboratory animals, exposure to background levels of PCB, PCDDs, and PCDFs is not consistently associated with negative effects on birth outcomes or thyroid function [64].

In children from a Dutch birth cohort examined at the age of 42 months, higher prenatal, but not postnatal, exposure to PCBs was associated with subtle changes in lymphocyte subset distribution and with decreased levels of serum antibodies to mumps and rubella [65]. Similarly, in routinely vaccinated children from two Faroe Islands birth cohorts, there was an inverse association between prenatal PCB exposure (assessed as maternal serum concentrations) and serum antibody levels for diphtheria toxoid at 18 months and for tetanus toxoid at 7 years of age [66]. Postnatal exposure (the child's own serum PCB concentration at the time of examination) showed similar associations, and early postnatal exposure in particular was an important predictor of diphtheria antibody levels at 18 months of age. In the Dutch cohort, increased postnatal PCB exposure (current serum PCB concentration) was associated with a higher incidence of otitis media, whereas gestational exposure was associated with less shortness of breath with wheeze. An association between perinatal PCB exposure and otitis media was also observed in some Inuit cohorts, along with an increased frequency of upper-respiratory infections, gastrointestinal infections, and infectious episodes overall. Although these findings suggest subtle effects on the developing immune system with possible clinical relevance, the results need to be interpreted with great caution due to a variety of methodological shortcomings [64]. Few studies have examined the immunological sequelae of dioxin and PCB exposure in adults. Although there are occasional reports of disturbed lymphocyte subset distribution and decreased serum concentrations of immunoglobulin and complement, the results are highly inconsistent and do not provide convincing evidence of immunotoxicity.

One of the greatest concerns over the continuing human exposure to PCBs and PCDD/Fs are their possible neurodevelopmental toxicities. Children of mothers exposed to high levels of PCBs, PCDFs, and thermal degradation products of these compounds due to the ingestion of highly contaminated cooking oil displayed developmental delay and impaired performance on behavioral and cognitive function tests. Numerous cohort studies have examined the effects of prenatal exposure to environmental background levels to PCBs on neurodevelopmental outcomes. Exposure was assessed as maternal or infant body burden, as reflected by concentrations in maternal serum or in cord blood. Overall, the results of these studies indicate that prenatal

PCB exposure is associated with subtle but significant delays in the early neurodevelopment of infants and children [67]. In some cohorts, these effects were persistent until at least 7 years of age, whereas in others they had disappeared as early as the age of 18 months. Note that PCB exposure was not associated with neurodevelopmental outcomes in some of the most recent cohorts [68,69], and it has been suggested that this is related to the slow decline in exposure and body burden seen over recent decades. Despite the much greater transfer of organochlorines from the mother to the infant via breast milk, most studies do not reveal any significant associations between postnatal exposure via breastfeeding and neurodevelopmental outcomes, although there are some notable exceptions. Nonetheless, WHO did not find sufficient evidence to change its endorsement of breastfeeding.

Substances arising from production processes

Hormones

Six steroid hormones are approved by the USFDA, namely estradiol, progesterone, testosterone, zeranol, trenbolone (acetate), and melengestrol acetate. The first three are natural female and male sex hormones, respectively. Zeranol is a synthetic form of α -zearalenol, an estrogenic metabolite derived from the mycotoxin ZEA. Trenbolone and melengestrol acetate are synthetic anabolic steroids.

In the Red Book containing data on 2007 surveillance activities of the FSIS/USDA, it was reported that testing for the three synthetic growth promoters did not reveal any residue violations in 261 veal samples tested for zeranol, 258 veal samples tested for trenbolone, and 309 heifers tested for melengestrol acetate. No nonviolative positives were seen for zeranol or trenbolone, but two samples tested positive at nonviolative levels for melengestrol acetate. The published literature on the tissue levels of hormones in animals treated with one of the natural or synthetic growth promoters indicates that the responses of individual animals are highly variable but that there generally is a dose-dependent increase in parent compound and metabolites. The magnitude of this increase strongly depends on the compound or metabolite and the tissue examined, for example ranging from 3.7 in muscle to 6.4 in fat for estradiol-17 β and from 1.2 in muscle to 1.9 in fat for estrone in animals with estradiol-containing implants [70]. Similar results have been reported for animals treated with other growth promoters [71,72]. Note that zeranol, i.e., α -zearalenol, is a minor metabolite in the tissue of untreated pigs if their feed is contaminated with the mycotoxin ZEA, whereas it was found to be undetectable in muscle or liver tissue of cattle that had received ZEA-contaminated feed.

The JECFA has set ADIs of 0–0.05 μ g/kg bw per day for estradiol 17 β , 0–30 μ g/kg bw per day for progesterone, and 0–2 μ g/kg bw per day for testosterone. Assuming a theoretical daily meat intake of 500 g (300 g muscle, 100 g liver, 50 g fat, 50 g kidney—which seems a very high estimate), it has been calculated that the contribution to the daily maximum intake of estradiol of 3 μ g (FAO/WHO ADI of 0.05 μ g/kg bw in a 60-kg adult) is 0.2% from untreated animals, 1.3% from animals treated according to the existing guidelines, and up to 6.7% from animals with off-label use (two implants) [73]. Note, however, that currently used methods are not designed to detect estradiol fatty acid esters, and it is somewhat unclear to what extent glucuronide and sulfate conjugates are hydrolyzed before analysis and subsequently measured [70]. Estradiol fatty acid esters have been found to exert stronger estrogenic effects compared to estradiol-17 β after oral administration, possibly because of slower more sustained absorption and higher bioavailability [74]. There are indications that the metabolism of trenbolone, melengestrol acetate, and zeranol yields a metabolite profile that is much more complex than previously thought. The biological activity of these metabolites has not been thoroughly investigated. Given these data gaps, exposure and risk assessment of hormone and synthetic growth promoter residues in the meat of treated animals is currently impossible. This would be highly desirable, however, given the increasing evidence that estrogens are carcinogens.

The issue of whether hormonal residues in meat, particularly beef, are a human health concern has led to a major trade dispute between the USA and Canada on one hand and the EU on the other hand because the EU refuses to allow the import and sale of meat from hormone-treated animals. In addition to the limited knowledge about metabolites and their relative potencies, the EU Scientific Committee on Veterinary Measures raised the issue of incorrect or off-label use. They deemed such use a common occurrence based on statements made by representatives of the US beef industry and on numerous scientific publications advocating multiple dosing. They further feared that incorrect placement of the hormonal implants could result in their being overlooked at slaughter and could result in contamination levels of whole batches of meat that would far exceed the MRL and, if they reached the customer, might be high enough to cause acute hormonal effects. Even if it could be assumed that hormone residues at the current tolerances and their metabolites are by themselves insufficient to affect human health, the question remains to what extent they interact with the numerous other hormone-like substances we have been and continue to be exposed to from food (and from the endogenous burden we have accumulated over a lifetime) and whether eliminating an entirely avoidable source of hormones would not be a good place to start reducing this exposure.

Antibiotics and other veterinary drug residues

The USFDA and similar regulatory agencies in many other countries require premarket safety and efficacy testing of veterinary drugs analogous to pharmaceuticals for human usage. In addition, in order to ensure the safety of meat products from drug-treated animals, the producers of veterinary drugs have to provide evidence that residues in such meat products do not cause harm to humans. These data are then used not only to establish ADIs where applicable and to set tolerances or MRLs but also to determine withdrawal times. The withdrawal time is the period that is required for the elimination of the antibiotic from the animal's tissue, i.e., the time that has to elapse after the last administration of a particular veterinary drug before the animal can be slaughtered. In the USA, tolerances for veterinary drug residues are listed in the Code of Federal Regulations 21CFR 556. The USA, the EU, and Japan agreed to harmonize their legislation on the requirements for registration of veterinary medicinal products. This has resulted in a series of new guidelines from the Center of Veterinary Medicine of the USFDA (www.fda.gov/cvm/guidance/published.htm) providing details on the genotoxicity, carcinogenicity, and developmental toxicity testing required in the safety evaluation of veterinary drugs. Note that the intestinal microflora has important functions in preventing the colonization of the gastrointestinal tract with pathogenic bacteria and in stimulating the gut-associated immune system. The effects of antibiotic residues in meat on the human intestinal microflora seem to have been rarely considered in earlier toxicological testing of veterinary drugs but are now included in the new harmonized guidelines.

The FSIS/USDA is responsible for inspecting domestic and imported meat for veterinary drug residues. As illustrated in the Red Book published by the FSIS, the detection of veterinary drug residues at levels exceeding their respective tolerances is a rather rare occurrence. For example, only four of 3,372 domestic samples contained antibiotic residues at levels exceeding the tolerance, while 193 (5.7%) were positive but did not violate existing guidelines. This suggests that exposure to antibiotic and other veterinary drug residues is unlikely to cause direct health effects in human consumers.

However, another more indirect effect on human health needs to be considered in the case of antimicrobial drugs. Antibiotic resistance has become a major cause for concern because there are now some microorganisms that are resistant to (almost) every antibiotic currently used in human medicine, making it increasingly difficult—and expensive—to treat certain diseases. It is feared that this development may reach a point where the emergence of antibiotic resistance in pathogenic bacteria outpaces the

development of new antimicrobial agents. Veterinary antibiotics are used not only for veterinary indications, i.e., to treat infections, but are routinely added to animal feed to increase feed efficiency and enhance growth in the USA and other countries. The EU banned all uses of antibiotics as growth promoters in 2006. Although in most countries, antimicrobials used as performance enhancers must not include products that are used for therapeutic purposes in humans and/or animals, they frequently are analogs of therapeutic antibiotics and show cross-resistance. There are numerous lines of evidence strongly suggesting that the high level of antibiotic use in animal husbandry is a major contributor to the emergence, selection, and dissemination of resistant bacteria [75]. As in humans, it has been shown that the introduction of a specific antibiotic into veterinary practice, particularly as an antimicrobial performance enhancer, results in an increase of resistance to this antibiotic not only in pathogenic bacteria but also in the bacteria of the intestinal microflora of the treated animals. It has further been demonstrated that such resistant bacteria can be transferred to humans either by direct contact with the animal, with its fecal matter or with meat products. There are also indications that resistance genes can be transferred between pathogenic bacteria and residents of the intestinal microflora. Thus, the intestinal microflora constitutes an important reservoir of resistance genes and every effort should be made to keep this reservoir as small as possible. It has been estimated that the EU ban on antimicrobials as performance enhancers would reduce antibiotic use in animals by 30–50%. And since 50% of all antibiotics used in the EU were given to animals before their ban as growth promoters, this would result in a considerable reduction of antimicrobial use overall [75]. The USFDA also is concerned that human exposure to antibiotic-resistant bacteria via the ingestion of animal-derived products could reduce or destroy the effectiveness of antimicrobial drugs used to treat human infections. Another one of the new USFDA guidance therefore deals with the types of premarket tests of new veterinary antimicrobials that are required in order to minimize this risk.

Pesticide residues

Organophosphate pesticides

The term pesticide is used to refer to herbicides, insecticides, fungicides, fumigants, rodenticides, and other chemicals designed to destroy or repel pests. According to data published by the USEPA, their use (as active ingredient at user level) exceeded five billion pounds worldwide and 1.2 billion pounds in the USA in each of the years 2000 and

2001. When chlorine/hypochlorites (2.5 billion pounds), wood preservatives, and specialty biocides were included, total usage in the USA alone was almost five billion pounds. At least 75% of this amount is used in agriculture, while the remainder is equally divided between home and garden use and commercial/industrial/government. Application of OP pesticides declined by nearly 45% between 1980 and 2001 but still amounted to 73 million pounds in 2001. This is the first group of pesticides we focus on in this section because it has been the focus of much regulatory attention in recent years, as will be discussed in more detail in the last section of this review.

Exposure to pesticides can occur via inhalation, dermal absorption, and ingestion. What limited data are available on the subject suggest that inhalation makes by far the greatest contribution to aggregate exposure of certain OP compounds (~85%) in adults, with much of the remainder stemming from pesticide residues in solid food. In children, however, solid food may constitute the major route of exposure. In contrast, in their assessment of aggregate (from all exposure pathways and routes) and cumulative (from all OP pesticides) exposure to OP pesticides, the USEPA concluded that food made the major contribution to overall risk in the population in general. However, their data also indicate that whether food, other oral exposure (e.g., hand-to-mouth behavior of small children), drinking water, or inhalation from residential application dominated total exposure depended on the age group examined, geographic region, and time of year and whether the 95th, 99th, or 99.9th percentile was considered. Within the category of residential exposure, inhalation represented the major route of exposure. Note that the USEPA considered only residential exposure arising from the application of pesticides in the home or garden or from pet collars but did not take into account that residents of homes situated near agricultural areas are exposed to significantly higher environmental and residential levels of pesticides, including OP pesticides [76].

Based on food consumption data from two large cohort studies combined with the data from the USFDA Total Diet study, it was estimated that mean daily dietary intakes of chlorpyrifos, diazinon, and malathion were 0.8, 0.5, and 5.5 µg/day for women and 0.9, 0.5, and 6.1 µg/day for men. Analysis of duplicate diet samples indicated an adult dietary chlorpyrifos and malathion intake of 0.5 and 1.3 µg/day, respectively, and a dietary chlorpyrifos intake in children of 0.263 µg/day. The mean dietary intake of total OP and carbamate pesticides expressed in chlorpyrifos equivalents was estimated to range between 5.7 and 13.6 µg/day in the average adult Dane, depending on whether samples below the detection limit were set to zero or one half the limit of quantitation. The corresponding values in the average child were 3.9 to 9.3 µg/day. Recent

estimates of total dietary OP exposure based on probabilistic approaches indicated that the median exposure to OP (in acephate equivalents) and carbamate pesticides (in oxamyl equivalents) was zero for adults and children. At the 99th percentile of exposure, adults consumed 3.9 µg OP per kilogram bw per day and 0.11 µg carbamates per kilogram bw (analogous to 273 and 7.7 µg/day in an adult weighing 70 kg) [77]. The corresponding figures in children aged 1–6 years were 7.9 µg OP per kilogram bw per day and 0.28 µg carbamates per kilogram bw per day.

Pharmacokinetics studies in human volunteers indicate that OPs are readily absorbed after oral administration and quickly metabolized to more polar metabolites, which are then eliminated in urine with half-lives ranging from 2 h for orally administered diazinon up to 27 h for chlorpyrifos. Therefore, biomonitoring potentially provides a method of estimating total exposure to OP pesticides, either by measuring urinary metabolites or by quantifying concentrations of the pesticides themselves and/or some of their metabolites in plasma. Problems associated with the measurement of urinary OP metabolites include that (a) only some of the pesticides in common use have specific metabolites whereas dialkyl phosphate (DAP) metabolites are nonspecific since they can be derived from a wide variety of OP compounds; (b) data on the fractional absorption and excretion are largely unavailable; and (c) urinary metabolites can arise either from a parent compound or from direct exposure to its metabolites. Furthermore, unless 24-h urine samples are obtained, urinary metabolite concentrations need to be corrected for dilution, but the appropriate method is still a matter of debate.

OP pesticides irreversibly inhibit the enzyme acetyl cholinesterase (AChE). Inhibition of AChE at neuronal junctions results in the accumulation of acetyl choline (ACh) and continued neurotransmission. Since the autonomic, somatic, and central nervous system all use ACh, the acute symptoms of OP-mediated AChE inhibition are manifold and include dizziness, headache, confusion, convulsions, blurred vision, respiratory distress, bradycardia and hypotension, fatigue, weakness, ataxia, muscle cramps, and increased lacrimation and salivation.

Neurodevelopmental toxicities In experimental animals, gestational or early postnatal exposure to OP pesticides at levels that inhibit AChE to a minor extent (~20%) and are not associated with overt systemic toxicity results in impaired neurodevelopment. In addition to brain AChE and choline acetyltransferase inhibition, a variety of mechanisms may contribute to the neurodevelopmental effects of OP pesticides. These include alteration of muscarinic receptor function and permanent reduction in the density of muscarinic cholinergic receptors, altered synaptic development and function that can persist into

adulthood, decreased expression and activity of multiple components of the adenyl cyclase cascade, impaired DNA and RNA synthesis, and reduced cellularity and brain weight in offspring.

The detection of pesticides or their metabolites in human amniotic fluid and meconium and strong correlations between OP pesticide/metabolite concentrations in maternal and umbilical cord plasma indicate that human exposure to these compounds can begin in utero. In recent years, the neurodevelopmental effects of OP pesticide exposure have been examined in several birth cohorts that recruited mothers with high levels of exposure due either to frequent pesticide applications in the home (as in two cohorts from New York City) or to their extensive use in surrounding agricultural areas (as in the CHAMACOS project in the Salinas Valley, CA, USA).

In neonates of the CHAMACOS cohort, maternal urinary DAP metabolites were significantly associated with an increased number of abnormal reflexes (failure to respond or hypoactive response) and with the proportion of neonates with more than three abnormal reflexes [78]. Interestingly, the association differed depending on the age at the time of the behavioral assessment. The association was negative in neonates examined after the age of 3 days but unexpectedly positive in infants assessed within the first 3 days of life. Although this suggests some caution in the interpretation of these results, they were essentially replicated in one of the New York cohorts, i.e., maternal urinary concentrations of DAP metabolites as well as malathion dicarboxylic acid (a specific metabolite of malathion) were significantly associated with the number of abnormal reflexes in neonates [79].

At 3 years of age, children from New York City with high levels of gestational OP pesticide (specifically chlorpyrifos) exposure were significantly more likely to exhibit mental and psychomotor developmental delays, attention problems and attention-deficit/hyperactivity disorder, and pervasive developmental disorder problems [80]. Note that the children's own exposure during the first 3 years of life was not taken into account in this study. In the CHAMACOS cohort, gestational OP pesticide exposure was associated with lower scores in assessments of mental development, the association reaching significance by the age of 24 months, but not at 6 or 12 months of age [81]. No association with attention problems was detected at any age. Interestingly, postnatal OP exposure (as assessed by metabolites in spot urine samples obtained at 6, 12, and 24 months of age) showed a positive association with mental development scores. The authors hypothesized that children with higher cognitive functioning may have more interactions with their environment that would expose them to higher pesticide levels or that better diets with higher levels of fruits and vegetables may result in improved

cognitive function despite the potentially higher pesticide residue intake. Most importantly, however, it needs to be taken into account that spot urine samples do not provide a good estimate of chronic pesticide exposure, as shown by the high day-to-day variability in most biomonitoring studies.

Of particular note, exposure to a variety of other compounds known or suspected to affect neurodevelopment was measured and controlled for in the CHAMACOS study, including PCBs, β -hexachlorocyclohexane, hexachlorobenzene, DDT, and its breakdown product DDE [81]. There was no statistically significant interaction between DDT and prenatal OP exposure (maternal urinary DAP metabolites), indicating that the effects of the two exposures are independent. This is of particular interest since a previous study in the same cohort had revealed an association between prenatal DDT exposure (maternal serum DDT levels) and mental development scores at 12 and 24 months of age [58].

Pregnancy outcomes There are several reports that OP pesticide exposure during pregnancy (as assessed by metabolite concentrations in one or two spot urine samples) is associated with decreased birth weight and length [82,83]. In contrast, in the CHAMACOS cohort, maternal urinary DAP metabolites were associated with a significant increase in head circumference and a marginally significant increase in birth length [84]. The dimethyl phosphate subset of DAP metabolites, but not total DAP, and cord blood cholinesterase activity were significantly associated with decreased gestational duration.

Contaminants formed during food production and cooking processes

A variety of chemicals are formed in the food production process or during cooking. These include polycyclic aromatic hydrocarbons, which occur mainly in smoked and grilled meat, i.e., in situations where combustion products come into direct contact with food. The highly carcinogenic benzo(a)pyrene is a major representative of this group. In addition, heterocyclic aromatic amines are formed in heated meat products and have been implicated in causing mammary and colorectal cancer. Furthermore, nitroso compounds, such as *N*-nitrosamine, *N*-nitrosamide, and related substances, result from the reaction of the preservatives nitrate or nitrite with secondary amines. Carnitine, choline, citrulline, creatine, creatinine, pyrrolidine, or trimethylamine all constitute relevant precursors for this reaction in foods. Many of the nitroso compounds are potent genotoxic carcinogens in laboratory animals. In the following, we focus on some other chemicals that arise

from cooking processes and that have been the particular focus of risk assessment efforts in recent years.

Acrylamide ($\text{CH}_2=\text{CH}-\text{CONH}_2$) is produced industrially to synthesize polyacrylamide, which is used as a soil conditioner, for water treatment, in grouting compounds, in gels used for electrophoresis, and numerous other applications in the paper, textile, and cosmetic industries [85]. In addition, it is present in cigarette smoke. In 2002, the Swedish National Food Administration and researchers from Stockholm University announced the discovery that acrylamide also can be formed when certain types of food are fried, deep-fried, or baked at high temperatures ($>140^\circ\text{C}$) [86]. Since then, it has become widely recognized that particularly high levels are present in French fries and potato chips. Considerable concentrations have also been found in bread, cookies, crackers, cereals, and also in coffee, but lower levels of acrylamide can be detected in numerous other foods. It is generally accepted that the acrylamide in food is mostly formed from the reaction of asparagine with reducing sugars. More recently, acrylamide has also been detected in prune juice and black olives, indicating that other pathways can lead to the formation of this substance. Since the discovery of acrylamide formation in foods, numerous western countries have collected data on the dietary intake of this chemical [87]. These exposure assessments were based on national food surveys that were not specifically intended to assess dietary acrylamide intake. The intake levels determined in these surveys were then linked with data on acrylamide concentrations in a limited number of food groups known to contain high levels of this compound. Therefore, the resulting data need to be interpreted with some caution. The USFDA estimated mean dietary acrylamide intake of adults in the USA to be $0.4\mu\text{g/kg}$ bw per day. This is in close agreement with estimates from other western nations, for example $0.2\text{--}0.4$ in EU countries overall, 0.5 in France, 0.45 in Sweden, or 0.48 in the Netherlands. The 95th (or 90th) percentile ranged from 0.6 to 1.1 . Note, however, that the mean (and 90/95th percentile) daily intake of children is generally estimated to be twofold to threefold higher, being 1.07 (2.31) $\mu\text{g/kg}$ bw per day in small children (aged 2–5 years) in the USA. Other subpopulation may also have high intakes, as indicated by the observation that the 15–18-year-old age group in Germany had a mean (95th percentile) dietary exposure of 1.1 (3.4) $\mu\text{g/kg}$ bw per day. Using a semiprobabilistic approach, the mean dietary exposure of the UK adult population was calculated to be $0.56\mu\text{g/kg}$ bw per day [88]. A probabilistic model yielded an estimate of $0.59\mu\text{g/kg}$ bw per day in the Irish adult population [88]. These numbers provide only rough estimates since there is considerable variability in acrylamide content between products of the same category of food and even between

lots of the same food because the formation of acrylamide depends on cooking temperature, cooking time, and the composition and other characteristics of the specific food. In addition, the analytical method has been found to have a significant influence on the test results.

What foods are the main contributors to dietary acrylamide intake in a particular population depends on eating habits and the way foods are processed and prepared, although a considerable portion derives from French fries and other potato products in most Western nations (e.g., 35% in the USA, 50% in the Netherlands, 30% in Norway, and 26% in Sweden) [87]. In contrast, acrylamide from coffee constitutes only 7% of total dietary intake in the USA but is the major contributor (39%) in Sweden.

Acrylamide is well absorbed by ingestion, inhalation, and after dermal application. Studies in mice and rats indicate greater bioavailability when administered as a bolus by gavage than when given as part of the diet, which is usually consumed over several hours. There are two major metabolic pathways: (a) conjugation to glutathione and (b) oxidation to its epoxide glycidamide, which in rodents is mediated by CYP2E1. Like acrylamide, glycidamide can be conjugated with glutathione. Both glutathione conjugates are excreted in urine as mercapturic acids.

Urinary metabolites can be used to assess acrylamide exposure over the last 24 h since acrylamide metabolites have half-lives of elimination between 14 and 26 h [89]. Both acrylamide and glycidamide form hemoglobin adducts, and these can be used to estimate total exposure over the last 2–3 months (the average lifetime of an erythrocyte). There is fairly good agreement between studies in various nonsmoking western populations that median excretion of urinary metabolites is approximately $30\text{--}40\mu\text{g/l}$ for acrylamide mercapturic acid and $3\text{--}9\mu\text{g/l}$ for glycidamide mercapturic acid. Median hemoglobin adduct levels in such populations range between 26 and 37 pmol/g hemoglobin for acrylamide and between 18 and 34 pmol/g hemoglobin for glycidamide. Estimates of dietary intake calculated from urinary metabolite excretion or hemoglobin adduct levels suggest median acrylamide exposures in a similar range as obtained in dietary survey studies, namely median levels of $\sim 0.45\mu\text{g/kg}$ bw per day from urinary metabolites and of $0.51\mu\text{g/kg}$ bw per day from hemoglobin adducts [90].

Glycidamide is genotoxic, whereas acrylamide itself is not, and glycidamide is thought to be responsible for the carcinogenic effects of acrylamide exposure in laboratory animals. The IARC has classified acrylamide as a probable human carcinogen (group 2B). Recently, and particularly in the last few months, numerous studies have been published on the possible association between acrylamide exposure as measured by dietary intake assessment and the risk of various cancers. For the most part, acrylamide exposure was not found to be associated with cancer of the

gastrointestinal tract (colorectal, gastric, pancreatic, or esophageal), brain, prostate, endometrium, or breast. One exception comes from a case-cohort that was part of a large prospective Dutch cohort study, in which dietary intake of acrylamide was associated with postmenopausal endometrial and ovarian cancer, but not breast cancer, particularly in nonsmokers [91]. In the same cohort, the highest compared to the lowest quintile of dietary acrylamide intake conferred a slightly but significantly increased risk of renal cell but not bladder or prostate cancer [92]. Note that many of these studies may have lacked the statistical power to detect the small increase in cancer risk that may be expected in association with acrylamide exposure. In addition, the wide distribution of acrylamide in foods and the high variability of its concentrations even within foods of the same category make exposure misclassification likely when dietary intake is used as a measure of exposure. In the only study to date to use hemoglobin adducts as a measure of acrylamide exposure, this exposure was positively associated with estrogen-receptor-positive breast cancer, though not with estrogen-receptor-negative breast cancer or breast cancer overall in a case-control study within a large prospective cohort study in Denmark [93].

As previously mentioned, glycidamide rather than acrylamide itself is thought to be responsible for acrylamide-induced carcinogenesis in rodents. It was recently shown that when humans and rodents received similar doses of acrylamide (corresponding to the mean daily intake in humans), only ~9% of the relevant metabolites excreted in urine were derived from glycidamide in humans, whereas they constituted at least 45% of the determined metabolites in rats [89]. If glycidamide is the proximate carcinogen of acrylamide, these results suggest that humans are at considerably lower risk of acrylamide-induced carcinogenesis due to this greatly reduced ability for acrylamide bioactivation. Note, however, that there appears to be considerable interindividual variation in the ratio of glycidamide mercapturic acid: acrylamide mercapturic acid excretion, as indicated by the finding that this ratio ranged from 0.004 to 1.4 with a median of 0.3 in 91 nonsmoking individuals with background levels of acrylamide exposure [90]. Of particular note, this ratio was found to be significantly higher in children aged 6–10 years than in adults, and they also had a significantly higher ratio of glycidamide hemoglobin adducts to acrylamide hemoglobin adducts.

Recently, physiologically based pharmacokinetic/pharmacodynamic modeling based on hemoglobin adduct formation in conjunction with probabilistic estimate of dietary exposure indicated that the lifetime excess cancer risks for mammary, mesothelioma, thyroid, central nervous system, and thyroid tumors were in the range of $1\text{--}4 \times 10^{-4}$ [94]. In addition, it is known from occupational exposure

that acrylamide is a neurotoxin, and this is also observed in animals. However, the results of physiologically based pharmacokinetic/pharmacodynamic modeling based on tissue concentration of acrylamide or its metabolite glycidamide indicated that the exposure levels arising from dietary consumption of acrylamide were not associated with significant human risk [94].

Acrolein Acrolein is an intermediate in the industrial production of acrylic acid but is also released in combustion processes (including tobacco smoking) and may be an intermediate in the formation of acrylamide in foods. It is formed from carbohydrates, vegetables oils, animal fats, and amino acids during the heating of foods. In particular, the heating of cooking oil to temperatures $>180^\circ\text{C}$ generates considerable amounts of acrolein, and it has been estimated that the emissions from commercial kitchens may exceed those of vehicles. Another important source of exposure is tobacco smoke. Furthermore, acrolein is produced endogenously, especially in situations of oxidative stress. The EPA estimates that human exposure stems mostly from the atmosphere, but set an oral Rfd of $5 \times 10^{-4} \text{ mg/kg}$ per day.

Furan Furan (not to be confused with the PCDFs sometimes referred to as “furans”) is another chemical that arises from heat treatment of certain foods, mostly in brewed coffee, but also detected in chili, cereals, salty snacks, soups containing meats, and a variety of other items. The USFDA estimates mean intakes to be $0.26 \mu\text{g/kg}$ bw in the general population, the 90th percentile of intake being $0.61 \mu\text{g/kg}$ bw per day. Infants are estimated to ingest a mean of $0.41 \mu\text{g/kg}$ bw per day, with the 90th percentile being $0.99 \mu\text{g/kg}$ bw per day. A European analysis of 273 baby food items indicated furan concentrations from below the detection limit to $112 \mu\text{g/kg}$. Assuming a daily consumption of 234 g of baby food, this would result in exposures ranging from <0.03 to $3.5 \mu\text{g/kg}$ bw per day in a 6-month-old baby.

Furan is a small volatile compound that can pass through cell membranes and is easily absorbed after dietary ingestion or inhalation and most likely also through the skin. It is metabolized by cytochrome P450 (CYP2E1) in the liver, the primary metabolite being carbon dioxide, whereas the key cytotoxic metabolite is *cis*-2-butene-1,4-dial, a reactive dialdehyde. Effective first pass metabolism and subsequent excretion are thought to largely prevent furan from being distributed to other tissues, but after administration of radiolabeled furan, about 15% of a single dose is detected in tissues, mostly in liver, lower amounts in kidney, still less in large intestine, and some in small intestine, stomach, blood, and lung. Within 24 h after administration, about 80% of the dose is eliminated via lung, urine, and feces, and radiolabel becomes essentially undetectable 1 week after a single dose. After repeat

administration, furan derivatives accumulate mostly in the liver and kidney.

The main reactive metabolite of furan, *cis*-2-butene-1,4-dial, binds to proteins and nucleosides and is mutagenic and genotoxic in vitro, whereas furan itself is only genotoxic. Orally administered furan is clearly carcinogenic in rats, resulting in dose-dependent increases in hepatocellular adenomas and carcinomas as well as cholangiocarcinomas. In rats, it also dose-dependently induces mononuclear leukemia. A number of alkyl furans, dihydrofurans, furanones, and tetrahydrofurans can occur in food, but their metabolism is thought or has been shown not to yield reactive dialdehydes similar to *cis*-2-butene-1,4-dial. They are therefore deemed unlikely to exhibit similar toxicities as furan. The oral Rfd established by the USEPA is 0.001 mg/kg bw per day.

Chloropropanols The Chloropropanols, 3-chloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropanol (1,3-DCP), were first detected in acid-hydrolyzed vegetable protein, which is frequently used in soups, gravy mixes, stock cubes, and savory snacks. These chemicals are a by-product of the production process of hydrolyzed vegetable proteins, which involves hydrochloric acid treatment at high temperatures, resulting in the formation of chloropropanols from fats and oils. In addition, 3-MCPD has been detected in soy sauce and a variety of other foods, including toasted bread, grilled cheese, and fried batters. Their formation in these foods is not well understood.

Recently, the EU asked member states to assess the dietary exposure to 3-MCPD of their inhabitants. This revealed that consumption from soy sauce alone ranged between 0.108 and 1.08 µg/kg bw in different countries, and in some states, soy sauce contributed 70% of total 3-MCPD intake. In other member states, sauces were the main source of 3-MCPD exposure, and in Denmark, bread constituted one third of total dietary intake. 3-MCPD is carcinogenic in rats at high doses, but not via genotoxic mechanisms. The EU set a TDI for 3-MCPD of 2 µg/kg bw per day. In contrast, 1,3-DCP is a genotoxic carcinogen for which the FAO/WHO recommends keeping the levels in food as low as technologically achievable.

Contaminants arising from the packaging or packaging process

Phthalates

Phthalates are dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid. They are used as plasticizers in plastic and PVC products, which have a wide range of applications, including floor tiles, vinyl upholstery, toys, disposable medical examination and surgical gloves, medical

tubing, blood storage bags, food wraps, and components of paper and paperboard. In addition, phthalates are used as fixatives, detergents, lubrication oils, and solvents, in cosmetics and personal care products, and in medications. Since phthalates are not covalently bound to plastic and PVC-based products, they leach and vaporize from these materials over time. As a result, these chemicals are ubiquitous, and contamination of laboratory equipment makes it difficult to obtain reliable measurements of phthalate concentrations in food, other consumer products, air, and dust. Determination of phthalate metabolites in urine has become the method of choice for assessing total phthalate exposure, but this makes it impossible to establish what route and what products make the major contribution to total exposure. In addition, extrapolation of daily phthalate intake from urinary excretion of their metabolites is hampered by several factors. Urinary phthalate metabolite levels show high day-to-day and month-to-month variability. Depending on the phthalate metabolite, up to four samples obtained 1–3 months apart may be necessary to classify exposure with a reliability of 80%. Furthermore, data on the fractional excretion of urinary phthalate metabolites are extremely limited, and the use of different published fractional excretion factors can result in up to tenfold differences in the median daily exposure estimate of specific phthalates.

Phthalate exposure can occur via ingestion, inhalation, and dermal absorption. What limited data are available on the subject suggest that dietary intake generally represents the major route of exposure [95], with fatty foods, such as dairy, fish, meat, and oils containing the highest levels. Inhalation may contribute considerably to exposure to certain phthalates, such as di-*n*-butyl (DBP), benzyl butyl (BBP), and diethyl phthalate (DEP) [96]. Estimates of daily phthalate intake derived from biomonitoring studies are summarized in Table 6, and the tolerable intake values from various regulatory agencies are provided in Table 7. Note that German children were recently found to exceed the TDI set by the EFSA for DBP, whereas none exceeded the TDI of 500 µg/kg per day or the USEPA Rfd of 200 µg/kg per day for BBP [97].

Phthalates appear to be readily absorbed after gut lipases and esterases hydrolyze them into their more easily absorbable monoesters. The relatively polar and low molecular weight phthalates are conjugated to glucuronides, whereas a large proportion of the monoesters of phthalates with higher molecular weights, such as di-(2-ethylhexyl) phthalate (DEHP), di-*n*-octyl phthalate, and di-isononyl phthalate (DINP) undergo rather extensive oxidation before glucuronidation. Despite their lipophilic nature, phthalates are metabolized and excreted via urine and, to a lesser extent via the feces, within 3 days; consequently, bioaccumulation is not thought to occur.

Table 6 Estimated median or geometric mean (95th percentile) daily phthalate intake in microgram per kilogram body weight per day

Phthalate	US, NHANES [212]	Germany, general population [213]	Germany, children (2–14years) [97]	Germany, students [214]	Germany, duplicate diet [215]
DEHP	0.60 (3.05)	10.3 (38.3)		2.4 (5.7)	2.43 (3.95)
DEP	12.34 (93.33)	2.32 (22.1)			0.11 (0.18)
DBP	1.56 (6.87)	5.22 (16.2)	4.07 (14.9)	1.9 (5.3)	0.26 (1.35)
BBP	0.73 (3.34)	0.60 (2.52)	0.42 (2.57)	0.22 (0.910)	0.23 (0.38)
Di- <i>n</i> -octyl phthalate		< LOD (0.42)			
DINP	0.21 (1.08)			0.4 (1.5)	

Reproductive toxicity It is extensively documented that certain phthalates exhibit toxicities in the male reproductive system of rats, mice, rabbits, and to a much lesser degree in hamsters. In particular, gestational exposure to DEHP, BBP, DBP, dipentyl phthalate (DPP), and, with far lesser potency, DINP induces defects of the male reproductive organs. These defects have been called the “phthalate syndrome,” which consists of hypospadias, cryptorchidism, gubernacular agenesis, testicular atrophy, underdeveloped or absent epididymis, irreversible degeneration and atrophy of seminiferous tubules, reduced anogenital distance, and retained nipples/areolae. Many of these effects are attributable to the marked decreases in testicular and systemic testosterone production that are also observed in rats that were exposed to phthalates in utero and that constitute one of the most sensitive end points in the assessment of the male reproductive toxicities of phthalates. Other sensitive indicators include decreases in sperm count and motility, and an increased incidence of morphologically abnormally sperm. Of particular note, phthalates are unique in that they are the only group of endocrine disrupting chemicals investigated to date that causes agenesis of the gubernacular cords. The development of this tissue depends on insulin-like hormone 3 (InsI3), a hormone that is produced by Leydig cells. It has been shown that gestational exposure to phthalates with male reproductive toxicity results in pronounced reductions

in InsI3 mRNA and protein production. Effects on reproductive parameters have also been observed in rats following administration during prepuberty, while adult exposure results in adverse effects on the male reproductive system only at very high doses. Interestingly, testosterone production may be affected differentially depending on the exact timing of exposure.

Human relevance The proximate developmental toxicants of phthalates in rats and mice are thought to be their respective monoesters, although recent data suggest that some of the secondary oxidative metabolites may exhibit even greater toxicity. Earlier data indicated that, at equivalent doses, primates had lower serum concentration of mono(2-ethylhexyl) phthalate (MEHP, the monoester of DEHP) than rats. From these data, it would be expected that humans would also be exposed to lower internal doses after oral DEHP intake compared to rats. However, this was not confirmed in a study of a single human volunteer. Comparison of the results obtained in this volunteer with data from marmosets and rats suggests that human tissues may actually be exposed to higher levels of MEHP.

It has been proposed that the unconjugated monoesters are the mediators of reproductive toxicity in rats since monoesters undergo little glucuronidation in this species. In contrast, in humans, the majority of phthalate monoesters and even the secondary metabolites are present in the form of glucuronides in urine and possibly in serum. However, there are indications that the percentage of free phthalate monoesters rises with increasing exposure within the range found in the general population. In addition, there seems to be considerable variability between subjects in the degree of conjugation. In conclusion, there is reason to believe that at least certain more highly exposed subgroups of the general population and/or those with low capacity for glucuronidation may be at risk for adverse health effects from exposure to certain phthalates. Accordingly, numerous regulatory agencies have affirmed that the animal data on reproductive toxicity of certain phthalates are likely to be relevant to humans.

Further confirmation comes from studies investigating the correlation between phthalate exposure and a variety of

Table 7 TDI values for phthalates in microgram per kilogram body weight

Phthalate	SCF/EFSA	EPA (oral Rfd)	Health Canada
DEHP	50	20	44
DBP	10	100 ^a	63
DEP	200	800	
BBP	500 ^b	200	
DMP (dimethyl phthalate)	50		
DINP	150		

^a 300 are proposed, but not yet finalized

^b Increased from a previous TDI of 100 µg/kg bw

health outcomes. In adult men, an inverse association has been reported between exposure to certain phthalates, measured as their urinary phthalate metabolite levels, and semen quality, such as sperm concentration and motility and frequency of DNA damage in sperm [98–100]. Changes in reproductive hormone levels in association with urinary phthalate metabolite levels have also been observed, but the results are inconsistent, and the associations with several hormones exhibited unexpected patterns and directions [101,102]. In another study, maternal urinary concentrations of four phthalates metabolites (monoesters of DEP, BBP, DBP, and di-isobutyl phthalate) during pregnancy were inversely associated with anogenital distance in their male children examined at the age of 2–36 months [103]. Somewhat surprisingly, another group of researchers observed a significant inverse association between amniotic fluid phthalate metabolite levels (monobutyl) and anogenital distance only in female, but not in male, neonates [104]. In addition, increased urinary phthalate metabolite concentrations have been associated with decreases in the thyroid hormone thyroxine, which prompted an investigation of a possible association between maternal phthalate exposure during pregnancy and behavioral outcomes in neonates [105]. Prenatal phthalate exposure was significantly associated with decreased quality of alertness and orientation scores in female neonates only.

Note that almost all of these studies used single measurements of urinary phthalate metabolite levels. Given the relatively high day-to-day variability of this parameter, a single spot urine sample is unlikely to accurately reflect chronic phthalate exposure. This may account for the unexpected patterns and directions of some of the reported associations. At the same time, it raises some doubts concerning the validity of the other observed correlations. Nonetheless, a somewhat more reliable indication that phthalates can affect human sex hormone production comes from a study that investigated possible associations between six phthalate monoester concentrations in mothers' breast milk collected as additive aliquots during months 1–3 postnatally and serum concentrations of reproductive hormones in their male children [106]. The observed correlation of certain monoesters with sex hormone-binding globulin and with the ratio of luteinizing hormone to free testosterone suggested that neonatal phthalate monoester exposure via breast milk has subtle but significant and dose-dependent effects on the levels of reproductive hormones in male infants.

Bisphenol A

Bisphenol A is a high-volume chemical used as the monomer to synthesize polycarbonate plastic and epoxy

resins, which have numerous applications, including polycarbonate baby feeding bottles and other plastic storage and heating containers for food and beverages and epoxy lining of metal cans. It is an environmental pollutant that can be detected in water, including drinking water, soil, house dust, and residential air. However, the limited data available on aggregate exposure estimates suggest that the vast majority of human exposure occurs via oral intake of foods contaminated with bisphenol A leaching from plastic food and beverage containers [107]. Note, however, that there are indications that the bisphenol A concentrations in human blood are much higher than would be expected from what is known about rodent and human metabolism and clearance of this chemical. This suggests that not all sources and routes of human exposure have been fully accounted for [107].

There is enormous variability in the estimates of bisphenol A exposure from food alone based on food intake data combined with values measured in certain types of food. Data collected by the USFDA indicate an intake of 0.183 $\mu\text{g/kg}$ bw per day in US adults, which is lower than estimates reported from the EU (0.37 to 1.4), Japan (0.002 to 0.43), or New Zealand (4.8 $\mu\text{g/kg}$ bw per day). Exposures of infants and children are generally much higher, with the USFDA estimate for infants being 1.75 $\mu\text{g/kg}$ bw per day, similar to the 1.6 $\mu\text{g/kg}$ bw per day reported from the EU, whereas older infants and children aged 4–6 years were estimated to ingest 0.8 and 1.2 $\mu\text{g/kg}$ bw per day, respectively. Estimates based on urinary excretion of bisphenol A after hydrolysis of conjugates suggest considerably lower daily intakes of 0.047 $\mu\text{g/kg}$ bw per day for the US population overall, 0.065 in children aged 6–11 years, and 0.71 in adolescents [108].

The bioavailability of bisphenol A is much lower after oral ingestion than after intravenous or subcutaneous administration in rodents and primates. The concentrations of unconjugated (free) bisphenol A measured in human tissues and fluids range from 0.3 to 4.4 ng/ml even excluding studies that used enzyme-linked immunosorbent assay (ELISA; which is considered to yield overestimates due to unspecific binding) [107,109]. Of particular note, this chemical can be detected in a vast majority of fetal cord blood samples at rather high levels (up to 4 ng/ml in studies using chromatographic methods). Free bisphenol A concentrations in human breast milk of 0.61 to 1.3 ng/ml have been reported, with total bisphenol A levels in these and other analyses between 0.46 and 3.41 ng/ml. Note that some of the variability may be due to differences in the analytical methods.

In humans, other primates, and rodents, there is extensive first-pass metabolism of bisphenol A yielding mostly glucuronide conjugates with a minor amount of sulfate conjugates and little free parent compound remaining in the circulation. In rodents, there is extensive

enterohepatic recirculation resulting in prolonged exposure to bisphenol A, including the free form. In contrast, humans and other primates do not show enterohepatic recirculation but instead rapidly excrete bisphenol A metabolites in urine. Some regulatory agencies consider this to indicate that adult exposure to the low levels of bisphenol A in foods is not a major concern. However, there are indications that human serum concentrations of free bisphenol A are in the same range as those measured in rats after acute dosing [107]. In addition, the enzymes involved in the detoxification of bisphenol A are not fully developed in the fetus and neonate so that exposure in this age group may result in higher levels of free parent compound and increased toxicity.

Toxicities Bisphenol A is a selective estrogen receptor modulator that is several orders of magnitude weaker than the endogenous estrogen 17 β -estradiol in some outcomes but shows equal efficacy and potency in others. Earlier studies with bisphenol A at high doses indicated the lowest observed adverse effect level of 50 mg/kg per day. However, there are now >100 studies in rodents on the neuroendocrine and reproductive toxicity of prenatal, postnatal, and adult exposure to lower doses of this compound [110]. “Low dose” refers to doses within the range of typical (nonoccupational) human exposures or doses below the current USEPA oral Rfd of 50 μ g/kg bw per day. The results of these studies indicate that gestational and lactational exposure to bisphenol A, including via the oral route, can result in a number of organizational effects. The term “organizational effect” refers to persistent alterations of an organ or system that result from exposure solely during organ development. The disturbances arising from prenatal or perinatal bisphenol A exposure include alterations in brain structure and chemistry that are associated with impaired learning, mating, and maternal behavior. There is also extensive evidence that gestational exposure to low-dose bisphenol A affects the male reproductive organs, decreasing testicular testosterone levels, the weights of epididymis and seminal vesicles, and daily sperm production but increasing the weights of prostate and preputial glands. Decreased testosterone and sperm production have also been observed in juvenile and adult rodents treated with bisphenol A. In addition, there are some studies showing effects of gestational bisphenol A exposure on the female reproductive tract, in particular early onset of puberty. More limited and at times controversial data suggest that bisphenol A can affect a variety of immune parameters. Specifically, this chemical was found to increase antigen-specific interleukin (IL)-4 and IL-10 production in animals primed with a variety of antigens and to also increase serum concentrations of antigen-specific IgE. Results on other cytokine and antibody responses were somewhat more conflicting, possibly

reflecting differences in the route, timing, and duration of exposure. Of particular note, dietary bisphenol A was found to decrease IFN- γ synthesis of mitogen-stimulated splenic mononuclear cells in association with reduced IgG2a production and partial protection from glomerulonephritis in the NZB/WF1 model of lupus.

Several governmental or supragovernmental agencies have commissioned risk assessments of bisphenol A, including the USA (CERHR Expert Panel for the National Toxicology Program (NTP)/National Institutes of Health (NIH)/Department of Health and Human Services), the EU (EFSA), and Japan. In addition, the National Institutes of Health/Department of Health and Human Services together with the USEPA recently sponsored a meeting on bisphenol A health risk assessment. The conclusions largely depended on the interpretation of the available data on pharmacokinetics in laboratory animals and humans and on the numerous low-dose studies conducted in recent years.

The EFSA concluded that the low-dose effects reported in some studies were not robust and reproducible. It further decided that recent data on major species differences between rodents and humans in the metabolism of bisphenol A justified reducing the uncertainty factor from 500 to 100 and therefore of establishing a fivefold higher full TDI of 0.05 mg/kg per day instead of the previous temporary TDI of 10 μ g/kg bw per day. The NTP concluded that there was minimal to negligible concern over adult exposures but expressed some concern over possible neurological, behavioral, and prostate gland toxicities of bisphenol A in fetuses, infants, and children.

In marked contrast, the participants of the meeting sponsored by the NIH and USEPA deemed the evidence convincing that the mean concentrations of unconjugated bisphenol A in humans correspond to the circulating levels of bisphenol A seen in rats after acute low-dose oral exposure. They expressed the same level of confidence in data showing that concentrations in the fetal mouse after a maternal dose that produced adverse effects in numerous experiments (25 μ g/kg bw) were within the range of unconjugated bisphenol A concentrations in human fetal blood. They further felt confident that the currently available data provide evidence for organizational changes in the prostate, breast, testis, mammary gland, brain structure, and chemistry and behavior after prenatal and/or neonatal exposure and for substantial neurobehavioral and reproductive effects after adult exposure in laboratory animals. The experts also deemed certain other effects of bisphenol A “likely but requiring confirmation.” These included the reported effects of adult exposure to low-dose bisphenol A on the immune system, brain, and female reproductive system [110]. Whether and how these findings should be used in setting a TDI for bisphenol A was not addressed at this meeting.

Semicarbazide

Azodicarbonamide is used in the manufacture of foamed plastic seals in the metal lids of glass jars and is used as a blowing agent in other food packaging materials. This chemical partially degrades during heat processing to form semicarbazide. In addition, semicarbazide is a known metabolite of the illegal veterinary drug nitrofurazone and has been detected in seaweed derived products (e.g., carrageenan), but these sources are unlikely to make major contributions to dietary exposure. Semicarbazide is detectable in essentially all foods sold in glass jars, but baby foods were found to contain the highest concentrations (up to 54 µg/kg). The dietary exposure from baby food was estimated to be 0.5 µg/kg bw per day for 0–12-month-old babies in the USA; 0.4 and 0.66 µg/kg bw per day for Canadian babies aged 1–3 months and 6–9 months, respectively; and between 0.23 and 0.53 µg/kg bw per day for European babies [111]. In laboratory animals, semicarbazide exposure in utero or postnatally inhibits bone mineralization and leads to skeletal deformities. In addition, it is weakly carcinogenic. Various regulatory agencies, including the USFDA, Health Canada, and WHO, do not consider the levels of dietary exposure as a human health threat since they are three to four orders of magnitude lower than the NOAEL in animal studies. Nonetheless, since baby foods from glass jars may lead to high exposures in infants, who eat more food per unit body weight, it is strongly recommended that azodicarbonamide be replaced by other suitable substances. The EU banned the use of azodicarbonamide in the sealing of food jars until more is known about the toxicology of semicarbazide.

Food allergens

Food allergies are adverse immunologic reactions to otherwise harmless food constituents, almost always proteins, and are most commonly IgE-mediated but also include cell-mediated delayed hypersensitivity responses, most frequently gluten-sensitive enteropathy that affects just under 1% of the population (celiac disease). The prevalence of food allergies, excluding celiac disease, has been estimated to be 5–8% in children under the age of 4 years and 3–4% in adults [112].

For those affected by severe, life-threatening food allergies in which even a trace amount of a food may trigger an anaphylactic reaction, food allergy is a significant food safety issue. Approximately 150 people are believed to die from food allergy each year in the USA, but accurate numbers are not available. Food allergy is the most common cause of emergency room visits for anaphylaxis. A wide variety of foods have been shown capable of

eliciting IgE-mediated allergic responses, but approximately 90% of food allergies are due to eight foods: cow's milk, hen's egg, peanut, soy, wheat, tree nuts, shellfish, and fish. However, the most commonly implicated foods will vary by geographic region based on dietary preferences and even pollen counts, since some food allergy to fruits, vegetables, or nuts is due to IgE antibodies cross-reacting with pollen allergens, with the pollen as the primary sensitizer. For example, in Switzerland, hazelnut allergy is the most common food allergy in adults and is due to cross-reacting IgE to a birch pollen pathogenesis-related protein [113]. In Israel, where sesame is incorporated prominently in the diet, sesame seed is the most common cause of anaphylaxis in young children, and peanut allergy is rare [114]. In Singapore, edible bird's nest soup is commonly implicated in pediatric food allergy, while in Japan, buckwheat is an important cause of food allergy in school-age children [115,116]. Thus, although there are some regional differences, internationally, the Codex Alimentarius Commission recommended in 1999 that member countries adopt the list of eight common foods and take steps to ensure that manufacturers within member nations list these foods or ingredients derived from these foods on labels [117]. These eight foods were the focus of subsequent US legislation, known as the Food Allergen Labeling and Consumer Protection Act [118], that took effect in 2006. Under this legislation, all products containing "Big Eight" foods require clear, "plain English" labeling designed to help consumers navigate the confusing world of ingredient lists. For example, "non-dairy soy cheese" made from soybean, but also containing added casein, was previously allowed to list "casein" as an ingredient without disclosing that this is a cow's milk protein. To a parent of a child diagnosed with severe cow's milk allergy, this might have appeared an excellent substitute for regular cheese—with horrific consequences. The European Union, Australia, New Zealand, Canada, and Japan have additionally published labeling guidelines designed to ensure the safety of the food-allergic consumer [119–122]. Table 8 lists the requirements in the different countries or economic blocks.

Exceptions to the US labeling rule are allowed by a petition and notification processes, similar to exceptions approved within the evolving framework of the recent EU Directive (2003/89/EC) on allergen labeling that went into full effect in November 2005 [121]. For example, in the EU, by a provisional exemption, casein, fish gelatin, and fish isinglass (a product derived from fish collagen) will continue to be used as clarifying agents in beer, cider, or wine without labeling since there is no evidence that such products pose a risk, while further research is performed. It would be quite confusing for consumers to read a wine label and find that it contains fish or cow's milk after

Table 8 International food allergen labeling requirements [119–121]

Country/block	USA	European Union	Australia–New Zealand	Canada	Japan
Cow's milk	Yes	Yes	Yes	Yes	Yes
Hen's egg	Yes	Yes	Yes	Yes	Yes
Wheat	Yes	Yes	Yes	Yes	Yes
Soy	Yes	Yes	Yes	Yes	No
Peanut	Yes	Yes	Yes	Yes	Yes
Tree nuts	Yes	Yes	Yes	Yes	No
Fish	Yes	Yes	Yes	Yes	No
Crustaceans	Yes	Yes	Yes	Yes	No
Molluscs	No	No	Yes	Yes	No
Sesame seed	No	Yes	Yes	Yes	No
Mustard seed	No	Yes	No	No	No
Celery	No	Yes	No	No	No
Buckwheat	No	No	No	No	Yes

consuming the product with no adverse reaction for years. A full exemption will be granted when the industry has satisfactorily demonstrated to a scientific panel that no allergen capable of eliciting an allergic reaction is present in the finished products [123]; the literature in this area is growing [124–126].

Although the “Big Eight” foods account for about 90% of clinical reactions in children with atopic dermatitis in referral centers in the USA, the pattern of foods causing reactions prompting emergency department visits is somewhat different. In a recent multicenter study of emergency department visits for food allergy, which gives a national perspective, peanuts and tree nuts accounted for 21% of visits, crustaceans 19%, and fish 10%, but fruits as a group were significant and represented 12% of allergic reactions [127]. However, the “Big Eight” remains highly useful for labeling rules because the vast majority of individuals with food allergy will be helped, and specific fruits or vegetables are easier to avoid once the allergy is identified, and the patient receives appropriate education on avoidance. Fruits and vegetables are not as commonly found as “hidden” ingredients in processed foods but rather pose problems in restaurants or when dining away from home. On the other hand, edible seeds (e.g., sesame, mustard, coriander, sunflower), which are sometimes associated with potentially life-threatening allergies, are often found in trace amounts in a food and do not require disclosure in the USA, but sesame and mustard must be declared in the EU [114,128]. It would be prudent for food manufacturers to list such ingredients, even if not required by a law, for the public good.

At the current time, the focus is on labeling food allergens and maintaining good manufacturing practices to avoid cross-contact and contamination of unlabeled food with an allergen. Such cross-contact can result in expensive food recalls or even severe food allergic reactions. Some

manufacturers use provisional labeling, such as “may contain peanuts” or “processed in a plant that also processes peanuts,” but this is confusing for consumers and should be avoided. Risk assessment as derived from toxicology practice is also applied to food allergy, and NOAELs and LOAELs are being reported for food proteins involved in IgE-mediated food allergy and for gluten in celiac disease, where there is more data. For example, in the EU, by 2012, only foods with gluten levels by ELISA less than 20 ppm may carry a “gluten-free” label [129,130].

Genetically modified foods

Genetically modified (GM) foods are foods made from plants or animals that have been given specific traits by the insertion of one or more modified gene(s) or gene(s) from another organism using the technique of genetic engineering. Although genetically engineered animals also belong in this category and the USFDA is in the process of developing a guidance on regulations of foods derived from such animals, the focus here will be on GM food crops.

GM crops have gained rapid and widespread acceptance in the farming communities around the world, with the area dedicated to the cultivation of such crops increasing from 1.7 million hectares in six countries in 1996 to 125 million hectares in 25 countries in 2008. Nonetheless, the USA and Argentina account for two thirds of all commercial GM crop plantings and only five countries (USA, Argentina, Brazil, India, and Canada) account for 85% of them. Consumer acceptance of GM foods has not been equally enthusiastic, particularly in many European countries because of fears that these foods represent risks for human health and the environment that are difficult to foresee accurately.

The approach of the USFDA to regulating GM foods is product-oriented, not focused on the process by which the

product was created. It is based on the concept that GM organisms are not fundamentally different from nonmodified ones and that existing laws at the time of their first introduction were for the most part adequate for regulation of GM products. Except for a comprehensive scientific review of a GM tomato in 1992, the USFDA has reviewed GM foods via a voluntary consultation process during which the developer of the GM food presents data on the modification and the possible allergenicity and toxicity of the expressed product, the composition and nutrient profile, and, in some cases, the results of feeding studies comparing the GM food to its nonmodified counterpart. Although in 2001 the USFDA proposed a rule to make this process mandatory, we have been unable to find a finalized rule on this issue.

It seems probable that a majority of consumers are unaware to what extent “conventional breeding” of plants relies on extensive manipulation of the plant genome. This includes techniques that have been in commercial use since the 1920s, such as interspecific and intergeneric hybrids and accelerated mutagenesis (i.e., the use of chemical and radiation treatments to induce mutations and chromosome rearrangements), to mention but a few [131]. This raises the question of whether risk assessment should be confined to GM foods. Interestingly, Canada appears to be the only country where regulatory oversight is the same for all new agricultural commodities, regardless of the process by which they are created (www.agbios.com/static/cscontent/REGCanada-USAEN_printer.html). GM foods are subsumed under the category of novel foods, i.e., products that do not have a history of safe use as a food or foods that are manufactured in ways that significantly change their properties. The category “plants with novel traits” encompasses plants that are neither familiar nor demonstrate substantial equivalence to established plants, regardless of whether they arise from recombinant DNA techniques or are produced by chemical mutagenesis, cell fusion, and even conventional crossbreeding. The safety assessment is based on a comparison of the new food to a traditional counterpart.

In all other countries, it is not the product but the process of genetically modifying a plant that triggers regulatory oversight. However, the concept of “substantial equivalence” also forms the cornerstone of the safety assessment of GM foods required for their authorization. This concept was first introduced by the OECD and has since been adopted and elaborated by various international agencies, such as the FAO/WHO, the International Life Sciences Institute, and others. It means that a comparative approach is applied in the risk assessment of GM foods, which is not intended to guarantee absolute safety but to ensure the same level of safety as provided by traditional foods. The safety assessment of GM foods usually follows a stepwise procedure that focuses on two main categories of potential hazards: (1) those related to the intended properties and

functions of the introduced trait and (2) possible unintended effects resulting from the insertion of the transgene into the plant genome. Unintended effects can arise because it is impossible to predict where in the genome a transgene becomes inserted, how many copies are inserted, and whether the transgene is rearranged in the process. Depending on the place of insertion, the introduced gene can alter metabolic pathways or induce the expression of previously silent genes, possibly resulting in increased expression of toxins, antinutrients, or allergens or reduced levels of essential nutrients. Therefore, key components of the comparison of GM foods to their mostly isogenic counterparts are compositional, molecular, phenotypic, and agronomic analyses. The compositional analysis focuses on macronutrients and micronutrients, antinutrients, and toxicants. In order to compare the composition of GM foods to their traditional counterparts, it is absolutely essential to have information on the natural ranges of variation of the key components that should be analyzed. Remarkably, it took almost 10 years to develop consensus documents on the natural composition and its variability of important GM crops, such as maize, soybean, canola, potato, sugar beet, and bread wheat [132]. Others are still being developed.

Whether further data are called for before a GM product can be marketed largely depends on whether or not the molecular and compositional analysis demonstrates substantial equivalence and is decided on a case-by-case basis. Generally, it is only if the results of these analyses provide indications that unintended effects have occurred (e.g., that the composition is modified substantially) that animal feeding trials may be required. In such cases, most regulatory agencies require only 90-day feeding trials in a single species. Such trials have revealed relatively subtle but significant effects on growth/weight gain, hematological and immunological parameters, and organ histopathology with numerous GM foods [133]. These significant differences compared to the included control groups are often dismissed as being within the normal range for that species, raising the question of why anybody bothers to include a control group at all.

Allergenicity assessment of GM foods

The safety evaluation of GM foods contains a unique feature: it requires assessment of the potential allergenicity of the transgenic protein(s) and possible alterations in the allergenicity of the whole food. Increased allergenicity can result when (1) the protein encoded by the introduced gene is identical or cross-reactive with an existing allergen; (2) expression of the transgene results in the *de novo* creation of a food allergen (either because the transgene encodes a novel protein not previously encountered in the human food chain or because the transgenic protein is rendered

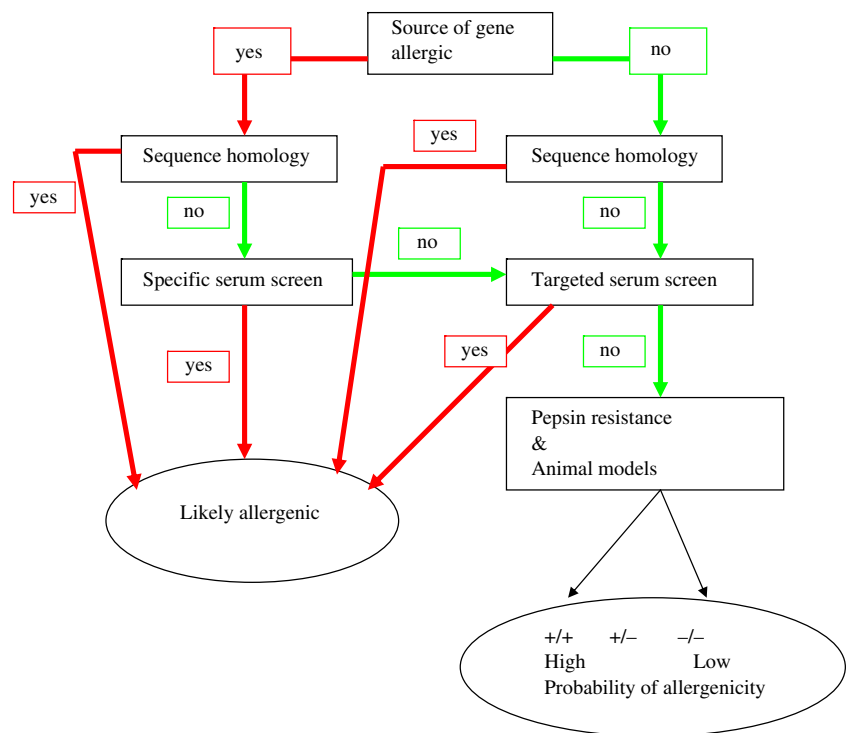
allergenic by alterations in the gene sequence or posttranslational modifications in the host), or (3) the introduction of the transgene increases the levels of—and exposure to—an endogenous antigen due to unintended effects on plant metabolism. In addition, there are ongoing attempts to apply genetic engineering to the elimination of allergens from staple foods such as potatoes, rice, tomatoes, and apples or highly nutritious foods such as peanuts [134].

A very limited number of items accounts for most food allergies, with cow's milk, egg, peanut, soy, wheat, and fish representing the most common allergens in children and peanut, tree nuts, fish, and shellfish being the predominant allergens in adults. While allergy to cow's milk, egg, soy, or wheat can range in severity from mild to severe, most cases are mild and are outgrown by age 5, while allergies to peanuts, tree nuts, fish, and shellfish are usually persistent. Only about 20% of children with peanut allergy will outgrow it by age 5 to 6 [135]. Peanut or tree nut allergy is often severe and together accounts for 90% of reported fatalities to foods in a US case series reported by the Food Allergy and Anaphylaxis Network [136,137]. This suggests that there is a broad spectrum of allergenic potencies ranging from the inability to cause sensitization at one end to the capability to persistently sensitize and provoke severe allergic reactions at the other end. Further evidence that all proteins are not equally allergenic comes from the observation that allergenic proteins share certain functional and biochemical properties. Specifically, they belong to a small proportion of all known sequence-based (2%) or structural protein families (5%) and most frequently exhibit hydrolase, metal, or lipid binding and transport activity or are involved in cytoskeleton organization and biogenesis [138]. Food allergens in particular most often are members of the prolamin, cupin, or cysteine protease superfamilies, and many of them belong to the seed storage or pathogenesis-related proteins. In addition, allergenic food proteins commonly, but not always, are abundant in their respective foods, fall into the size range between 10 and 70 kDa, and are glycosylated, heat stable, and resistant to acid and enzymatic proteolysis.

Nonetheless, predicting the allergenicity of novel (transgenic) proteins remains difficult since, to date, no unique feature or set of features that confers allergenic properties to proteins has been identified. Note, however, that new approaches are becoming available. There now is a structural database of allergenic proteins (<http://fermi.utmb.edu/SDAP/>) that includes 3-D models of all cataloged allergens and provides a variety of analytical and computational tools that take some of the known features of allergenic proteins into account [139,140]. The application of these tools is thought to hold great promise for better predicting the allergenic potential of proteins than the currently available approaches [141].

Several expert bodies and regulatory agencies, including the FAO/WHO, Codex Alimentarius Commission, USFDA, and the European EFSA, have published guidelines for conducting the assessment of potential allergenicity of transgenic proteins. They all recommend a step-by-step decision tree process that uses a weight-of-evidence approach (see Fig. 1 for the FAO/WHO decision tree). It includes consideration of whether the source of the gene to be transferred is known to be associated with allergic reactions. The FAO/WHO discourages the use of genes from common allergenic foods. Regardless of whether the source is a known allergen or not, the next step should be a sequence comparison between the transgenic protein(s) and known allergens in order to detect possible significant sequence homologies suggestive of cross-reactivity. According to the FAO/WHO guidelines, cross-reactivity needs to be considered if there is (a) identity of six contiguous amino acids or (b) more than 35% identity in the amino acid sequence of the expressed protein over any stretch of 80 amino acids. The first criterion is now considered to result in an unacceptably high number of false positives [141], and common practice is to use identity of eight rather than six amino acids. The second approach is also likely to yield very conservative estimates of potential allergenicity, but whether applying more stringent criteria (e.g., 50% homology) would improve the prediction without unduly increasing the number of false negatives remains to be established. It has been argued that the epitopes recognized by IgE are more likely to be conformational and that the use of significant homology over larger stretches of the protein would yield more relevant information than identity over six or eight amino acids. Note, however, that food allergens are generally believed to be partially denatured or digested by the intestinal tract before coming into contact with the immune system, especially in the initial immune response. As a result, IgE binding to linear epitopes is likely to be of much greater importance in severe food allergy than recognition of conformational epitopes [138]. Hence, some investigators feel that global sequence similarity may not be suitable for predicting cross-reactivity among food allergens and favor sequence homology of linear epitopes for assessing potential allergenicity. However, data are incomplete on the importance of conformational epitopes in different food allergens, in large part because their study has been technically difficult to carry out [142]. Current guidelines also frequently recommend serum screening in the case that the sequence comparison of the transgenic protein with known allergens did not reveal significant sequence homology. Specific serum screening refers to testing of whether serum IgE from individuals with documented clinical food allergies to the source of the transgene recognizes the novel protein (either because it is an as yet

Fig. 1 FAO/WHO decision tree for the assessment of the allergenic potential of foods derived from biotechnology (www.fao.org/ag/agn/food/pdf/allergygm.pdf)



unknown allergen or is cross-reactive with a protein to which the patient produces IgE). Targeted serum screening is considered useful either when specific serum screening yields negative results or when the transgene is derived from a source with unknown allergenic potential and encodes a protein product that does not show significant sequence homology with known allergens. Targeted serum screening refers to testing the protein of interest with sera from patients sensitive to substances that are broadly related to the source material of the gene. (The FAO/WHO distinguishes six source groups: yeast/molds, monocots, dicots, invertebrates, vertebrates, and “others”).

Conducting serum screening in a manner that accurately predicts allergenicity is considered to remain “extremely challenging” [141]. One of the major obstacles lies in obtaining sufficient amounts of well-characterized specific donor sera. In addition, it is crucial to use an appropriately validated IgE assay in which precautions are taken to prevent the much greater abundance of specific IgG antibodies or the presence of clinically irrelevant cross-reactive carbohydrate determinants from obscuring the results [141]. It also needs to be kept in mind that sensitization (the presence of IgE) can commonly exist in the absence of clinical allergic symptoms. Ideally, double-blind, placebo-controlled food challenges should be performed in order to demonstrate biologically relevant allergenic activity. However, this may not be possible, particularly if the novel protein exhibits similarity to a known allergen that is associated with severe or even life-

threatening reactions. Therefore, currently available studies on the allergenicity of novel proteins often complement serum screening with skin prick testing (SPT). The release of histamine from the rat basophilic leukemia line 30/25, transfected with the human IgE receptor, may represent a valid *in vitro* alternative [141].

If sequence homology analysis and serum screening have not yielded any indications of allergenicity, it is recommended to examine the susceptibility of the transgenic protein to pepsin digestion. The rationale is that peptides large enough to be recognized by the immune system need to survive the digestive process in order for allergic sensitization to occur. Consistent with this hypothesis, a majority of food allergens associated with clinically severe reactions are resistant to pepsin degradation *in vitro*, even though there are examples of pepsin resistant food proteins that do not elicit food allergies.

Most guidelines for allergenicity assessment of isolated food proteins (or whole foods) also recommend the use of animal models but point out that no generally accepted and validated models are currently available. An appropriate animal model should reproduce the major features of human allergy, i.e., sensitization and provocation of allergic symptoms, at physiologically relevant doses. The oral route of exposure is thought to be most pertinent, although humans may also develop food allergies due to cross-reactivity with inhaled allergens or after transdermal exposure. In fact, transdermal sensitization to peanut in murine models is extremely effective [143], and current

human prospective studies suggest that household environmental exposure to peanut, especially in children with atopic dermatitis, is a risk factor for peanut allergy while early oral ingestion may be protective [144]. However, as in humans, animals generally develop oral tolerance to ingested proteins, necessitating the use of adjuvants when the test substance is given orally or by an alternate route of exposure.

The need to assess the allergenicity of GM foods has provided a major impetus for the development of appropriate animal models. Several mouse strains, including Balb/c, A/J and C3H/HeJ, and the Brown Norwegian rat represent high IgE responder strains and thereby resemble the human phenotype that is susceptible to developing IgE-mediated allergies. Other models include dogs and neonatal pigs, which offer some advantages because of their larger size but are thought to lend themselves more readily to mechanistic studies rather than routine safety assessments. Recent efforts have focused mainly on mouse models because of the ready availability not only of inbred strains but also of reagents for characterizing their immune responses [141]. It is recommended to not only examine specific IgE production (sensitization) but to also obtain a readout of clinically relevant end points, such as allergic manifestations after challenge. In addition to sensitization, the ability to escape the induction of oral tolerance is an important prerequisite for allergic responses, and its assessment has been suggested to offer valuable insights [141]. Of note, recent experimental data have provided evidence that pepsin stability is an important predictor of sensitization but that oral tolerance induction requires resistance to digestion by both pepsin in the stomach and trypsin in the intestine. There are now several indications that the food matrix can affect the allergenicity of individual food constituents by altering their digestibility and solubility. This raises the question of whether the use of purified (and often microbially expressed) proteins represents the most appropriate approach to the allergenicity assessment of GM foods. Food matrix effects are but one of several issues that need to be clarified before any of the currently available animal models can be validated. It should be kept in mind that animal models by themselves cannot capture all traits of human food allergies and should not be expected to do so but may yield important hints as to possible allergenicity of GM (and more conventionally bred novel) foods. However, in the context of risk/safety assessment, animal models always need to be used in conjunction with other methods.

Note that the guidelines concerning the allergenicity assessment of GM foods generally are focused exclusively on the transgenic protein(s), even though it is recognized that unintended changes in plant metabolism may result in qualitative or quantitative changes in existing allergens. The European EFSA guidelines are a notable exception in that

they specifically call for an assessment of the allergenicity of the whole GM product (www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775770.htm). However, they provide essentially no details on how such an assessment should be conducted except to say that the allergen repertoire of the GM food should be compared to that of its non-GM counterpart using modern “profiling techniques.” We would also like to emphasize again that currently the assessment of potential allergenicity is only required for transgenic proteins (foods). However, more conventional breeding methods result in more pronounced changes in the expression of untargeted genes compared to transgene insertion [145]. In addition, novel proteins (resulting from novel open reading frames) that are present in certain conventional food crops show a similar number of sequence homologies with known allergens and also of identical stretches with indications that they are true epitopes as do transgenic foods [146].

Applications of the assessment of potential allergenicity

Relatively early in the commercial development of GM foods, there was an attempt to improve the nutritional quality of soybeans, which are relatively deficient in the essential amino acid methionine. For this purpose, a methionine-rich Brazil nut protein (2S albumin) was inserted into the genome of a soybean variety. Even though the allergenicity of Brazil nuts was well known at the time, information on the major allergens in this food was still lacking. Using a variety of techniques, including radioallergen sorbent (RAST) assays, sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting, and SPT, it was clearly demonstrated that 2S albumin was recognized by serum IgE from subjects with proven Brazil nut allergies and probably represented a major Brazil nut allergen [147]. Commercialization of this methionine-rich transgenic soybean was abandoned.

In most other published studies, serum screening was not conducted during the premarket allergenicity assessment but as a form of postmarketing surveillance. The methods included RAST inhibition, immunoblots, and/or ELISA on sera of subjects sensitized to the non-GM food and, in some cases, from potentially sensitive subjects with a variety of other food or inhalant allergies. In several instances, serum screening was combined with SPT or histamine release. These investigations provided no evidence that the allergenicity of transgenic crop plants, including soybean, maize, tomatoes, and potatoes, differed significantly from that of their conventional counterparts. There are, however, isolated examples of differential reactivity to wild-type and GM foods and at least one case where SPT reactivity was confined to GM soybean, but these assays were done in individuals with sensitization to soy, not clinical allergy,

and the wild-type soy was not the parent cultivar of the GM soy [148,149].

However, the well-publicized case of StarLink corn provides an example of the detection of a potential novel food allergen through the use of pepsin and trypsin digestion in conjunction with other considerations. This transgenic corn was generated by inserting a gene derived from *Bacillus thuringiensis* (Bt) that encodes an insecticidal toxin belonging to the crystal protein family, Cry9c. The original sequence of this gene was modified to yield the truncated active toxin rather than the bacterially encoded protoxin that needs to be cleaved by trypsin into its active form. It was also modified to resist further trypsin digestion to an inactive form by a single amino acid substitution that eliminated the relevant trypsin cleavage site. Because Cry9c was found to be resistant not only to trypsin but also to pepsin digestion and heat treatment, it was considered a potential allergen even though it did not show any sequence homology with known allergens. As a result, the USEPA approved StarLink corn for animal feed use only, but not for human consumption [150]. In studies submitted by the company at a later time point, specific serum screening indicated that the allergenicity of StarLink corn did not significantly differ from that of conventional corn. On the other hand, some undigested Cry9c could be detected in the blood of animals that were fed the pure protein. In addition, it was capable of inducing allergic reactions in Brown Norway rats, but these results were not considered in the evaluation by the USEPA because this animal model had not (and has not) been validated for assessing the allergenic potential of transgenic proteins [151]. Unfortunately, StarLink corn did get into the human food supply and 51 people claimed allergy to it, but no clinical food allergy was ever confirmed [152,153].

Of note, since it proved difficult to isolate the protein from transgenic corn, the USEPA allowed the microbially produced trypsinized active toxin to be used as a test substance. This is problematic since the resulting Cry9c would not be glycosylated, whereas it might be subject to glycosylation in plants [150]. There are numerous indications that IgE binding to an allergen that is solely due to the recognition of cross-reactive carbohydrate determinants is clinically irrelevant. However, in the case of specific allergens, it has been shown that the carbohydrate moieties of certain plant glycoproteins are required for IgE recognition of allergenic epitopes and/or activation of helper T cells, whereas in other cases such carbohydrates can inhibit specific T cell responses. Glycosylation may also influence the thermostability and resistance to proteolysis of proteins.

A more recent study further underscores the potential importance of glycosylation [154]. It was shown that the transgenic expression of a bean protein (α -amylase inhibitor-1, α AI) in peas resulted in structural modifications of the

transgenic protein that altered its immunogenicity. Specifically, the transgenic pre-pro-peptide underwent different posttranslational processing (glycosylation and possibly cleavage) in the pea compared to the bean. Intragastric administration of bean or nontransgenic pea seed meal did not provoke a specific IgG1 response even after intratracheal or subcutaneous challenge with purified α AI. In contrast, oral administration of transgenic pea seed meal induced immunoreactivity to α AI (increased α AI-specific IgG1 levels) and failed to induce oral tolerance as assessed by significant delayed-type hypersensitivity responses to foot-pad challenge and airway hyperreactivity and pulmonary eosinophilia after intratracheal challenge with purified α AI. Importantly, extensive boiling of transgenic peas did not abrogate their ability to prime for pulmonary hyperreactivity, eosinophilia, and mucus secretion after intratracheal challenge. Most notably, oral administration of transgenic pea seed meal also resulted in enhanced IgG1 responses to several other pea seed proteins. Furthermore, concomitant oral administration of transgenic pea seed meal and ovalbumin (OVA) resulted in cross-priming, as evidenced by significant increases in OVA-specific IgG1 levels along with airway hyperreactivity and pulmonary eosinophilia compared to OVA treatment alone (which does induce oral tolerance). Unfortunately, α AI-specific IgE was not determined in this study. Although it has been suggested that antigen-specific IgG1 represents a surrogate of IgE in the mouse [155], the two are regulated differentially, and in particular antigen-induced airway hyperresponsiveness has been shown to occur in animals with specific IgG1 responses in the absence of significant specific IgE production. Therefore, it cannot be determined whether transgenic pea meal induced classical IgE-mediated hypersensitivity. Nonetheless, these results underscore that transgenic expression of a nonallergic protein (endogenous α AI in beans) in a different host crop can exhibit altered immunogenicity, most likely due to differential posttranslational modifications. They further illustrate that such an altered transgenic protein can enhance the immunogenicity of concomitantly administered food antigens. This is an aspect that is not addressed at all in the safety assessment of GM foods.

Whether GM foods are safe for humans, animals, and the environment cannot be determined from the existing data. It has been calculated that the margin of safety between animal intake in 90-day feeding trials and human intake is generally greater than 100 [133]. This might be reassuring if one could have confidence in the quality of the studies on which these calculations were based. It certainly does not promote consumer confidence in the approval process or the safety of GM foods to find out that the reanalysis of data originally submitted for approval of MON863 corn revealed indications of sex-dependent liver and kidney toxicity in mice fed with this product for 90 days [156],

whereas the original publication of the results from this experiment considered the significant differences they found not biologically meaningful. It is also not reassuring that most regulatory agencies require only 90-day feeding trials in a single species—and only in cases where a feeding trial is indicated by the compositional and molecular analyses.

Many consumer protection groups in the USA and Europe demand more extensive safety testing of GM products. As pointed out above, it may be more appropriate to take the Canadian approach and include not only GM foods but other novel foods derived from more conventional plant “breeding” techniques in such safety assessments. Whether the “substantial equivalence” concept proves tenable in the safety assessment of GM foods remains to be established. As Millstone et al. [157] pointed out, developers of GM foods want to have the best of all possible worlds by arguing that their foods are sufficiently novel to justify patenting them, but not so novel as to require risk assessment. They further underline that the “substantial equivalence” approach provides “an excuse for not requiring biochemical or toxicological tests.”

Ironically, severe food allergy to an unregulated soy substitute, lupin seed, has emerged in markets where there is distrust over GM soy. Lupin, previously mainly used as an animal feed rather than human food, has been promoted since about 1997 as an excellent protein source, high in fiber and able to replace egg and butter in some products. It can be added to breads and pastas instead of soy flour. However, there is significant clinical cross-reactivity with peanut, and the first cases of anaphylaxis were reported in 1999. A recent study has shown that approximately 4% of children with peanut allergy are clinically allergic also to lupin [158]. The 2S albumin seed storage proteins from lupin have significant homology with the counterparts in peanut. Thus, the potential for new food allergens exists not only for GM foods and foods derived from traditional plant breeding that enhance desirable characteristics (and thus might increase certain allergens) but also with unmodified foods newly introduced to a population.

Emerging food safety issues

Nanomaterials

It has been conjectured that the impact of nanotechnology in the twenty-first century will surpass that of the industrial revolution during the nineteenth century. The prefix nano refers to a measure of 10^{-9} units. Nanotechnology refers to the design, production, and application of structures, devices, and systems by manipulating shape and size at the atomic, molecular, or macromolecular scales. Nanomaterials can result from “top-down” or “bottom-up”

techniques. There is no generally accepted definition of “nanomaterials.” The Scientific Committee on Emerging and Newly Identified Health Risks, an advisory committee of the EU Commission, proposed the definition of “any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less.”

It has been estimated that close to 400 manufacturer-identified nanotechnology-based consumer products were on the market by 2007. In the food production chain, nanotechnology products are (claimed to be) used as nanosensors for the detection of contaminants, toxins, or microorganisms; in water purification filters; pesticides; food packaging (either as nanosensors for the detection of food deterioration, in nanosized silver sprays with antibacterial action, or for some packaging materials themselves); and also in certain functional foods or food supplements.

Typically, the biological activity of particles increases with decreasing particle size. This is because there are a greater number of particles with a larger surface area per unit mass, resulting in increased potential for biological interactions. There are already indications that certain nanomaterials can generate reactive oxygen species, induce oxidative stress, alter mitochondrial function, resulting in apoptosis or other forms of cell death, interact with proteins in ways that alter their immunogenicity, damage DNA, and interfere with cell cycle regulation [159].

Whereas the inhalation and, to a lesser extent, dermal routes of exposure to certain nanomaterials have been investigated to some extent, there is little information on the fate of nanoparticles after ingestion [159]. What few data are available suggest that the uptake from the gastrointestinal tract after oral administration may be quite efficient for some types of nanomaterials, whereas the bioavailability of others may be very limited. A recent study investigated the toxicology of orally administered silver nanoparticles, which are extensively used as antimicrobial films in food wraps and a variety of other applications [160]. Dose-dependent accumulation of silver in all tissues examined suggests at least some bioavailability after oral administration. Interestingly, significantly higher levels of silver were detected in the kidney of female compared to male rats at all doses. For most other nanomaterials, information on their absorption, tissue distribution, metabolism, and excretion is incomplete or absent.

Since the exposure to nanomaterials of the general population is bound to increase dramatically in the recent future, detailed knowledge of nanomaterial absorption, distribution, metabolism, and excretion after oral and other routes of exposure and nanomaterial toxicology must become the focus of intense research. Their impact on the environment is another topic that urgently needs to be addressed. Such research faces numerous challenges. The

physicochemical properties of nanomaterials may differ fundamentally from their macroscale counterparts, as may their toxicological profile [161]. Therefore, the safety of a macroscale compound does not provide any indication of the potential toxicities of its nanoscale counterpart. Nanomaterials come in various shapes, including nanosheets, nanorods, nanotubes, nanodots, nanowires, or nanofibers, each of these structure having its own individual characteristics. These include shape, size (surface area, size distribution), mass, chemical composition, surface structure, surface chemistry, surface charge, porosity, solubility, and aggregation/agglomeration state. These characteristics may all influence the behavior, including the biological activity, of nanoparticles and should therefore be described in any testing of nanomaterials. There is uncertainty over whether mass, particle number, or surface area is the most meaningful dose metric [162]. This currently needs to be evaluated on a case-by-case basis, and it is recommended to express the dose response in terms of all three parameters whenever possible in order to enable quantitative interpretation of the collected data and allow them to be compared to other results.

Because of these difficulties, there is as yet little to no information on the extent of our exposure to nanomaterials or their potential hazards, the two fundamental requirements for risk assessment. Several consumer protection groups have called for a moratorium on all marketing of nanomaterials until it has been shown that their production and use does not present a significant risk to human health and the environment. Many scientists consider this to be excessive as long as all the relevant regulatory agencies evaluate whether existing regulations provide sufficient protection and stay alert to the possible requirement for new regulations as new applications arise.

Autoimmunity and food

One subject that has not been discussed independently is the issue of foods in autoimmunity. We have discussed select data on mercury and autoimmunity, and the purpose of this review is not an exhaustive discussion of foods and, for example, inflammatory bowel disease. However, we would be remiss if we did not note that not only foods, i.e. milk, but also the possibility of food contaminants can elicit significant immune reactions [163]. These are exemplified by detection of antibodies against raw and processed foods, the role of gluten in celiac disease, the potential impact of probiotics on the immune response, the developing data on vitamin D and immunity, and the influence of fatty diets and the immune response [167–173]. In addition, and perhaps of greater interest, is the potential role of food as an initiator of autoimmune diabetes. This is an issue that has attracted attention for several decades but still remains elusive. Recent literature on diabetes has continued to

define the role of T cell reactivity to various insulin and GAD65 peptides, but whether such observations reflect direct causation with food is still very much unclear [174–183]. Indeed, it is abundantly clear that further study of the mucosal immune system, and including Peyer's patches, is critical for dissecting mucosal tolerance [184–186]. Lastly, we should note the role of the innate immune system, including the potential of food and food additives to promote inflammation [187]; discussion of innate immune responses is beyond the scope of this review, but recent literature is cited [188–195].

Mixture toxicology

The current risk assessment paradigm—hazard identification, exposure assessment, and risk characterization—is generally applied to single chemicals via a single route of exposure. In order for this approach to be valid, each one of the multitude of natural and synthetic chemicals to which we are exposed would have to act independently of all the others. If confirmation was needed that we are indeed exposed to multiple chemicals everyday, it certainly has been provided by studies showing the presence of metabolites of numerous phthalates in urine [196] or the simultaneous detection of various PCB congeners, DDT, DDE, hexachlorocyclohexane, and hexachlorobenzene in serum of women who also excreted OP pesticide metabolites in their urine [58]. Furthermore, the focus on a single route of exposures would only be justifiable if all other routes made negligible contributions, which does not appear to be the case for numerous compounds.

In recent years, there has been increasing recognition that information on the cumulative risk from multiple chemical exposures is urgently needed, and the field of “mixture toxicology” has emerged. First, it needed to be determined what substances would constitute good candidates for mixture studies. The combined toxicological effects of mixtures of compounds can take one of three forms: independent action (also called response addition), dose addition, or interaction. Substances with diverse modes of action will each exert their individual effects whether alone or present in combination with other substances, i.e., they will display independent action. Dose addition is commonly seen with substances acting via a common mode of action and represents a situation where one compound in the mixture can be replaced by another one, provided it is present at an equally effective dose level. The term interaction refers to mixture effects that are greater (synergistic, potentiating) or smaller (antagonistic) than predicted on the basis of dose addition. Once a group of compounds with a common mode of action has been identified, it needs to be determined whether these

compounds exhibit dose additivity. Most commonly, this is done by establishing dose response curves for each of the individual compounds, then using these data to predict their combined effects under the assumption of dose addition, and finally establishing whether there is agreement between the predicted and the observed results.

There are several methods for conducting cumulative risk assessment for substances that have been shown to act in a dose-additive manner [197]. One of these approaches, the use of toxic equivalency factors (TEFs), was originally developed by the USEPA for the toxicity of mixtures consisting of dioxins and dioxin-like compounds. It has since been adopted and recently updated by the WHO [62], and WHO TEFs are used widely for the assessment of human exposure to dioxins and related substances. It expresses the potency of chemicals relative to that of an index compound (TCDD in the case of dioxins and dioxin-like compounds). The exposure to each of the chemicals in the common mode of action group is then multiplied by the TEF in order to express all exposures as a fraction or multiple of the index compound. The resulting values are summed to provide the total exposure in TEQs.

Applications In 1996, the US Congress passed the Food Quality Protection Act (FQPA), which requires the USEPA, which is responsible for setting tolerances for pesticide chemical residues in food, to assess the cumulative human health risk from simultaneous exposure to pesticides that have a common mechanism of toxicity and to consider aggregate exposure, i.e., exposure from all conceivable pathways (food, drinking water, residential activities) and routes (ingestion, inhalation, and dermal absorption). The USEPA is specifically directed to take into account the special susceptibility of infants and children and the behavioral patterns that may put them at increased risk from disproportionately high levels of exposure.

Following the mandate of the FQPA, the USEPA conducted cumulative and aggregate risk assessments for OP pesticides, *N*-methyl carbamate pesticides, and a subset of triazine pesticides that share the ability to cause neuroendocrine and endocrine-related developmental, reproductive, and carcinogenic effects. It employed the TEF approach for the cumulative exposure assessment, using chlorpyrifos as the index compound for OP pesticides because the most extensive data were available for this particular substance.

There are indications that the choice of the index compound can have marked effects on the resulting exposure estimates [198,199]. For example, estimates of the high end of cumulative exposure to OP and carbamate pesticides in the Dutch population amounted to 13.4 µg/kg bw per day with acephate as the index compound but 27.6 µg/kg bw per day with phosmet as the index compound [198]. If determined under ideal conditions,

the TEF approach should yield identical results, regardless of the index compound used [197]. Therefore, the observed dependence of the exposure estimates on the index compound reflects the incompleteness and uncertainty associated with the database on which the TEF values were based. In its cumulative risk assessment of OP pesticides, the USEPA assumed that the effects of these compounds would be characterized by dose addition. However, even studies cited by the USEPA show that certain combinations of OP pesticides can have synergistic effects particularly in the low-dose range, although the synergism was of relatively small magnitude [200–202]. These synergistic effects are likely to result from the ability of certain OP pesticides to also inhibit carboxyl esterases, which are involved in OP pesticide detoxification.

Together, these findings underscore that, although the USEPA used tremendous amounts of resources to conduct its cumulative risk assessment of certain groups of pesticides, the validity of the conclusions remains somewhat doubtful. Despite these uncertainties, however, a cumulative risk assessment approach is certainly preferable to the single chemical approach and has already resulted in the revocation of 3,200 tolerances and the modification of another 1,200 by the USEPA.

Several other types of pesticides are classic antiandrogens in that they bind to the AR and thereby inhibit androgen binding and induce malformations in the developing male reproductive system in rodents. These pesticides include the herbicide linuron and the fungicides vinclozolin, procymidone, and prochloraz. A group of Danish researchers provided evidence that the combination of vinclozolin and procymidone [203] or a mixture of those two pesticides plus flutamide (an antiandrogenic pharmaceutical) induces male reproductive malformations and changes in prostate gene expression in a dose-additive manner [204–206]. According to comments made in 2000, the USEPA has been well aware of the possibility that vinclozolin and a subgroup of the dicarboximide class of fungicides (which includes procymidone) may modulate androgens by a common mechanism of toxicity. At the time, however, it considered the available evidence insufficient to determine whether this was indeed the case.

Not only pesticide residues but also phthalates belong to the chemical compounds to which consumers are exposed on a daily basis. In addition, all phthalates that affect male reproductive development have been shown to do so via a common mechanism of action, namely a reduction in testicular testosterone production and in Leydig cell *Insl3* expression. When DBP and DEHP were combined, each at half of the effective dose (ED) predicted to cause a 50% incidence of epididymal agenesis, dose addition was found to provide the best model for predicting several of the examined outcomes [207]. In addition to the incidence of

epididymal agenesis, these included decreases in the anogenital distance and the incidence of areolae retention and testis malformation. Note, however, that the frequencies of hypospadias, gubernacular agenesis, and seminal vesicle malformations were greater than predicted by dose additivity, suggesting a synergistic effect of the two phthalates. In an extension of this study, the male reproductive toxicity of a mixture of five phthalates (BBP, DBP, DEHP, DPP, and di-isobutyl phthalate) combined at their respective ED50 values was investigated [208]. Again, the dose addition model accurately predicted fetal mortality and fetal testosterone production.

The same group of scientists, which incidentally includes researchers from the USEPA, then began investigating the effects of phthalates combined with pesticides that act as AR antagonists. Initially, they showed that exposure during the critical window from gestational days 14 to 18 to the herbicide linuron and the phthalate BBP individually and in combination reduced testicular testosterone production and concentrations in GD18 rat fetuses [209]. The greatest effect was seen with the combination. Whereas the herbicide and the phthalate alone were not associated with reproductive malformations at the selected doses, the combination induced significant incidences of incomplete preputial separation, cleft prepuce, cleft phallus, hypospadias, and vaginal pouch. A low incidence of malformations of internal reproductive tissues (adhesion or agenesis of ventral prostate, agenesis of seminal vesicle and epididymis, undescended testis) was seen after exposure to BBP or linuron alone but was significantly increased in the mixture group. For most of these effects, the two antiandrogens with mixed mechanisms of action were found to act in a cumulative dose-additive fashion. More recently, a mixture of seven antiandrogens was tested, namely vinclozolin, procymidone, linuron, and prochloraz and the phthalates BBP, DBP, and DEHP [210]. After careful construction of dose response curves for each of these substances, using several of the typical male reproductive malformations as end points, each compound was used at one seventh of its respective ED100 (i.e., they would be expected to contribute equally to the overall effect). The TEF model performed at least as well as the dose addition model or even better in predicting the observed reproductive malformations. This is somewhat surprising since the TEF approach is a specific application of dose addition. In a more recently published study, the effects of the same antiandrogens with both similar and disparate mechanisms of toxicity were tested singly and in pairs. Regardless of the combination used (two AR antagonists, two phthalates, or AR antagonist plus phthalate), the binary mixtures containing each individual compound at a dosage level of one half of its ED50 for hypospadias induced dose-additive effects.

It is worth underscoring again that the mechanisms by which the various compounds induce male reproductive toxicity are different. Vinclozolin and procymidone are AR antagonists, while the phthalates are not. Conversely, the phthalates act by reducing fetal testosterone and *Ins13* mRNA expression and protein production, which the two fungicides do not. Linuron affects male reproductive development by multiple mechanisms, including AR antagonism and reduction in fetal testosterone production, but it does not alter *Ins13* expression. Similarly, dose addition was found to predict the effects of a mixture of PCDD/Fs, dioxin-like PCBs, and non-dioxin like PCBs, which individually all disrupt thyroid hormone homeostasis but via different mechanisms [211]. The fact that dose additivity can be observed with mixtures of compounds that act by distinct molecular mechanisms but produce similar effects in a given target organ highlights that regulatory agencies need to define the term “common mechanism of action” quite broadly rather than choosing a narrow focus on identical molecular mechanisms when it comes to identifying compounds that need to be evaluated for the cumulative risk they pose.

What constitutes a “common mechanism of toxicity” has been a matter of much debate, but several scientific bodies endorse a rather broad definition, where the focus should be on a common adverse outcome in a specific target tissue rather than on a common molecular target or molecular pathway, possibly involving the same toxic intermediate ((National Research Council (NRC), <http://www.nap.edu/catalog/12528.html>), [197]). In particular, the NRC has urged the USEPA to assess the human health risk resulting from cumulative exposure not only to all the phthalates with reproductive toxicities but also pesticides such as linuron, vinclozolin, and procymidone that act by distinct mechanisms but result in very similar reproductive malformations. The NRC also emphasizes that, in order for cumulative risk assessment of phthalates to become possible, it will be imperative to address three key issues: (a) characterize the full spectrum of phthalate metabolites, (b) determine which metabolite is most appropriate for use as a biomarker of human exposure, and (c) identify the most important sources of human phthalate exposure.

Incidentally, such an assessment would be well within the mandate of the FQPA, which specifically directs the USEPA to provide for the testing of all pesticide chemicals for their estrogenic activity “or such other endocrine effects as the Administrator may designate.” In addition, the FQPA authorizes the USEPA to “provide for the testing of any other substance that may have an effect that is cumulative to an effect of a pesticide chemical if the Administrator determines that a substantial population may be exposed to such a substance” (our emphasis).

Not only the USEPA but also the USFDA and other governmental departments would do well to rethink their

approach to regulating food contaminants. In an article posted on the USFDA website, Healthcare without Harm (www.noharm.com) criticized that chemical policy in the USA is severely “balkanized.” Phthalates were used as an illustration: the USFDA is responsible for food contaminants (including phthalates), drug ingredients (including phthalates), medical devices (including phthalate-containing PVC products), and cosmetics (including phthalates). In addition, the Consumer Products Safety Commission regulates phthalates leaching from toys, while the EPA is in charge of phthalates in pesticide formulations. Each of these agencies and even each of the divisions within the USFDA work in isolation from all the others. This means that each one considers only the exposure resulting from the application that comes under its jurisdiction, without ever taking into account the cumulative and aggregate exposure resulting from all of these applications. This underscores that a more coordinated approach both within the USFDA and between the USFDA and other agencies involved in the regulation of chemicals in their various applications is urgently needed.

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