COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Making Decisions about the Risks of Chemicals in Foods with Limited Scientific Information

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ABSTRACT: On occasion, food safety managers may detect an undesirable chemical contaminant or unanticipated chemical substance in a food commodity, ingredient, or finished product, thereby warranting an assessment of the health impact of the substance at the level detected. Many times, such an assessment must be made with limited scientific information. In such situations, food safety managers must expeditiously evaluate the available data and other information and make decisions such as whether to implement a food product recall to protect public health and maintain integrity of and confidence in the food supply.

Under such circumstances, making decisions about risk can be very complicated by the interactions of a number of issues. Interpretation of scientific and public policy can cause confusion as a result of insufficient data for conducting a risk assessment, conflicting data, uncertainty stemming from toxicological issues or temporal constraints, emerging nature of the state of the science, and regulatory constraints (for example, zero tolerance). A user-friendly conceptual framework would aid food safety managers faced with making decisions about the risks of newly detected, undesired chemical substances in foods—whether naturally occurring toxins, direct or indirect food additives, substances arising through food processing, or other substances.

The Institute of Food Technologists (IFT) convened a group of experts to (1) examine the complexities that challenge timely decision-making about such substances when available scientific information is limited and (2) define and develop a workable tool to guide food safety managers in effectively and knowledgeably evaluating available scientific evidence pertinent to assessing the risk from exposure to a chemical substance to make timely decisions. This Expert Report delves into the legal U.S. underpinnings of the risk management of chemical substances in foods, international considerations, risk-benefit evaluation, importance of the food matrix to risks and benefits, risk assessment and management, and the need for a new approach to timely decision-making with limited scientific information. This report includes case studies that demonstrate (1) the various complexities and how sound decision-making with sufficient available pertinent data is reinforced as additional supportive data subsequently become available and (2) the importance of assessing and balancing consideration of risks and benefits from a whole food perspective.

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Introduction

Safety has no mixed connotation and, as it applies to food and health, is nonnegotiable. The food industry views food safety as its highest priority. Cooperation among producers, ingredient suppliers, food scientists, processors and other food technologists, distributors, and federal, state, and local regulatory officials is critical in ensuring the safety of the global food supply and maintaining consumer trust and confidence. There have been many efforts to improve risk assessment. Virtually all of them stress the importance of acquiring additional data. In contrast, this report deals with risk assessment on the basis of currently available and often less than adequate data. Facing the dilemma of the need to make a decision about the level of risk presented by a chemical substance associated with food, perhaps arising through the discovery of traces of a chemical, with limited or less than a desired amount of data triggers a host of questions: Is it safe? How did it get in the food? What can be done? What should be done? According to the Toxic Substances Control Act list by the U.S. Environmental Protection Agency (EPA), there are more than 75000 known chemicals in our environment, many of which may come in contact with food via soil, air, or water. The presence of chemicals such as acrylamide and polycyclic aromatic hydrocarbons (PAHs), which form upon normal food preparation and heating, may be discovered through advances in analytical methods and techniques. Other chemical substances may be intentionally added, such as direct food additives, unintentionally added, such as indirect food additives migrating from packaging components to food, naturally occurring or more deliberative, arising from accidental contamination or malicious adulteration.

Product safety is the foundation of consumer trust. However, consumer trust in the food supply can be undermined by food recalls, which may sometimes constitute the end stage of food safety assessments and decision making. An online poll of 2563 adults indicated that almost 8 in 10 adults (79%) are aware of food

recalls in the United States (Harris Interactive 2007). Moreover, more than 86% of those polled mentioned at least some concern with food recalls, and 29% indicated that food recalls were a serious concern.

Food safety issues quickly become news that is rapidly disseminated to distant regions and countries at the speed of an e-mail or broadcast journalist's report. While they are the most public aspect of apparent food safety failures, food recalls is just 1 component of the complicated food safety decision-making process. Critical and challenging decisions are made prior to a recall. However, real-time information is difficult to obtain and is often limited and/or difficult to interpret. Undertaking food chemical safety assessments requires detailed knowledge of the chemistry and toxicology of the substances in question. Invariably, deficiencies or gaps in the underlying science become evident. The context of the level of exposure to a chemical substance in question also is critical in making meaningful interpretations of available information. This is particularly true when confronted with deficiencies and gaps in available scientific data. Consequently, the decision-making process in making food safety assessments can be very difficult.

This report has 3 main sections. The first section sets forth the U.S. legal framework and international institutions and measures that govern the safety of the food supply. The 2nd section deals with risk analysis—that is, how the nature and size of real or potential risks are determined or, more frequently, estimated. This section discusses in some detail ways to use the available information to best advantage, especially when that information is less complete than desirable. And the third section of the report covers how that information on the nature, size and probability of a risk can be applied in making appropriately conservative and balanced decisions. This section particularly emphasizes the need to weigh carefully information about the risk(s) of an unavoidable food component against the benefits of the food(s) in which that component is found.

For the purpose of this report, the term "risk" is defined as "the possibility of loss, injury, disadvantage, or destruction" (Gove 1993). Risk is not the reality of being deprived or in a state of disadvantage; instead, it is the threat of becoming so. Health risks are a subset of what are often called vital risks because they have a major impact on life itself. Similarly, the term "benefit" is "something that guards, aids, or promotes well-being" (Gove 1993). Secondary meanings connote something good, or producing a positive outcome. Thus, the terms "risk" and "benefit" are not opposites: Risk always and explicitly includes the element of chance; benefit does not.

U.S. Legal Framework

An extensive legal framework involving key federal statutes, such as the Federal Food, Drug and Cosmetic (FD&C) Act, provides a strong foundation for U.S. food safety policy and decision making that incorporates precaution and science-based risk analyses (FDA-USDA 2000). This report focuses on food safety decision-making relating to chemical substances; thus, the legal framework presented herein excludes aspects of the regulatory authority for meat and poultry by the U.S. Dept. of Agriculture (USDA). This report also does not address the legal framework for rDNA biotechnology-derived foods (see the IFT Expert Report "Biotechnology and Food" for information on biotechnology).

The safety of food products other than meat, poultry, and certain egg products is regulated in the United States by the FD&C Act, as interpreted and applied by the FDA and, on occasion, federal courts. The FD&C Act prohibits introducing any food that is adulterated into interstate commerce and the adulteration of any food already in interstate commerce. An adulterated food is subject to a civil seizure action in a U.S. district court, and corporations or individuals responsible for introducing adulterated food into interstate commerce food are subject to an injunction proceeding and/or to criminal prosecution in the U.S. district courts. A food may be deemed adulterated for a number of different reasons, the following of which are relevant to this report:

- the presence of any poisonous or deleterious substance that may render a food injurious to health unless the substance is not an added substance and present in an amount that does not ordinarily render it injurious to health;
- the presence of any unavoidable added poisonous or deleterious substance, other than a pesticide residue, a food additive, a color additive, or a new animal drug unless the quantity of such substance does not exceed an applicable FDA-designated tolerance level;
- the presence of a pesticide chemical residue at levels that exceed an applicable tolerance level or that is not subject to an exemption;
- the presence of an unapproved food additive or unapproved color additive;
- the presence of a new animal drug (or conversion product thereof) unless the drug residue falls within an applicable tolerance for such drug;
- the dietary supplement or a dietary ingredient therein that presents a significant or unreasonable risk of illness or injury under recommended or labeled conditions of use, or if no such recommendations or labeling, under normal conditions of use; or
- failure to submit to FDA a new dietary ingredient premarket notification at least 75 days prior to marketing a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered (21 USC §§ 331–333, 342, 350b).

The intended use of an item determines its regulatory status under the FD&C Act, and its regulatory status dictates the standard that is to be applied in evaluating its safety. Whether an article is regulated as a food or falls within a different regulatory classification depends on its intended use, rather than its source, biochemical composition, or ingestive status. Items classified as food are presumed safe, and the FDA bears the burden of establishing by a preponderance of the evidence that a particular food is injurious to health. However, this presumption of safety does not extend to most food components, the vast majority of which fall into specific regulatory classifications with attendant safety standards that must be met by manufacturers. Such food components include food additives, color additives, processing aids, substances that migrate into food from processing equipment or packaging materials, dietary ingredients in dietary supplements, pesticide residues, and animal drug residues. Circumstances requiring expert decision-making with respect to the safety of a food or food component range from the introduction of a new food ingredient or a new food use to the presence of an unapproved food additive or color additive and the intentional or unintentional addition of a contaminant. (See 21 USC § 321 (f); *Nutrilab Inc. v. Schweiker*, 713 F.2d 335, 337–8 (7th Cir. 1983); *Jones v. Rath Packing Co.* 430 US 519 (1997); *U.S. v. O.F. Bayer & Co.*, 188 F.2d 555 (2nd Cir. 1951).)

Introduction of new substance, new use

The Food Additives Amendment of 1958 added food additive provisions to the 1958 FD&C Act. The amendment established a broad definition for food additives and required FDA premarket approval of such substances. Exempted from food additive status were substances used in accordance with a

sanction or approval granted by either the FDA or the USDA prior to the enactment (known as prior-sanctioned substances), generally recognized as safe substances (commonly referred to as GRAS substances), color additives, pesticide chemical residues, pesticide chemicals, and new animal drugs. The Dietary Supplement Health and Education Act (DSHEA) of 1994 later exempted certain dietary supplement ingredients from the food additive definition. Of the substances exempted from regulation as food additives, only prior-sanctioned substances, GRAS substances, and certain dietary supplement ingredients do not require FDA approval or notification prior to use. However, exemption from food additive status does not exempt a substance from other provisions of the FD&C Act relating to safety. If the FDA were to conclude that a substance, which was prior-sanctioned or believed to be GRAS at a time when certain risks were not perceived, subsequently became known to be dangerous for use, the agency could object to use of the substance as an added poisonous or deleterious substance.

Food additives. The FD&C Act defines a food additive as "any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food . . . , if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used . . . prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use" (21 USC § 321(s)).

A substance that may become a component of food, either directly or indirectly, is regulated as a food additive unless the substance is considered a GRAS substance or falls within a category of substances exempt from regulation as food additives. Use of a food additive is prohibited unless FDA first approves the substance and publishes a regulation prescribing the conditions under which the additive may be safely used in foods (21 USC §§ 331, 342, 348).

The practical effect of classifying a product as a food additive rather than a food is twofold: (1) FDA premarket approval is required, and (2) the burden shifts to the manufacturer to demonstrate safety. Predictably, disputes have arisen as to whether a particular product constitutes a food or a food additive. The courts have held that whether a substance is a food additive is not dependent on quantity. Substances present in both large and small quantities can be food additives, and a substance may be both a food and a food additive regardless of whether the substance ordinarily would be considered a food.

However, not every component of food is a food additive. In the court case *U.S. v. Two Plastic Drums . . . Vipointe Ltd*, black currant oil was encased in a capsule made from gelatin and glycerin to facilitate swallowing. Seeking to condemn the component, the FDA claimed that the black currant oil was a food additive because the combination of black currant oil, glycerin, and gelatin created a food and merely being a component of food rendered the black currant oil a food additive. The 7th Circuit U.S. Court of Appeals disagreed with the FDA's broad interpretation of the food additive definition, subsequently concluding that "to be a food additive, a substance must not only be added to food, but it must also have the purpose or effect of altering a food's characteristic" (Vipointe at 818). Shortly thereafter, the first Circuit U.S. Court of Appeals confronted the issue again in the court case *U.S. v. 29 Cartons . . . an Article of Food* (Oakmont Investments), which also involved capsules of black current oil, finding that the gelatin capsule itself was not a food additive within the meaning of the FD&C Act. The court wrote, "The proposition that placing a single-ingredient food product into an inert capsule . . . converts that food into a food additive perverts the statutory text, undermines legislative intent, and defenestrates common sense." (See *U.S. v. Two Plastic Drums . . . Vipointe Ltd*, 984 F.2d 814 (7th Cir. 1993); *U.S. v. 29 Cartons . . . an Article of Food* (Oakmont Investments), 987 F.2d 33, 35 (first Cir. 1993).)

The FD&C Act provides that the FDA shall issue no food additive regulation if "a fair evaluation of the data . . . fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe" (21 U.S.C. § 348(c)(3)(A)). This is the general safety standard applied to food additives. For this purpose, the FDA defines safe as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21CFR § 170.3(i.)). The FDA recognizes that "it is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance" (21 CFR § 170.3(i)). In its risk assessment of food additives, the FDA must consider, among other relevant factors, the estimated consumption of the additive and of any substance formed in foods due to use of the additive, the cumulative effect of such substances and any chemically or pharmacologically related substances in the diet, and the appropriate safety factors for interpretation and extrapolation of animal data. In applying animal experimental data to man, the FDA typically uses a safety factor of 100 unless scientific evidence justifies a different safety factor (21 CFR § 170.22).

The 1958 amendment added the food additive Delaney Clause, which is also known as the anti-cancer clause, to the FD&C. The food additive Delaney Clause provides that no food additive shall be deemed safe if "it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal" (21 USC § 348(c)(3)(A)). The FDA has no discretion to permit use of a food additive for human food use once a finding of carcinogenicity is made regardless of the degree of risk involved. However, the Delaney Clause applies to the food additive itself and not to constituents of the additive. As the FDA has acknowledged, if a food additive has not been shown to cause cancer even though it contains a carcinogenic impurity, then the food additive Delaney Clause does not apply and the additive is properly evaluated under the general safety standard using risk assessment procedures to determine whether there is a reasonable certainty that no harm will result from the proposed use of the additive (FDA 2003). (See *Les v. Reilly*, 968 F.2d 985 (9th Cir. 1992); *Scott v. FDA*, 728 F.2d 322 (6th Cir. 1984)).

GRAS substances. GRAS substances are not regulated as food additives and do not require FDA approval prior to use. Shortly after enactment of the 1958 amendment to the FD&C Act, FDA published a list of many, but not all, substances believed to be GRAS for use in food (FDA 1958, 1959). These regulations are known informally as the FDA's GRAS list. However, the agency explicitly recognizes that it is impracticable for the FDA to publish regulations identifying all substances that are GRAS for use in food. The FDA also acknowledges that "a substance that is GRAS for a particular use may be marketed for that use without agency review and approval" (FDA 1997). Additionally, FDA recognizes that companies "have the right to make independent GRAS determinations on food substances" (FDA 1988). Manufacturers may notify the FDA of their GRAS determinations, but such notifications are completely voluntary (FDA 1997). (See 21 CFR Parts 182, 184, and 186; 21 CFR §§ 170.30(d), 182.1(a).)

A determination of the GRAS status of the use of a substance may be based either on common use in food prior to January 1, 1958 or on scientific procedures. Establishment of GRAS status through common use is ordinarily based on generally available data and information and does not require the same quantity or

quality of scientific evidence needed for food additive approval. The common use in food may occur outside of the United States, provided that documentation and independent corroboration of such use is both widely available in the country of use and readily accessible to interested, qualified scientific experts in the United States. However, in *Fmali Herb Inc. v. Heckler*, the 9th Circuit U.S. Court of Appeals cautioned that "evidence of foreign use of an ingredient, standing alone, may rarely or never be enough to establish safety."(See 21 CFR §§ 170.35(c)(1), 170.35(c)(2); *Fmali Herb Inc. v. Heckler*, 715 F.2d 1385, 1390–1 (9th Cir. 1983)).

Establishing GRAS status through scientific procedures requires both technical evidence of safety and evidence of common knowledge or general recognition among qualified experts. As to technical evidence of safety, the FDA's position is that "a GRAS substance is neither more nor less safe than an approved food additive" (FDA 1997). Therefore, the same quantity and quality of scientific evidence is required (at least in theory) to demonstrate safety of a substance that is GRAS based on scientific procedures as is required for a food additive. The common knowledge element is an important distinguishing factor between a substance that is GRAS based on scientific procedures and a safe food additive. To satisfy the common knowledge requirement, the data and information relied on to establish the safety of the substance must be generally available to the scientific community, and there must be a basis to conclude that a consensus exists among qualified experts about the safety of the substance for its intended use. (See 21 CFR § 170.30(b).)

A conclusion that a substance is actually safe is not sufficient to support the GRAS status of a food ingredient. Rather, the conclusion of safety must be supported by evidence that the substance is generally recognized among experts as safe under the conditions of intended use. While unanimity is not required, there must be evidence of a consensus among qualified experts and pivotal safety data must be publicly available. In this respect, it is a much higher standard than the general safety standard applied to food additives. (See *United States v. An Article of Food . . . Coco Rico Inc.*, 725 F.2d 11 (first Cir. 1985); *United States v. Articles of Food . . . Coli-Trol*, 518F.2d 743 (5th Cir. 1975); *United States v. Articles of Food . . . Sodium Pangamate*, (1978 to 1979 Transfer Binder) Food Drug Cosm. L. Rep. (CCH) ¶ 38,226.)

Color additives. The Color Additive Amendment of 1960 added FDA premarket approval and, for certain dyes, certification, requirements to the FD&C Act. A color additive is defined in the FD&C Act as a material that

- is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source, and
- when added or applied to a food . . . is capable (alone or through reaction with other substance) of imparting color thereto: except that such term does not include any material which (FDA), by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring. . . . The term color includes black, white, and intermediate grays. (See 21 USC §321(t).)

There are no exemptions from the color additive definition, and GRAS does not apply to color additives. Even if a color additive is generally recognized among qualified experts as safe for use as a color additive, it must first be approved and listed by the FDA before it may be used as a color additive in food.

The FD&C Act provides that the FDA "shall not list a color additive for a proposed use unless the data before (the FDA) establish that such use, under the conditions of use specified in the regulations will be safe . . . " (21 USC § 379e(b)(4)). For purposes of determining safety of a color additive, safe means that "there is convincing evidence that establishes with reasonable certainty that no harm will result" from such use in foods (21 CFR § 70.3(i)). Among other factors pertinent to safety, the FDA must consider the probable consumption of the color additive or any substances formed in food from the additive; the cumulative effect, if any, of the additive in the diet of man or animals, taking into account background dietary exposure and chemically or pharmacologically related substances; appropriate safety factors for application of animal data; and availability of practicable methods of analysis for identifying and quantifying the color additive in foods. (See 21 USC §§ 342(c), 379(e), 379e(b)(4),379e(b)(5).)

The Color Additive Amendment included an anti-cancer Delaney Clause for color additives. The clause states that an additive shall be deemed unsafe and shall not be listed by the FDA if the additive is found "to induce cancer in man or animal." No *de minimis* exception to the color additive Delaney Clause exists; once a color additive has been found to induce cancer in man or animal, the FDA cannot list the additive for use in human foods even if exposure to the substance in foods would present a toxicologically insignificant trivial risk. The courts have upheld the FDA's interpretation of this clause as applicable only to the color additive as a whole, not to its carcinogenic impurities. Thus, the FDA may approve a color additive shown not to cause cancer even though the additive contains trace amounts of a known carcinogenic impurity. The FDA will evaluate such substances applying risk assessment methods under the general safety clause. (See *Public Citizen v. Young*, 831 F.2d 1108 (D.C. Cir. 1987); *Scott v. FDA*, 728 F.2d 322 (6th Cir. 1984); 21 USC § 379e(b)(5)(B).)

A DES Proviso exception for colors used in feed for food-producing animals was added to the color additive Delaney Clause by the 1962 Drug Amendments. The DES Proviso exception allows the FDA to approve a color shown to induce cancer when ingested by man or animal for use in an animal feed only if 2 conditions are met: the color must not adversely affect animals consuming the colored feed, and FDA-approved or prescribed analytical methods would find no residue of the color in edible portions of the animal after slaughter or in food yielded by or derived from the living animal. (See 21 USC § 379e(b)(5)(B).)

Food contact materials. Without an established GRAS status or a prior sanction for the intended use, a substance present in food contact materials that could reasonably be expected to migrate into foods may be regulated as a food additive requiring FDA premarket approval. However, most substances used in food contact materials are not regulated as food additives. In 1979, the U.S. Court of Appeals for the District of Columbia Circuit held that the FDA must determine with a fair degree of confidence that such a substance migrates into food in more than insignificant amounts to regulate it as a food additive (*Monsanto v. Kennedy*, 613 F.2d 947 (D.C. Cir. 1979)). The court noted that the FDA may decide, based on scientific evidence, that the level of migration into food of a particular chemical is so negligible or *de minimis* as to present no public health or safety concern (*Monsanto v. Kennedy* at 955). In subsequent notice-and-comment rulemaking, the FDA concluded that numerous food additives used in the production of food contact articles would be expected to migrate to foods at trivial levels presenting no safety concerns and established a procedure for granting a threshold of regulation (TOR) exemption from food additive regulation, thereby exempting such substances from the premarket approval requirement (FDA 1993, 1995). A TOR exemption will be granted if "the substance has not been shown to be a carcinogen in humans or animals, and there is no reason, based on the chemical structure of the substance, to suspect that the substance is a carcinogen." In addition, the substance may not contain any carcinogenic impurities, or if it does, the impurity must have a TD_{50} (the dose that causes cancer in

50% of test animals) of less than 6.25 mg/kg body weight/d. The intended use of the substance must result in dietary concentrations ≤ 0.5 parts per billion (≤1.5 μg/person/d), or if it is already approved by FDA for direct addition to food, the dietary exposure must be less than 1% of the FDA's acceptable daily intake (or ADI). Finally, the substance may not have any technical effect in or on the food to which it migrates, and the substance must not adversely affect the environment. (See *Monsanto v. Kennedy*, 613 F.2d 947 (D.C. Cir. 1979); 21 CFR §§ 170.39, 170.39(a)(1), 170.39(a)(2), 170.39(a)(3)–(4), 174.6.)

The FDA Modernization Act of 1997 amended the FD&C Act to create a premarket notification (FCN) procedure as the primary method by which the FDA regulates substances that are used in production of food contact materials. The FD&C Act defines a food contact substance as any substance intended for use as a component of materials used in manufacturing, packing, packaging, transporting or holding food if such use is not intended to have any technical effect in such food. (See 21 USC §§ 348(h), 348(h)(6); 21 CFR §§ 170.100-170.106.)

The FD&C Act states that the FCN process shall be used for authorizing the marketing of a food contact substance unless the FDA determines that submission of a food additive petition is necessary to provide adequate assurance of safety. The FDA may require a food additive petition if (1) the use of the FCN increases cumulative daily dietary concentration to 200 ppb (0.6 mg/person/d) or more for a biocide or to 1 ppm (3 mg/person/d) or more for a nonbiocide, or (2) there exists a bioassay of the FCN which the FDA has not reviewed and in which the results are not clearly negative for carcinogenic effects. The FDA must apply the food additive general safety standard in its risk assessment of a food contact substance. (See 21 USC §§ 348(c)(3)(A), 348(h)(1), 348(h)(3)(A); 21 CFR § 170.100(9c)(1).)

Dietary supplements and dietary ingredients for dietary supplements. The DSHEA amended the FD&C Act to define dietary supplements and prevent regulation of such products as food additives. Consequently, a dietary supplement is a product (other than tobacco) intended to supplement the diet that contains 1 or more of the following dietary ingredients:

- vitamin;
- mineral;
- herb or other botanical;
- amino acid;
- dietary substance for use by man to supplement the diet by increasing the total dietary intake; and
- concentrate, metabolite, constituent, extract, or combination of any of the aforementioned. (See 21 USC § 321(ff)(1).)

Dietary supplement products must also be intended for ingestion as a tablet, capsule, powder, soft gel, gel cap, or liquid; labeled a dietary supplement; and not be represented for use as a conventional food or as a sole item of a meal or diet. (See 21 USC §§ 321(ff)(2); 350(1)(B).)

The DSHEA also added new safety standards to the FD&C Act specifically for dietary supplements. A dietary supplement or a dietary ingredient therein is deemed adulterated if the supplement "presents a significant or unreasonable risk of illness or injury . . . under . . . conditions of use recommended or suggested in labeling, or . . . if no conditions of use are suggested or recommended in the labeling, under ordinary conditions of use" (21 USC §§ 342(f)(1)(A)). The FDA bears the burden of proof in court if it asserts that a dietary supplement is adulterated under this safety standard. In banning ephedrine alkaloids, the FDA interpreted the term "unreasonable risk" in the safety standard to mean "a relative weighing of the product's known and reasonably likely risks against its known and reasonably likely benefits" (FDA 2004). Furthermore, the FDA concluded that "in the absence of a sufficient benefit, the presence of even a relatively small risk of an important adverse health effect to a user may be unreasonable" (FDA 2004). However, the agency acknowledged that some evidence of risk is required to meet this standard.

A dietary ingredient not marketed in the United States before October 15, 1994, is considered a new dietary ingredient. A dietary supplement containing a new dietary ingredient is deemed adulterated unless either (1) the supplement "contains only dietary ingredients which have been present in the food supply as an article used for food in a form in which the food has not been chemically altered," or (2) there is a "history of use or other evidence of safety establishing that the dietary ingredient when used under the conditions recommended or suggested in the labeling . . . will reasonably be expected to be safe" (21 USC § 350b(a)). In the latter case, the manufacturer or distributor of the dietary ingredient or dietary supplement must, 75 days prior to marketing, submit to the FDA information (including citations to any published articles) that constitutes the basis upon which the manufacturer or distributor concludes that a product will reasonably be expected to be safe. (See 21 CFR § 190.6.)

In addition, a dietary supplement or dietary ingredient therein may also be deemed adulterated if the Secretary of the Dept. of Health and Human Services declares that it poses an imminent hazard to public health or safety. The authority to declare a dietary supplement to be an imminent hazard must be exercised by the secretary and may not be delegated to the FDA. The secretary must thereafter promptly hold a formal hearing to assemble data "to affirm or withdraw the declaration" (21 USC § 342(f)(1)(C)). As with the significant or unreasonable risk of illness or injury standard, the government bears the burden to show that the dietary supplement or dietary ingredient is adulterated. The net effect of these new standards is significantly less demanding than the reasonable certainty of no harm under the conditions of intended use and wholly at variance with the legal framework for food and color additives addressed earlier.

Pesticide chemical residues. Before a pesticide chemical may be sold or distributed in the United States, the EPA must evaluate the pesticide and determine whether to grant a registration permitting such sale and distribution under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The EPA also establishes a tolerance (that is, maximum residue level), or an exemption from the requirement of a tolerance, for the pesticide chemical prior to its use on food crops. Under the FD&C Act, any pesticide chemical residue in or on a raw agricultural commodity is deemed unsafe, thereby rendering the food adulterated, unless exists a tolerance or an exemption governing such use of the pesticide chemical exists. Minute residues of the pesticide chemical may be present in processed foods produced from treated agricultural commodities. A pesticide residue in a processed food does not render the processed food adulterated if the pesticide was applied to the raw agricultural commodity pursuant to a tolerance, the residues in or on the commodity were removed to the extent possible under GMP, and the residue level in the processed food does not exceed that allowed for the commodity. Residues of metabolites or other degradation products of pesticide chemicals may not render a food adulterated as long as certain conditions are met. If the pesticide applied to the commodity is subject to an exemption, its residues in a processed food do not render the processed food adulterated. While the EPA is responsible for establishing, modifying, and revoking or suspending pesticide residue tolerances, the FDA is responsible for enforcing such tolerances in domestic and imported foods other than meat, poultry, and certain egg products. (See 21 USC §§ 342(a)(2)(B), 346a(a)(1), 346a(a)(2)(A).)

The 1996 Food Quality Protection Act (FQPA) amended both the FIFRA and the FD&C Act to establish a safety standard for pesticide chemical residues in food, and required EPA to reassess

existing pesticide chemical tolerances and exemptions under the new safety standard:

> . . . 'safe,' with respect to a tolerance for a pesticide chemical residue, means that . . . there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information. (See 21 USC §§ 346a(b)(2)(A)(ii), 346a(c)(2)(A)(ii).)

To apply the FQPA safety standard, the EPA refined its existing risk assessment methods and added some new risk assessments. For example, the EPA considers the following to establish, modify, or revoke a tolerance or an exemption:

- the validity and reliability of available data from studies on the pesticide or its residues;
- any toxic effects shown in such studies;
- the relationship of pertinent study results to human risk;
- the dietary consumption patterns of consumers and major identifiable subgroups;
- the cumulative effects of such residues and other substances having a common mechanism of toxicity;
- the aggregate exposure levels of consumers and major identifiable subgroups to residues and related substances, including dietary exposure under tolerances in effect or from nonoccupational sources;
- the variability of sensitivities of major identifiable consumer subgroups;
- information on possible effects similar to estrogenic or other endocrine effects; and
- safety factors considered appropriate for reliance on animal data by food safety experts. (See 21 USC § 346a(b)(2)(D)(i)-(ix).)

In particular, the EPA must also assess any risk to infants and children associated with aggregate exposure to the pesticide residue. With respect to use, the EPA must consider whether use of the pesticide protects consumers from adverse health effects that would pose a greater risk than dietary risk from the pesticide residues and whether use of the pesticide is necessary to avoid significant disruption in domestic production of an adequate, wholesome, and economical food supply. (See 21 USC §§ 346a(b)(2)(B)(iii), 346a(b)(2)(C).)

Animal drug residues in food-producing animals. In general, the FDA must approve new animal drugs as safe and effective for the intended use in animals, and food produced from such animals must be safe for human consumption. The new animal drug approval provisions in the FD&C Act include a Delaney Clause prohibition of FDA approval of animal drugs shown to induce cancer in man or animals, as well as a DES Proviso exception from the prohibition. As mentioned earlier, the DES Proviso allows FDA approval of use of a carcinogenic compound in food-producing animals, notwithstanding the food additive, color additive, and animal drug Delaney Clauses, if the compound does not adversely affect the animal under the conditions of intended use and if no residue of the compound would be found in the edible tissues after slaughter or in food from the live animal by an FDA-approved analytical method. (See 21 USC §§ 360b, 360b(d)(1)(I).)

For a carcinogenic compound to be approved under a DES proviso, any residue in the target tissue must be nondetectable or below the limit of detection (LOD) of the approved regulatory method (FDA 2002). The FDA defines the LOD as the lowest concentration of analyte that can be confirmed by the approved regulatory method, and the "regulatory method" as the aggregate of all experimental procedures for measuring and confirming presence of the marker residue of the carcinogenic compound in the target animal tissue (21 CFR § 500.82(b)). The FDA uses the "no significant risk" level (maximum lifetime risk of cancer of 1 in 1 million) determined through appropriate toxicological testing as a benchmark for assessing acceptability of a regulatory method. (See 21 CFR § 500.84(c).)

Presence of poisonous or deleterious ingredients

The FD&C Act provides a fail-safe provision that enables the FDA to act against substances that are not subject to premarket approval by or notification to the agency and that present a risk to human health when present in food. The FD&C Act deems a food adulterated if it bears or contains any poisonous or deleterious substance that may render it injurious to health. However, in case the substance is not an added substance, such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health. (See 21 USC § 342(a)(1).)

The FDA has frequently relied on this provision to regulate the presence in food of environmental contaminants such as lead, mercury, dioxin, and aflatoxin (FDA 1992). There are thus 2 separate and distinct safety standards that may be applied, depending upon whether a substance has been "added" to a food or not. If an added substance, a poisonous or deleterious substance causes the food to be adulterated if the substance may render the food injurious to health. If the substance is not added, it renders the food adulterated if the quantity that is present would ordinarily render the food injurious to health. The FD&C Act also deems unsafe and therefore adulterated foods containing an added poisonous or deleterious substance (other than a pesticide chemical residue, a food additive, a color additive, or a new animal drug) at levels that exceed an FDA-established tolerance level for such substance. (See 21 USC §§ 342(a)(2)(A), 346.)

Added substances. While the FD&C Act does not define the term "added," the courts have interpreted the term to require some demonstrable human intervention to render a substance added. In *United States v. Anderson Seafoods Inc.*, the 5th Circuit U.S. Court of Appeals held that the term "added" as used in 21 USC § 342(a)(1) means artificially introduced or attributable in some degree to the acts of man. The court determined that some mercury in fish is an added substance because it is not naturally produced by fish and becomes a component of the fish through contamination of its habitat by man. The court acknowledged that part of the mercury in fish may occur naturally and is thus not added but concluded that where some portion of a toxin has been introduced into the food by man, the entirety of that substance in the food will be treated as an added substance for purposes of the safety standard. (See *United States v. Anderson Seafoods Inc.*, 622 F.2d 157, 159–61 (5th Cir. 1980); *U.S. v. Blue Ribbon Smoked Fish Inc.*, 179 F. Supp. 30 (E.D. N.Y. 2001); *Continental Seafoods Inc. v. Schweiker*, 674 F.2d 38 (D.C. Cir. 1982).)

FDA regulations define an added poisonous or deleterious substance as one that does not naturally occur. In contrast, a naturally occurring poisonous or deleterious substance is "an inherent natural constituent of a food and is not the result of environmental, agricultural, industrial, or other contamination" (21 CFR § 109.3(c)). If the presence of a naturally occurring poisonous or deleterious substance is increased to abnormal levels through mishandling or other intervening acts, the substance may be rendered an added substance to the extent of such increase. The FDA "regards any substance that is not an inherent constituent of food or whose level in food has been increased by human intervention to be 'added' within the meaning of section (342(a) (1))" (FDA 1992). The agency views "a naturally occurring toxicant whose level is unintentionally increased by the genetic modification, as well as an unexpected toxicant that first appears in the food as a result of pleiotropic effects" to be added substances (FDA 1992).

For an added substance, the FDA need only show that the substance could render the food injurious to health. The "could render" standard means that the possibility of injury to the consumer is reasonable. It does not mean that there must be absolute certainty that no one under the most extreme circumstances could be harmed. The U.S. Supreme Court acknowledged in the court case *U.S. v. Lexington Mill* that "(a) very large majority of the things consumed by the human family contain, under analysis, some kind of poison," but the determination of whether the substance is dangerous depends upon the quantity and combination. The Supreme Court further stated that if a food could not by any possibility injure the health of any consumer, such food, though having a small addition of poisonous or deleterious ingredients, could not be condemned under the FD&C Act. The safety standard is one of a reasonable possibility of injury to health. (See *U.S. v. Lexington Mill & Elevator Co.*, 232 U.S. 399 (1914); *United States v. Anderson Seafoods Inc.*, 622 F.2d 157, 159-161 (5th Cir. 1980); *U.S. v. Anderson*, 447 F. Supp. 1151, 1155 (N.D. Fla. 1978).)

If an added poisonous or deleterious substance—other than a pesticide chemical residue, food additive, color additive, or new animal drug—is required or unavoidable under current good manufacturing practice, and if the FDA has established a tolerance level for use of such substance, then food containing the substance will not be deemed adulterated, provided such substance is present in the food in an amount below the tolerance level. The FDA has discretion in deciding whether to establish a tolerance level, which requires formal notice-and-comment rulemaking and prefers to employ informal guidance levels (also known as action levels) instead. Action levels are enforcement discretion guides that do not have the force of law, but rather inform industry that FDA generally will not enforce the FD&C Act's adulteration provisions with respect to an affected substance provided that the level of the necessary or unavoidable added poisonous or deleterious substances present in food remains below the established action level. Both tolerances and action levels are based on unavoidability; they do not establish a permissible level of contamination when such level is avoidable (21 CFR § 109.7(a)). (See 21 USC § 342(a)(2)(A); *Young v. Community Nutrition Inst.*, 476 U.S. 974 (1986).)

Not-added substances. Because the FDA defines a naturally occurring poisonous or deleterious substance as an inherent natural constituent of the food that is not present due to environmental, agricultural, industrial, or other contamination, bones in fish and oyster shell fragments are deleterious substances within this category. Potentially poisonous amygdalin, which is present naturally in apricot kernels and numerous other fruits, vegetables, grains, and seeds, is another example of a not-added substance. The safety standard applied to such nonadded substances is whether its presence ordinarily renders the food injurious to health. Courts interpret the standard to mean ordinary uses under ordinary conditions. This standard is more relaxed than the "render injurious" standard applicable to added substances. (See *U.S. v. 1232 Cases American Beauty Brand Oysters*, 43 F.Supp. 749 (W.D.Mo. 1942); *Millet, Pit and Seed Co. Inc. v. United States*, 436 F. Supp. 84 (E.D. Tenn. 1977).)

International Framework

Although the FD&C Act provides the fundamental framework for food chemical safety assessments in the United States, the food industry must consider a more global perspective because it serves and operates in international markets. The globalization of the food supply and the safety of foods and their constituents warrants that international commerce be addressed by agencies and organizations that keep pace with scientific advances, technological innovations, and international food standards. As consideration of food and food ingredient safety is increasingly broadened to the international stage, questions arise: Who undertakes food safety matters? Through what procedures and under what authority does this occur? How are food chemical safety determinations applied across multiple countries?

Codex Alimentarius Commission

In 1963, the United Nations' Food and Agriculture Organization (FAO) and the World Health Organization (WHO) created the Codex Alimentarius Commission, an international food standards setting organization that develops international food standards and codes of practice to protect consumers' health and facilitate fair practices in international trade in food. These various standards and practices comprise the Codex Alimentarius (the food code). It includes standards for food additives, contaminants, and toxins in foods that contain both general and commodity-specific provisions as well as guidance on a variety of food subjects and issues (FAO-WHO 2006) (Table 1). The United Nations recognized the importance of the Codex Alimentarius in addressing food safety internationally in 1985 when it passed Resolution 39/248, which states, "When formulating national policies and plans with regard to food, Governments should take into account the need of all consumers for food security and should support and as far as possible adopt . . . standards from the Codex Alimentarius" (CAC 2009). More recently, the Sanitary and Phytosanitary Measures Agreement (SPS Agreement) of the Uruguay Round of GATT requires World Trade Organization (WTO) members to base their sanitary and phytosanitary measures on international standards and to participate in Codex. The SPS Agreement specifically recognizes Codex texts as the international benchmark. The revised Technical Barriers to Trade Agreement also references Codex texts. Thus, the Codex Alimentarius and standards within it directly affect food safety and international food commerce and are an integral part of the legal framework within which international trade is being facilitated through harmonization (FAO-WHO 2006).

Codex is truly global in nature since membership is open to all member nations and associate members of FAO or WHO. Member nations span the range from those that are highly developed

Table 1—Standards and other guidance comprising the Codex Alimentarius.

Type of Guidance	Number of Texts
Commodity standards	186
Commodity-related texts	46
Food labeling	9
Food hygiene	5
Food safety risk assessment	3
Sampling and analysis	15
Inspection and certification procedures	8
Animal food production	6
Contaminants (maximum levels, detection, prevention)	12
Food additive provisions	1112 (addressing 292 additives)
Food additive-related texts	7
Pesticides (maximum residue limits)	2930 (addressing 218 pesticides)
Veterinary drugs (maximum limits)	441 (addressing 49 veterinary drugs)
Regional guidelines	3

Source: FAO-WHO 2006.

to newly emerging. Nearly 99% of the world's population is represented in the commission through approximately 174 member countries and 1 member organization (the European Community) (FAO-WHO 2006). In addition to government delegations in Codex matters, nongovernmental organizations (NGOs) also contribute to deliberations, participating as observers. NGO delegations represent consumer organizations, the food industry, and scientific associations (for example, IFT).

The Codex system involves standing committees that are general subject (such as Codex Committee on Contaminants in Foods) or commodity (such as Codex Committee on Fish and Fishery Products) in nature, regional coordinating committees, ad hoc task forces, a variety of collaborative activities, expert consultations, and expert advisory committees. To develop standards and other guidance texts such as those addressing the safety of food additives, Codex follows a transparent, defined, and often lengthy 8-step process upon acceptance of a specific proposed project by the commission or executive committee. This process is somewhat akin to a notice-and-comment rulemaking procedure used in the United States. To be sure, the scope and amount of information arising on food chemical safety topics can be formidable and far-reaching. This is often the case when Codex considers food ingredient standards and key characteristics, such as maximum use levels and the presence of undesirable components such as contaminants. During deliberations, questions often arise regarding whether the interpretations of the cited studies are accurate and whether the quality and quantity of science are sufficient to support the recommended positions potentially emanating from Codex. In addition to safe use levels and material purity, methods of analysis within a food matrix must be addressed.

In utilizing the committee structure, Codex subsidiary bodies draft texts ultimately yielding monographs that are then circulated to member countries, NGOs, and other interested parties. If warranted from the reviews, revisions are generated, which often involve further deliberation in preparation for final adoption. Although the Codex philosophy embraces harmonization, consumer protection, and facilitation of international trade, it can be difficult for countries to accept Codex standards in the statutory sense because of different legal formats, technical infrastructure, administrative systems, and various political systems (FAO-WHO 2006).

Expert advisory committees. Of great importance to Codex deliberations and national regulatory authorities is the work of scientific advisory bodies to the Codex Alimentarius Commission, FAO, and WHO and expert consultations. Of the 3 main FAO/WHO expert advisory bodies, 2 relate to food chemical evaluation and assessment. The Joint Expert Committee on Food Additives (JECFA), established in 1955, advises primarily through the Codex committees on food additives, contaminants, and residues of veterinary drugs in foods. These committees determine the food additives, contaminants, and veterinary drug residues JECFA should assess, enabling the work leading to their incorporation into Codex standards. Established in 1963, the Joint FAO/WHO Meetings on Pesticide Residues (JMPRs) recommend maximum residue limits (MRLs) for pesticide and environmental contaminants in specific food products to ensure the safety of foods containing residues and methods of sampling and analysis. The JMPRs work closely with the Codex Committee on Pesticide Residues, which identifies substances that need evaluation prior to considering and determining acceptable MRLs for the commission to consider adopting.

The input provided by these expert bodies is thus important when the Codex commission considers safety evaluations of food additives, food contaminants, animal drug and pesticide residues, and other food components and when it develops specifications and adopts acceptable methods of analysis. Codex derives great value from the JECFA and the JMPRs since a broad cross-section of technical disciplines—biochemistry, chemistry, food science and technology, physiology, pharmacology, toxicology, and pathology—is represented on the advisory groups, and selected experts are preeminent, impartial, and objective.

JECFA safety assessments and evaluations. During the last 50 years the JECFA has evaluated more than 1500 food additives, around 40 contaminants and naturally occurring toxicants, and residues of about 90 veterinary drugs (FAO-WHO 2006). Two important points about food chemicals are considered during JECFA safety evaluations: (1) most food components and additives are consumed at low levels in humans, possibly over an entire lifetime; and (2) toxicology studies are conducted at exaggerated doses that use concentrations, routes of exposure, and shorter periods of exposure that may not realistically reflect the anticipated human experience with the food chemicals in question. The JECFA, the JMPRs, and the Intl. Programme on Chemical Safety (IPCS) have developed principles) for the safety assessment of food additives and contaminants and toxicological assessment of pesticide residues in food.

The JECFA evaluates the toxicology and broad composite scientific information that is available and extrapolates the findings to arrive at an exposure that is considered to be safe for humans. This composite of information includes data and information submitted by interested parties in response to specific calls for data published by the JECFA. It is incumbent on interested parties to compile and submit for evaluation comprehensive scientific data for JECFA review. Such information includes the following:

- composition of the material(s) in question, including specifications for purity and identity that reflect the material that is subject to toxicology testing;
- appropriate safety studies from humans and experimental animals; and
- identification of food chemical applications, associated food use levels, and estimations of probable human intake.

The JECFA commonly applies safety factors, as the FDA does in its safety assessments, to account for uncertainties and limitations surrounding available scientific data as well as the experimental design limitations associated with the conduct of safety testing regimens. For chemical contaminants, the JECFA introduced the margin of exposure (MOE) approach developed by the European Food Safety Authority (EFSA) (2005). When considering intentional food additives, safety factors of at least 100 are commonly applied to a no-observed-effect level (NOEL) that is determined experimentally. The hundredfold safety factor reflects a tenfold variation in sensitivities within a given test species along with another tenfold variation in extrapolating from test animal experiences to man. In the absence of satisfactory safety data, the JECFA may also impose an additional safety factor as prompted by any safety concerns and judged against the available scientific data.

By considering the accumulated animal data and any available human studies, the JECFA determines an ADI that is usually expressed in terms of milligrams of substance per kilograms of body weight per day. If the accumulated scientific information is insufficient or incomplete, the reliance on safety factors larger than 100 may be required, which, in turn, may yield an ADI for the subject material that is below the estimated consumer exposure. When this occurs, either the proposed food uses must be restricted to reduce the estimated consumer exposure to a level that is less than the ADI or additional toxicology testing must be conducted to support an increased ADI that would exceed the estimated consumer exposure to the subject additive. However, care must be exercised when considering the reduction of the proposed additive use level to better align with a calculated ADI

since doing so could result in too low a level to achieve the intended technological effect.

In addition, the JECFA estimates the consumer exposure or probable daily intake of the substance in question, which is subsequently compared to the ADI. If the ADI is higher than the consumer exposure estimate, the additive is deemed to be safe for its intended uses. The JECFA findings are shared with the CCFA, which translates the JECFA assessment into a maximum allowable concentration of substance in food. Alternatively, the Codex committee may determine that a maximum level is unnecessary.

On occasion, the JECFA safety deliberations may yield an ADI that is not specified. Such a finding does not mean that the additive in question may be used in foods in any amount; instead, the JECFA and Codex ascribe to the principle that additives are to be incorporated into foods at the minimum levels needed to achieve desired technological effects and no more. Those additives that receive assigned ADIs of not specified exhibited very low toxicities on the basis of available science and the projected total dietary intake of the substance when added to foods at the minimum level that achieves the desired technological effect. Such use does not constitute a hazard to consumer health. Consequently, the JECFA regards the establishment of a numerical ADI as unnecessary for these additives.

The European Union

Many governments around the world maintain national control systems that affirmatively address chemical food safety matters based on their own laws and regulations. The European Union (EU) has been particularly active in setting laws and standards on chemical food safety. During recent years, the differing laws and degrees of law on food safety that existed in individual EU member countries, or member states, have been harmonized under common European law. These laws have set standards for food produced in and imported into EU member states. Other countries around the world have been influenced by these standards, whether for compliance of foods and food ingredients for export to EU member states or because national governments elsewhere decided to develop similar standards for local use. EU standards often set a precedent for wider discussions on global standards such as Codex.

Regulation 178/2002 consolidated an integrated farm-to-fork approach as a general principle for EU food safety policy. This general food law regulation recognizes international obligations and takes into account international standards. However, international standards do not always exist or may be insufficient for EU consumer protection expectations.

The EU Food Law adheres to the 3 well-recognized components of risk analysis: risk assessment, risk management, and risk communication. Risk management actions are based on a scientific risk assessment but also take into consideration a wide range of other factors such as consumer information, prevention of misleading practices, feasibility of controlling a risk, most effective risk reduction actions for the relevant part of the food supply chain, practicalities, socio-economic effects, and environmental impact.

The risk assessment process in the EU was transferred in 2002 from the European Commission and its Scientific Committee on Food to the EFSA under the EU Food Law. This gave risk assessment more independence and visibility in the EU. The EFSA has a number of independent scientific panels made up of technical experts in various fields. When an issue arises that requires scientific risk assessment, the risk managers at the European Commission forward a technical request to the EFSA or the EFSA will self-task for an assessment by the respective scientific panel. The European Commission then uses the EFSA assessment as the scientific basis for determining the extent of risk management actions that are necessary.

Transparency and effective consultation with stakeholder groups are essential elements for regulatory authorities to build external confidence in their actions. Consumer awareness and demands on food matters are high in European countries. Communication, evaluation, and explanation of potential risks, including full transparency of scientific opinions, are important for EU authorities and are expected by EU consumers. Consumer confidence is a primary goal for EU actions on food. Therefore, the EFSA has established guidance on general principles of scientific transparency for the scientific aspects of risk assessment. In addition, the EFSA follows the general risk assessment principles of Codex and has elaborated its guidance while also consulting with external stakeholders.

The European Commission develops EU legislation for managing food safety risks in conjunction with government representatives of its member states and consults with stakeholders such as industry and consumer groups. The European Commission proposes framework legislation to the European Council and Parliament. Within frameworks, the European Commission may often develop separate technical legislation without further consulting the council and parliament. The framework approach for regulating chemical contaminants is a good example to consider. Chemical contaminants are regulated under the framework of Council Regulation 315/93, which gives the European Commission the authority to establish separate legislation within the framework, including setting maximum levels on specific contaminants. In the EU, a number of regulations have addressed levels of contaminants in foods and these regulations are consolidated in Commission Regulation 1881/2006.

Determining acceptable safe levels is an issue facing all regulatory authorities. Credible levels must be based on sound principles. Scientific and other considerations are necessary to determine what satisfies the risk assessment advice and what is feasible and reasonably achievable in commercial practice. The EU follows the as-low-as-reasonably-achievable (ALARA) principle of decision-making. A difficulty and point of reservation for some groups on the ALARA principle is how to define "reasonably" in each case to ensure that reasonably achievable levels are indeed "reasonably achievable," based on good practices that are possible in commercial practice. For stakeholder consultations, the availability of data for ALARA-based levels is imperative. This ensures that regulators have information to address any practical difficulties, and supports food safety protection without presenting an unreasonable burden for food production.

For risk assessors, one category of food chemicals that is not straightforward when providing useful advice to risk managers is genotoxic carcinogens. Genotoxic carcinogens are considered to have no threshold of safety, potentially causing direct adverse effects on DNA. Therefore, no tolerable exposure levels can be set, which would otherwise help determine safe levels of dietary intake. To promote more useful advice on genotoxic contaminants and substances found in food, the EFSA developed an MOE approach. This considers the margin between doses shown in experimental studies to cause adverse effects and the amounts eaten by consumers. This gives an indication of how close human dietary intake is to levels shown to cause toxic effects in the laboratory. With this logic, it is important to ensure that the available data set on sources of dietary exposure is as complete as possible. Dietary exposure estimates must be based on consumers of the food and not on entire populations, as many people may never eat the food substance in question. Moreover, consumers of average levels must be taken into account as well as consumers who regularly eat higher amounts of the foods.

If the MOE is small, then risk management action is more likely to be necessary, particularly if all major sources of dietary intake are not understood. In some cases, even if the MOE is high overall but poor practices are known to cause high contamination in certain products, then risk management action is likely to be necessary to address problems. For example, direct heating from inappropriate fuel sources in poorly controlled environments caused very high levels of polycyclic aromatic hydrocarbons. In response, the EU developed maximum levels for polycyclic aromatic hydrocarbons. Clearly, for undesirable chemical contaminants not intended for use in food production, no authorization process is involved in their regulation. However, for substances that are intended for use in food production, different regulatory processes apply.

Additives have technological functions in food and their need and safety must be demonstrated. They must therefore be authorized for specific uses at predetermined levels based on functionality and safety assessment data. A complicating factor with additives is that there is often need for further safety evaluation even after authorization. Re-evaluation is sometimes necessary to respond to new scientific publications—hence the fluctuating views on some commonly used additives that can sometimes be raised in the media. Similarly, pesticides are controlled based on their agricultural function and safety to ensure that residues in food are kept to a minimum. The threshold of safety for intake of a given pesticide is often considerably higher than the likely exposure based on the legal MRLs authorized in food materials. This approach aims to encourage good agricultural practices (GAPs) and control the application of pesticides and the total residues in food. MRLs also help indirectly control residues in the environment; however, specific legislation applies for environmental residues. Environmental residue limits cannot be transposed to food items because the science and practicalities are very different. Whenever MRLs are set for any pesticide in food, it is important that they are calculated based on GAPs in the growing region for the particular raw ingredients to be consumed.

If any country decides to adopt a standard similar to one set by another country, the precise context for the standard, local implications, and feasibility of adopting that standard must be understood, based on what is known and what needs to be known about local products. For example, when the EU sets a standard, it takes into account thorough investigation of available data and good production practices for the implicated food substance as consumed in the EU. However, for the same food substances consumed in a different country, there could be differences in the way the substance is used, produced, or consumed that may have implications on what is reasonable to achieve locally. Local authorities must understand such differences and generate local data. When common international standards are being developed, countries can use the global Codex forum to input their local information to develop and promote standards that would be reasonably achievable on a global front, taking into account their local knowledge.

The precautionary principle

When the Rome Treaty was adopted in 1957, the regime of food products was influenced by 2 main sets of rules, the Common Agricultural Policy (CAP) and the general principle of free movement of goods within the European Community. Technically, there was no food law; however, ensuring safety of the consumer was contemplated both by the CAP, which included hygiene and veterinary rules, and by the enforcement of the free movement of goods principle, which allowed member states to prohibit imported products for the protection of public health. For this reason, some European Community directives were aimed at harmonizing the laws of the member states on matters related to consumer safety, as was the case with additives.

This legal background changed significantly in 1993 when the Maastricht Treaty modified the EC Treaty of 1957 by adding several new titles, including public health, consumer protection, and environment. A new general obligation was also imposed on the commission: to "take as a base a high level of protection" in all its proposals concerning health, safety, environmental protection, and consumer protection (article 100.3(95.3), EC Treaty). At the same time, article 130r(174) relating to environment was introduced. This article states, "Community policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Community. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should *a priori* be rectified at its source and that the polluter should pay." Although the precautionary principle was initially stated only in provisions of the treaty related to environment, it was eventually implemented as a basic principle of food law, backed by the European Court of Justice (ECJ), and is now well-embedded in food law.

The first step in applying the precautionary principle to foods occurred in the context of the bovine spongiform encephalopathy (BSE) crisis that erupted in 1996. In the case Natl. Farmers Union, the ECJ concluded that "where there is uncertainty as to the existence or extent of risks to human health, the institutions may take protective measures without having to wait until the reality and seriousness of those risks become fully apparent." The ECJ further stated, "That approach is borne out by Article 130r(1) of the EC Treaty, according to which Community policy on the environment is to pursue the objective *inter alia* of protecting human health." Article 130r(2) provides that the policy is to aim at a high level of protection, be based on the principles of preventive action, and integrate environmental protection requirements into the definition and implementation of other community policies. (See Natl. Farmers Union, ECJ C-157/96 (May 5, 1998).)

Several other subsequent judgments confirmed this approach and explicitly quoted the precautionary principle in areas other than the environment, particularly in food and feed. For example, in the case *Pfizer Animal Health*, which was related to an authorization procedure for an animal feed substance, the Court of First Instance (CFI) asserted that "the Community Institutions may, by reason of the precautionary principle, take protective measures without having to wait until the reality and seriousness of those risks become fully apparent." This judgment along with court decisions rendered during the same time period and subsequently by the ECJ and the CFI founded all the basic rules for the enforcement of the precautionary principle and the conditions for its use. (See Pfizer Animal Health, CFI 1-13/99 (Sept. 11, 2002).)

In parallel to the ECJ and CFI decisions, European Community institutions adopted a basic text on food law, Reg. 178/2002, in which for the first time, food as well as food law were defined, establishing the general principles and requirements of food law, the EFSA, and procedures in matters of food safety. Accordingly, food safety has become one of the cornerstones of food law regulation, and the precautionary principle has become one of its basic principles in the EU.

Noting that "Food Law shall pursue one or more of the general objectives of a high level of protection of human health and the protection of consumer's interests" (article 5.1, EC Treaty), the regulation documents the principles of risk analysis and mentions the precautionary principle as a tool for risk management. Articles 6 and 7 must be interpreted together to understand how European Community law now regulates decision-making regarding food safety when the science is incomplete. As to risk analysis, the treaty states, "Risk assessment shall be based on the available

scientific evidence and undertaken in an independent, objective and transparent manner"(article 6.2, EC Treaty), and "risk management shall take into account the results of risk assessment, and in particular, the opinions of the EFSA referred to in article 22, other factors legitimate to the matter in consideration and the precautionary principle where the conditions laid down in article 7.1 are relevant, to achieve the general objectives of food law established in article 5" (article 6.3, EC Treaty). Is it therefore clear that the precautionary principle is a tool for risk management and does not intervene at the risk assessment stage, which is entirely within the competence of science?

As to the precautionary principle and the conditions of its use, article 7 states,

> In specific circumstances where, following an assessment of available information, the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures necessary to ensure the high level of health protection chosen in the Community may be adopted, pending further scientific information for a more comprehensive risk assessment.
>
> Measures adopted on the basis of paragraph 1 shall be proportionate and no more restrictive of trade than is required to achieve the high level of health protection chosen in the Community, regard being given to technical and economic feasibility and other factors regarded as legitimate in the matter under consideration. The measures shall be reviewed within a reasonable period of time, depending on the nature of the risk to life or health identified and the type of scientific information needed to clarify the scientific uncertainty and to conduct a more comprehensive risk assessment.

This text was adopted in 2002, several years after the precautionary principle was first mentioned in the context of food law and after long debates within the EU and internationally, particularly in Codex. Much of the debate has subsided since the text of Regulation 178/2002 takes into account the case law of the ECJ and the CFI and delineates clear limits to the use of the precautionary principle. However, not all problems and misunderstandings have vanished.

According to Regulation 178/2002, the precautionary principle is a tool for risk management to be used by those who make food law (that is, regulatory authorities). It does not create obligations bearing directly on private operators. The responsibilities of private operators are addressed in articles other than articles 6 and 7 in Reg. 178/2002 (see art. 14 to 21). It is obvious that since the precautionary principle may govern decisions made by the public authorities, private operators must remain cognizant of it and would be well advised to take it into account when making management decisions. However, there is an ongoing debate as to whether failure to act according to the precautionary principle would provide a basis for suing or condemning an operator. Thus far, there is no precedent in the case law that would impose a penalty on an operator based on the precautionary principle.

By definition, the precautionary principle does not apply when there is any doubt whatsoever about the safety of a product. The precautionary principle is the response to the problem of incomplete science and not just lack of information or doubt regarding the accuracy of a process. Therefore it would be inappropriate for an operator to rely on a precautionary principle alone to justify a recall or restrictive measure as a way to benefit from the situation when the science is incomplete. Moreover, risk management measures taken on the basis of the precautionary principle are provisional, and further scientific information is necessary for a more comprehensive risk assessment. This means that the legality of a measure based on the precautionary principle depends not only on the fact that uncertainty persists but also that the research goes on even after said measure has been taken. Furthermore, article 7.2 of the EC Treaty explicitly states that the measure should be reviewed "within a reasonable period of time." A permanent precautionary measure would be illegal if no effort were made to clarify the scientific situation.

The measures taken under the precautionary principle must be proportionate and constitute the strongest protection against misuse of the principle. Proportionality is a general principle of European Community law in which any measure imposing obligations on operators should be limited to that which is strictly necessary to achieve the objective of a measure (provided also that the objective itself is recognized as legitimate). This general principle is a very effective tool enforced by the ECJ and the CFI for controlling measures by public authorities under European Community law. In assessing the proportionality of the measure, regard should be given to technical and economic feasibility. Technical and economic feasibility are not the only elements to be taken into account; importantly, the objective of the measure should be to ensure a high level of protection. The level of protection is of a political nature and therefore cannot be challenged as such in court whereas the adequacy of the measure undertaken to ensure this protection can be challenged in court.

Pursuant to separation between risk assessment and risk management authorities, risk assessment authorities are not entitled to invoke the precautionary principle. Their duty is to indicate when science is incomplete, but they should not suggest the measures to be undertaken. Under European Community law, these measures are undertaken by the authorities and can be based on all legitimate factors, including scientific risk assessment. The other legitimate factors are not scientific but include considerations of environmental or public order. It would therefore be illegal for an authority to invoke the precautionary principle in support of a measure undertaken on the basis of legitimate factors other than scientific uncertainty. Indeed, for any measure to be legal, it should be clearly motivated and consistent with its motives.

Risk Analysis

Risk science has emerged as a powerful tool to aide in safety assessment decision-making, and application of risk analysis techniques is progressing around the globe. The U.S. Natl. Academy of Sciences defines risk analysis as a paradigm involving 3 major steps: research, risk assessment, and risk management (NRC 1983). Since then, The Codex Alimentarius Commission and WHO have defined risk analysis as a process comprising 3 key components: risk assessment, risk management, and risk communication (CAC 1997, 2003a, 2007). In addition, Codex defined each of the 3:

- Risk assessment consists of 4 steps: hazard identification, hazard characterization, exposure assessment, and risk characterization.
- Risk management is the process, distinct from risk assessment, of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices and, if needed, selecting appropriate prevention and control options.
- Risk communication is the interactive exchange of information and opinions (about risk, risk-related factors, and risk perceptions) throughout the risk analysis process among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties (CAC 2003a, 2007).

Risk assessment

Recently, FAO and WHO initiated a joint project to update and consolidate principles of risk assessment of chemicals in foods and prepared a comprehensive report. The report outlines key approaches to follow when limited scientific information is available. In conducting a risk assessment, all available data on a potential chemical hazard must be evaluated even if data are incomplete or emerging. In general, such a systematic approach involves identifying and evaluating the following chemical data:

- chemical structure and properties, including pH, solubility in various solvents, chemical fate modeling, and octanol/water partition coefficient;
- structural alerts that may predict certain biological activities, possible metabolic pathways including detoxification and activation pathways;
- structurally related substances for which more data may be available that can be used to predict the potential toxicity and metabolic fate of the chemical of interest; and
- toxicological studies, including structurally related materials, which may help forecast potential toxicity (FAO-WHO 2009).

After this initial critical evaluation, more detailed investigations can be planned. While knowledge of predictive toxicology is considerable, it is not yet possible to predict with high accuracy the outcome of toxicological studies. Therefore, conducting at least some initial investigations of untested substances in experimental animals would be prudent. Obtaining the basic information needed to address hazard identification and characterization of an untested chemical substance requires a logical progression of investigation.

Structure–activity relationships. The use of chemical structures to predict biological activity has been applied in many settings, including pharmacology, industrial chemistry, and food toxicology. Reviewed by McKinney and others (2000), the overall hypothesis or theory of the use of structure activity relationships (SARs) in toxicology is that the chemical structure of a compound determines its physical and chemical properties, which in turn are responsible for the biological properties and toxicological effects of the compound. SAR analyses use mathematical models and databases to incorporate physical properties (that is, solubility, molecular weight, dissociation constants, melting points, and ionization potentials) and chemical properties (that is, steric properties, presence of functional groups, and electrophilicity) to predict biological properties of chemicals.

SARs are the result of combining chemical structure, biological activity, and statistics (Figure 1). There are 2 general categories of SARs: qualitative, such as identification of structural alerts (functional groups and substructures) related to biological activity, and quantitative, which predicts biological potency. For quantitative SARs, the assumption is that similarly structured chemicals will share a common rate-determining step and similar energy requirements for activity and that differences in reaction rates will give rise to observed differences in activity or quantitative potency (McKinney and others 2000). The science of SARs in toxicology continues to evolve because of the challenge of making risk assessments with limited toxicology data and resources and pressure to use fewer animals in safety evaluations.

Cramer and others (1979) incorporated features of chemical structure into their decision tree approach to classify chemicals into 1 of 3 classes of predicted toxicity: low, moderate, or severe. In research efforts led by Ashby and Tennant, 300 chemical structures were evaluated for electrophilic sites with the potential to react with DNA and for correlations with mutagenicity in *Salmonella* and carcinogenicity activity in rodents (Ashby and Tennant 1988, 1991; Ashby and others 1989). They found that most rodent carcinogens contain structural alerts and that most chemicals with structural alerts were mutagenic. Ashby and Tennant structural alerts are commonly used as predictors of mutagenic and carcinogenic potential.

The key is to identify which aspect of the chemical structure is responsible for the biological activity. The limitation of the use of SARs in toxicology is that many toxicological endpoints are poorly understood and poorly characterized, and it is not possible to relate the endpoint to a specific mechanism of activity or common chemical structures. Simon-Hettich and others (2006) noted the use of computer-based models, which (despite limitations) can play a role in predicting various toxicological effects of chemicals for which data are not available or toxicological testing is impractical due to lack of availability of sufficient compounds for testing and supporting the screening and subsequent prioritization of compounds for further testing.

Two types of SAR toxicity-predictive programs exist. The first consists of correlative or statistically based programs, which utilize a large group of dissimilar chemicals. In this type of program, SARs are extracted from the data using statistical analyses. Examples include TOPKAT and CASE/MultiCASE programs. The 2nd type of toxicity predictive program consists of rule-based approaches, which build relationships from small groups of chemicals then group similar-acting chemicals into classes using chemistry, mechanistic data, and expert judgment. The rule-based approaches are more limited in application but tend to have better predictive capability. Examples of rule-based programs are DEREK and oncologic.

Successful quantitative SAR models in toxicology tend to be based on receptor-mediated or protein-binding endpoints, such as those for estrogen, androgen, and dioxin receptors, and enzyme induction, such as that for P450 enzymes. For example, the Ah (aryl hydrocarbon) receptor binding capabilities of dioxin-like compounds established a common mechanism of action, which led to use of toxic equivalency factors (TEF) to predict concentrations of dioxin equivalents (TEQ) for various compounds. This approach has been further refined to incorporate nuclear magnetic resonance (NMR) spectral data, resulting in a quantitative spectrometric data–activity relationship (QSDAR) and allowing risk assessment of complex mixtures of both dioxin-like and nondioxin-like compounds (Wilkes and others 2008).

Use of SARs in food safety assessments. The FDA uses SARs in the food contact notification program (FCN) (Bailey and others 2005; Valerio and others 2007). The FCN uses a combination of

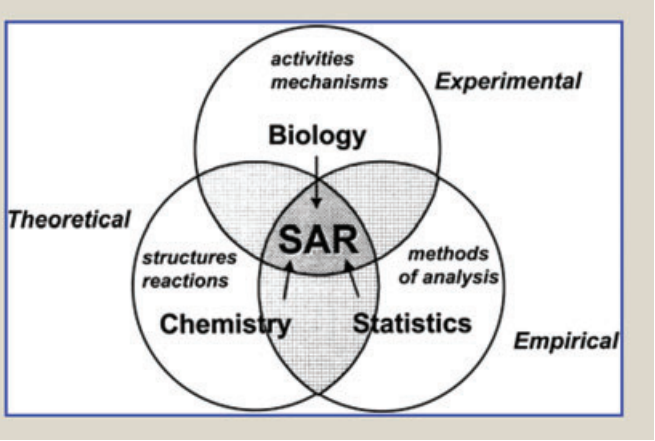

Figure 1—Diagram of structure activity relationships. Source: McKinney and others. 2000. The practice of structure activity relationships in toxicology, Toxicol Sci 56(1):8–17. Used with permission from the Society of Toxicology.

structural alerts identified by Ashby and Tennant and by Munro and others (1996) to identify structural alerts in a new compound. In addition, the overall results (weight of evidence approach) from a number of SAR programs are considered. These are Oncologic, MCASE-ES, TOXSYS, and LeadScope as well as in-house FDA databases (Bailey and others 2005).

The MDL-QSAR carcinogenicity module is a software program that predicts the carcinogenic potential of compounds using a predictive algorithm to flag high-risk compounds. The module includes the FDA's Center for Drug Evaluation and Research (CDER) carcinogenicity database of more than 1200 chemicals. Valerio and others (2007) reported a high sensitivity for detection of rodent carcinogens (97%) but a lower specificity for noncarcinogens. This program may be very useful when combined with experimental evidence and structural alert schemes for risk assessment and priority setting for untested natural food components.

Future of SARs. The need for improved computer-based testing is growing as the international community increasingly demands safety information for materials to become items of commerce, despite limited resources and time, constraints in use of experimental animals, inefficient available data, and/or the impracticality of toxicological testing due to lack of sufficient amount of the compound being available. Future development of high throughput screens for biological endpoints will allow evaluation of thousands of chemical structures for structural alerts of activity. For example, the GreenScreen HC GADD45α-GFP genotoxicity assay uses a cell line in which the DNA damage-inducible gene, GADD45α, is linked to a green fluorescent protein gene, which allows testing in the presence and absence of metabolic activation (Hastwell and others 2006). Thus, improved *in vitro* testing, which can be incorporated into quantitative SAR programs, has potential to improve computer-based testing.

Use of new technologies including genomic microarrays, differential gene-expression arrays, and protein-binding arrays in combination with computational biology are also being used to investigate toxicological responses and elucidate mechanisms of toxicity. The ability to incorporate these complex data sets into computer-based models for the purposes of predictive toxicology is a future challenge. To illustrate, the Molecular Libraries Initiative (MLI) by the Natl. Inst. of Health (NIH) will contribute significantly to the future of computer-based toxicology screening. The MLI has 3 components: the Molecular Libraries Screening Centers Network; cheminformatics initiatives, including a new public compound database (PubChem); and technology development initiatives in chemical diversity, cheminformatics, assay development, screening instrumentation, and predictive absorption, distribution, metabolism elimination, and toxicology (ADMET). One significant attribute of this project is the screening of compounds at multiple dose dilutions, which generate concentration-response curves, rather than only limited 1-dose results.

Nevertheless, because of the huge range of chemicals and possible interactions of chemical and biological systems, SAR models are unlikely to achieve absolute certainty of toxic outcomes. Still, SARs remain highly useful in situations where resources and data are limited. The concept of SARs continues to advance with integration of more chemicals, assays, and chemical analyses into highly complex models that have greater predictive capabilities for toxicological endpoints.

Surrogate compounds and metabolites. When data on the safety of a particular food chemical are limited or incomplete, seeking additional scientific information (surrogate data) from compounds that are chemically similar to the substances of interest is not uncommon. This can be done if the structure of the chemical is known. This approach is applicable to the evaluation of chemicals that may enter the food supply in minor amounts, such as food contact substances, processing agents, or impurities. Substances intentionally added to foods (for example, direct food additives, color additives, and GRAS substances) require chemical-specific data. However, it may be beneficial to conduct a preliminary evaluation of the safety of a new substance being considered for food use, such as a new synthetic flavor, using data on structurally similar chemicals, to screen potentially unsafe ingredients at an early stage in development. This approach often addresses safety concerns in a meaningful and prudent manner. However, the toxicology studies conducted on a related chemical may involve, for example, routes of administration and test material concentrations that are not representative of the new substance intended for human use. When attempting to augment the scientific studies for substances that have limited safety testing, caution must be exercised by judiciously relying on related safety studies that would realistically reflect the food chemical under consideration. This is true regardless of whether the food chemical is an intentional food additive, a contaminant, a processing aid, or a pesticide or animal drug residue.

Available through sources such as the EPA Robust Summaries for High Production Volume Chemicals and European Chemicals Bureau Intl. Uniform Chemical Information Database summaries, peer-reviewed literature, and unpublished reports often contain data for potential surrogate compounds whose structures are sufficiently similar to that of the target substance, metabolite, or impurity, so they might be useful in predicting the likely properties of the target. This can be true both for physical and chemical properties of the surrogate and also for its toxicological potential. It also may be possible to determine probable routes of metabolism from a surrogate's structure. For example, structures including ester groups are likely to be susceptible to hydrolysis. Therefore, structure searches can identify surrogates for the likely metabolites of a compound.

There are 2 main approaches to identifying structurally similar compounds. First, if the target contains a structural feature whose nomenclature is consistent and uncommon, then name or name-fragment searching may suffice. Second, structure searching can be employed. Structure searching is available in many of the files available on-line through STN Intl., which allows the search query to be defined in considerable detail. However, structure searching requires a high degree of proficiency to achieve the most effective outcome. For example, each atom in the search structure can be either open or closed to further substitution, and that substitution can be limited to certain classes/groups of substituents. Maximizing the retrieval of viable surrogate structures while minimizing the retrieval of irrelevant structures usually requires that the initial search strategy to be modified as the search progresses and then reviewed afterwards to confirm that no significant gaps in coverage were introduced.

Predictions based on physical/chemical data. In the absence of ADMET data, solubility and octanol/water partition coefficient properties of chemicals based on structure may be determined from models such as the Advanced Chemistry Development Inc. (ACD)/Aqueous Solubility and Log P version 7.04 model available from ACD/I-Lab. Chemical properties provide an indication of the potential biological fate of a compound. Generally, water-soluble compounds are readily excreted in urine after absorption. The log P (log D for charged molecules) provides an indication of the lipid-soluble potential of the chemical. Lipid-soluble compounds tend to be absorbed readily from the gastrointestinal tract by passive diffusion, with the exception of extremely lipophilic compounds that do not dissolve in gastrointestinal fluids (Rozman and Klaassen 2001). Compounds that are highly lipophilic may accumulate in fat. Data generated for any chemical entity should be assessed in conjunction with data generated for structurally

similar chemicals with known chemical properties to verify results.

Numerous models to predict pharmacokinetics and pharmacodynamics have also been developed; however, more comprehensive data are typically required for reliable pharmacokinetic/pharmacodynamic modeling. For example, a model incorporating maternal pharmacokinetics, maternal milk production, neonatal growth, and other characteristics has been developed to more accurately predict exposure of pups to test substances when delivered by gavage or orally (Yoon and Barton 2008). This model demonstrated that there are likely to be substantial differences between maternal and pup exposures during the course of an experiment and thus could provide improved risk assessments compared with use of maternal exposures only.

Toxicological evaluation. Consistency and predictability are the rule in chemistry, but this is not true for toxicological safety studies involving humans and other animals, which routinely display interspecies (between species) and intraspecies (within species) variations. Because toxicological safety studies evaluate chemical–biological interactions, food safety scientists face a major challenge in critically evaluating and extrapolating data from animals to humans. Sufficient sample size and appropriate statistical tests are crucial for meaningful evaluation. Equally as important as feeding tests are studies of the ADMET of a substance. It is necessary to know not only the end result but also how and why that result occurs to judge the relevance of a test result for humans.

Of the thousands of chemicals in commerce, toxicological databases are complete for only a small fraction of substances. Typically, classes of chemicals with robust data sets are limited to drugs, food additives, pesticides, important industrial chemicals (that is, those with multiple applications in different industries for which potential for human exposure is significant) and well-known toxic impurities (for example, dioxins and PCBs). In addition, the U.S. Natl. Toxicology Program (NTP) regularly identifies chemicals suspected to be carcinogenic or associated with significant human exposure and conducts 2-year cancer bioassays in rats and mice, using the most relevant route of exposure. After approximately 25 years in existence, the NTP has evaluated more than 500 chemicals for carcinogenic potential. When considered in terms of the amount of effort required to complete a single carcinogenicity bioassay and the number of chemicals used in commerce (which exceeds 80000), 500 is minimal. A chemical is considered to have a relatively complete oral toxicology database if results from the following studies are available: acute gavage study, 90-day oral subchronic study, oral teratology study, and *in vitro* genotoxicity assays. If the *in vitro* results are positive, data from *in vivo* genotoxicity assays or 2-year cancer bioassays in rats and mice may also be required.

For chemicals labeled as endocrine disruptors or estrogen-mimicking compounds, the decision of sufficiency of a data set for toxicological evaluation is complicated by the current lack of understanding of the significance of low-dose exposures to human health. For example, bisphenol A (BPA) is added to polycarbonate plastics and to the epoxy lacquer coatings of metal can interiors and bottle caps. Traditional studies with BPA and the lack of any adverse effects observed in humans despite 50 years of use indicate that the very low exposures from food would not be associated with adverse effects. However, a plethora of *in vitro* studies investigating endpoints such as receptor binding potential, as well as animal studies involving nonoral routes of exposure have raised questions about the safety of BPA even in the absence of direct evidence of harm to humans (NTP-CERHR 2008). As the significance of the findings in these studies is unclear, the precautionary principle could seem to dictate that banning of all chemicals with *in vitro* estrogenic activity be considered. Nonetheless, as of February 2009, the consensus of regulatory authorities in the United States, Canada, Europe, and Japan is that current levels of exposure to BPA through food packaging do not pose an immediate health risk to the general population, including infants and young children (FDA 2009).

Use of animal studies. Although SARs provide a good stepping stone, they do not substitute for classical ADMET studies. If the rationale is to use preexisting rat or toxicity data, then the objective would be to conduct the ADMET studies in the same species to show that the material is handled in the same way and that no other metabolites with potential safety concerns are produced. An alternative approach would be to conduct these studies in animals that would handle the material in a manner similar or identical to humans.

If SARs do not exist, then it is likely that some limited toxicity studies, such as a 28- or 90-day full toxicological study, would be required. If it can be shown that the compound is then handled in an identical manner in animals and humans, without the formation of additional metabolites in humans, then long-term studies may be considered unnecessary. These studies would require detailed knowledge of ADMET. To identify all potential metabolites, such studies should probably be conducted using radiolabeled material. Before studies can be conducted in humans, some limited toxicity data is required. However, the ethics or institutional review board would not likely approve investigations involving gathering of toxicity data from human subjects.

Classical toxicology studies are carried out in mice and rats. Food and food ingredient studies are administered in the diet normally at fixed percentages, normally a maximum of 5%. Values greater than 5% can cause nutrient dilution and are not recommended by the FDA. A fixed daily dietary intake over the lifetime of the study is not attainable with dietary feeding. Dosages start high and decrease on a milligram per kilogram body weight basis as the animals increase in body weight throughout their lifetimes. Issues with feeding studies include the effect of palatability and nutrient dilution; for example, studies with sweeteners have shown reduction in food intake coupled with a reduction in body weight gain. Even when adjustments occur for nutrient dilution, the effects on body weight gain cannot be fully explained. Animals that have a reduction in food intake at an early age never seem to catch up even though the food intake is the same. Because animals (rats) consume their diet throughout the night and then sleep during daylight, a feeding-associated dosage administration occurs during an extended period of time. Pair-feeding studies constitute attempts to address food intake palatability issues. Studies in which the substance is administered by gavage maneuver around the issue of palatability and food intake and body weight gain reduction.

The typical nonrodent alternative to the classic mouse or rat toxicology study is the dog. All of these animals are used for cost and ease of handling. Alternative animals such as mini pigs and marmosets, which might provide a better human model, are being investigated. The use of alternative animals can have issues such as lifetime exposures, but alternative animals might be preferable to rats, which produce tumors late in life because of increased weight gain. Also, there have been issues with substances such as saccharin, BHA, limonene, and several sugar alcohols that have caused tumors in rodents but were later determined safe for humans. Thus, relevancy of mechanism of action and site of effect need to be considered. The presence of carcinogenic animal tumors may, as a matter of law, prevent FDA approval of a food additive or a color additive, leaving evaluation under the GRAS safety standard as the only option in the United States.

The limitation of having general animal toxicology studies could lead to increased importance for mechanistic studies and

ADMET analysis, especially when good information on SARs is available. Similar to the current flavor safety paradigm, any new material can be initially assessed for toxicity, based on its structure and presence of reactive groups. If a material has a structure that is closely similar to one that was previously tested and the results from adequate, relevant studies exist in the public domain for the previously tested material, then an argument can be made that no toxicological studies are necessary as long as the new material is handled physiologically in a similar manner. Likewise, if it can be shown that a compound is metabolized rapidly to substances that occur endogenously or are formed systemically, then additional long-term toxicity studies are not required.

When evidence is insufficient to determine whether a substance is carcinogenic, a long-term carcinogenicity study may be required. The guidelines for conduct of carcinogenicity studies recommend a 2-year exposure period; however, the time of exposure for a carcinogenicity study has been debated. Davies and others (2000) analyzed 210 carcinogenicity monographs published by the Intl. Agency for Research on Cancer (IARC) to determine the time of onset of treatment-related tumorigenicity in long-term rodent studies for chemicals classified by IARC as having sufficient evidence of carcinogenicity in animals. The authors concluded that "termination of rodent carcinogenicity studies at 18 mo or earlier would greatly reduce the complications that arise in interpreting the findings in aged animals which often have defective hepatic or renal function and would also markedly reduce the time required for histopathological examination of dozens of tissues taken from the approximately 500 animals routinely employed in these studies" (Davies and others 2000). In contrast, the Ramazzini Foundation recommended extending the exposure period beyond 2 years until the animals die naturally (Huff and others 2008). Any efforts to increase the sensitivity of chronic bioassays also increase the need to assess even more carefully the relevance of the results to humans. Despite the debate over duration of exposure, most carcinogenicity studies conducted for food chemicals for regulatory purposes continue to be for the duration of 2 years as per FDA Redbook guidelines (FDA-CFSAN 2000).

In some cases, there are differences in toxicological outcomes between various species because of differences in metabolism (as with soy-isolate isoflavone) or differences in pharmacokinetics (as with cyclohexylamine and saccharin). The differences may be related to the formation of reactive metabolites, which would be highlighted in an interspecies comparison in ADMET, or how rapidly the substances are cleared from systemic circulation through either metabolism or excretion. Although mice and rats are the common species of choice for studying toxic effects of a variety of chemicals and carcinogens, these species metabolize substances differently than humans.

For example, B6C3F1 mice are much more sensitive to a variety of carcinogens than Fisher 344 rats (Beasley 1999). Numerous published reports showed that B6C3F1 mice developed more cancer tumors (45 reports) than Fisher rats (15 case reports); the mice developed more nongenotoxic tumors due to greater levels of preinitiated cells in the liver, allowing nongenotoxic agents to act as tumor promoters. Mice were shown to be very sensitive in the carcinogenic evaluation of 1,3-butadiene while rats were 1000 times less sensitive than mice and humans were similarly less sensitive. Moreover, Gu and others (2006) described major interspecies differences in the metabolizing of soy-isolate isoflavone among female Spraque–Dawley rats, Hampshire/Duroc Cross pigs, and cynomolgus monkeys. Isoflavones daidzen and genistein are metabolized and conjugated in the liver, forming aglycones, glucuronides, and sulfates with extreme differences in metabolites circulating in the plasma and excreted in the urine. Overall, the metabolic profile of isoflavones in pigs was determined to be closer to that of women than either rats or monkeys (Gu and others 2006).

To determine the rate of absorption and clearance, classical pharmacokinetic studies are necessary in which blood, plasma, and bile concentrations of a compound or its metabolites are analyzed over a period of time, usually up to 96 hours after administration. These results are then compared to the ADMET studies to determine the major form of excretion (urine or feces). Additionally, DNA repair varies in tissues and species (Beasley 1999). For example, kidneys are more sensitive to nitrosamines than livers in stimulating carcinogenesis because livers have more efficiently dispose of alkylated DNA molecules. Animals clear absorbed substances from their systems much more rapidly than humans.

And lastly, the FDA has used a hundredfold safety factor to establish safe levels of food additives, using the NOEL from a long-term animal study (Lehman and Fitzhugh 1954). This hundredfold safety factor takes into account a tenfold factor for differences in species (inter-species variation, allowing extrapolation from animal to man) and a tenfold factor for different sensitivities among humans (intra-species variation). The JECFA and the JMPRs adopted this framework in 1961 to define an ADI (Truhaut 1991). Nevertheless, over the last decade efforts have been made to adopt a more scientific approach to setting the appropriate safety margin based on the quality and quantity of animal and human data (Dourson and others 1996; WHO 1994, 1999, 2001). The more comparative data that exists between animals and humans the less of a safety margin may be required to be applied to set an ADI. If the accumulated scientific information is insufficient or incomplete, the reliance on safety factors larger than 100 may be necessary, which, in turn, may yield an ADI that is below the estimated consumer exposure. When this occurs, either the proposed food uses must be restricted to reduce estimated consumer exposure to a level that is less than the ADI, or additional toxicology testing must be conducted to support an increased ADI that would exceed estimated consumer exposure. However, care must be exercised when considering the reduction of the proposed additive use level to align more with the calculated ADI since doing so might result in too low a level to achieve the intended technological effect.

Statistical considerations. One of the important tools in risk assessment is statistical evaluation of a broad range of data from epidemiological observations, clinical trials, acute and chronic exposures via environmental and dietary routes, extrapolation procedures, pharmacokinetics, pharmacodynamics, dose–response relationships, and model hierarchal systems that predict clinical significance. Statistical evaluation consists of classic components such as fundamental analysis of variance (ANOVA) and determination of possible type I (false positive) and type II (false negative) errors to provide an initial statistical assessment. On the other hand, systematic reviews, meta-analysis, and confidence intervals convey an understanding of the findings for clinical relevance. An important aspect of risk assessment and statistical evaluation is the biological importance and clinical relevance of the measured outcomes and the efficacy and magnitude of a defined intervention. For example, an examination of obesity intervention and weight loss programs showed statistically significant weight loss among group consultation compared with individual therapy sessions (Renjilian and others 2001). Yet the clinical relevance of this weight loss is questionable when a weight loss of at least 10% is considered clinically important among overweight and obese individuals who followed a low-calorie diet for 6 to 12 months (NHLBI 1998).

Obviously, one of the challenges of statistical evaluation is that clinical studies are frequently too small in sample size and

the scope of the study is insufficient to detect true or relative differences between control and intervention groups. The reliability of effect and validity of measurements are other challenges that statistical evaluation presents. Moreover, too few studies discuss the clinical importance of the findings, thereby missing the implications for changes in public policy, clinical practice, or personal lifestyle. The interpretation of these applied statistical approaches has a significant impact on interpretation and application of statutory standards, public policy, and consumer health. For statistical evaluation to elicit effective decision-making, it must include the entirety of evidence for effective risk assessment (CAC 2003a, 2003b).

Perhaps the most challenging aspect of statistical evaluation is the extrapolation of toxicological data to humans. For example, aspartame is an FDA-approved nonnutritive sweetener food additive that is metabolized in the body to amino acids and a small amount of methanol (21 CFR § 172.804). Up to 10 ppm residual methanol may be present in FDA-approved food additives such as sucrose fatty acid esters and sucrose oligoesters and up to 5 ppm in the FDA GRAS-affirmed cocoa butter substitute (21 CFR §§ 172.859, 172.869, 184.1259). Methanol has relatively low toxicity (LD_{50} = 5628 mg/kg (rabbit, oral); NOAEL = 10000 pm (rats, oral)), and the metabolism and pharmacokinetics of methanol differ considerably among nonprimate and primate models (Clary 2003). Methanol is produced endogenously in humans, yielding a typical blood level of 0.15 to 0.6 ppm. It is also a by-product of ethanol metabolism; a nominal component of a variety of fruits, such as strawberries and cherries; and a product of metabolized pectin (Lindinger and others 1997). Pectin, a natural fermentable dietary fiber found in fruits, is also affirmed as GRAS by the FDA for use in foods with no limitations other than current good manufacturing practice (21 CFR § 184.1588). The recommended dietary fiber intake is 25 to 35 g per day, yet extrapolation of data from Grüner and others (1994) suggests that the acute consumption of this amount of pectin, which is equivalent to nearly 3.3 kg of apples, would produce a serum methanol concentration of as much as 150 ppm. This concentration is well above the normal baseline but very low compared to the amount produced by aspartame metabolism.

Additionally, addressing statistical significance and clinical relevance can be aided by a sequential approach, separating the questions of statistical significance and clinical importance separated (Snapinn and Jiang 2007). In such an approach, statistical significance is based on the most powerful approach (usually using the continuous variable), and clinical relevance is based on examination of the mean difference between groups and on response rates. The clinical usefulness of an intervention is often subjective, and the usefulness differs between group responses compared with individual response. Furthermore, clinical improvement over time is unrelated to intervention or chosen measurement. Yet there are specific circumstances during which there is a risk to the quality of life and health. For example, the link between sodium and hypertension remains controversial since many variables such as heart disease, diabetes, kidney disease, or other preexisting condition may contribute to increased blood pressure after sodium consumption (in the form of NaCl or table salt) and a decreased consumption (less than 2300 mg/day) may have a marginal decrease (up to 2 mmHg) in systolic blood pressure among healthy individuals. Statistics suggest that evidence is insufficient among normotensive populations that a 30% to 50% reduction in sodium intake would warrant an evidence-based public health policy (McCarron 2000).

Absolute safety, or the absolute absence of harm, is not possible nor is it required by the safety standards of the FD&C Act. On the other hand, it is not possible to have a full scientific understanding for ensuring no harm before developing and implementing a public health policy. The United Nations Rio Declaration on Environment and Development clearly implies this point in Principle 15: "Lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures" (UN 1992). Despite this biological variability, there are statistical windows of biological effectiveness that call for the establishment of standards such as upper limits of nutrients (for example, vitamin A, iron, nicotinic acid) above which acute and/or chronic consumption produces harm or undesirable effects. Regulatory officials worldwide are paying attention to the process for establishing upper levels of intake for nutrient substances, preferring approaches that rely on risk assessment to establish levels of intake below which no harm may occur (Munro 2006; Renwick 2006; Taylor and Yetley 2008).

Risk assessment combines direct and indirect data and observations to predict or assign a probability that an event may or may not occur. With typical distribution of events come measurement errors. A sense of confidence is also present because errors tend to follow predictable patterns or trends that are manageable through statistical probabilities. The concept of statistical significance, in which differences in measurements do not overlap, is often assigned an association or probability. Unfortunately, this association is often interpreted as a cause and effect instead of weighing the clinical relevance of the statistics and its biological importance. It is the clinical relevance, the assessment of mean differences and variances, response rates, and cumulative distribution of biological functions that remain paramount in assessing risk and developing public policy.

The Need for a New Approach

In the past, finding an unwanted chemical in a food often led to the prohibition of that chemical in food; none of the substance was considered allowable. The mere presence of the chemical was considered unsafe and adverse to health. Zero allowance of unwanted chemicals was the prevailing wisdom at the time. However, in actuality, zero was the LOD for the chemical, perhaps a part per million. The zero-allowance manner of managing the detection of low levels of chemicals in food is dynamic, changing as the sensitivity of analytical techniques increases. More specifically, a chemical once thought not to be present because it was not detectable may, if present, be recognized once the sensitivity of analytical techniques reaches detection capability, at parts per billion or parts per trillion. Analytical sensitivity has improved over time from parts per thousand and parts per million in the 1960s to parts per billion and parts per trillion in the 1980s and parts per quadrillion in the 2000s.

The increasing sophistication and sensitivity of analytical techniques warrants a new approach for managing low-level detections of chemicals. Because the mere presence of a chemical in a food does not mean that the substance necessarily poses a risk to health, a new approach is needed that empowers food safety professionals to use all available data in conducting a risk-based evaluation of the potential exposure, hazard, and toxicity of low levels of chemicals.

Human exposure thresholds

About 40 years ago, scientists began to realize that attaining zero unwanted chemicals was not only impossible but also unnecessary. In considering the dose–response relationships of a large number of chemicals, it became apparent that a safe level of exposure exists below which no toxicity is expressed even for the most toxic of compounds. The dose below which no evidence of toxicity or biological effect occurs is the threshold dose. The existence of a threshold is a result of chemical–biological interactions, often termed stimulus–response or dose–response

concepts. The dose–response concept is a toxicological principle credited originally to the 16th Century Swiss physician Paracelsus (Phillipus Aureolus Theophrastus Bombastus von Hohenheim), who made the following statement: "All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not a poison." The concept of specifying human exposure thresholds relies on knowledge of the range of toxicological risks for structurally related substances and on knowledge regarding the toxicological potency of relevant classes of chemicals for which good toxicity data exist (Munro and others 1999).

Professional opinion is divided, but most toxicologists agree that for noncarcinogens and nongenotoxic carcinogens a threshold dose exists below which no adverse effects occur. For genotoxic carcinogens, prudence requires that no such threshold dose be expressed; caution dictates that even very low doses might produce some adverse effect. However, there is broad agreement in regulation that there is a dose below which the risk is so low that it may safely be disregarded. That risk level is generally taken to be a lifetime risk of less than 1 in a million (10^{-6}) (FDA 1985, 1994). (See *Monsanto v. Kennedy*, 613 F.2d 947, 955 (D.C. Cir. 1979); *Scott v. FDA*, 728 F.2d 322 (6th Cir. 1984); *Public Citizen v. Hayes*, 831 F.2d 1108 (D.C. Cir. 1987).)

Development of the threshold of toxicological concern

Within the past several decades, scientists have been developing sophisticated models to address very low-level exposures; risks from these are so low they are likely negligible. For example, a decision tree exists to determine presumptive toxicity and aid priority setting for analytical testing of food ingredients. Other sophisticated decision tree models describe a threshold of toxicological concern (TTC) as "a principle which refers to the possibility of establishing a human exposure threshold value for all chemicals, below which there is no appreciable risk to human health" (Kroes and Kozianowski 2002). TTC is thus an insignificant value or a negligible risk standard.

Food safety professionals have long realized the need to manage trace levels of compounds. They have done so with an evolving perspective as advances in analytical methods have allowed detection of increasingly lower trace levels of compounds, thereby finding a greater number of compounds. For example, Frawley (1967) determined that except for certain substances (such as *Clostridium botulinum* toxins), no single organic chemical has advanced from the laboratory, through development, and into general commercial use with a toxicity to experimental animals at a dietary level of 40 ppm or less. He proposed that substances migrating from food packaging materials at a level of 0.1 mg/kg of human diet (which included a hundredfold margin of safety), equivalent to an intake of 150 μg/person/d, could be safely consumed. The basis for Frawley's proposal was analysis of 2-year chronic toxicity studies of 222 chemicals and categorization by dose in which no toxicological effects were observed. Munro (1990) developed a human exposure threshold value of up to 1000 ppt for substances migrating from food contact materials (1.5 to 3 μg/person/d, depending on assumptions regarding food intake) on the basis of a database of 350 substances. The FDA TOR exemption for noncarcinogenic substances migrating from food contact materials established an acceptable dietary exposure level at or below 1.5 μg/person/d (21 CFR § 170.39). Munro and others (1999) described a procedure for the safety evaluation of flavoring substances by integrating data on metabolism and toxicity of substances within structurally related groups, structure–activity relationships, and daily intake. More recently, Blackburn and others (2005) evaluated and found TTC applicability for ingredients in consumer products, and Müller and others (2006) found similar applicability for impurities in pharmaceuticals.

The TTC provides an acceptable high probability of health protection and practicality. A TTC evaluation can be an efficient screening and prioritizing tool for the decision-making process, particularly when data are incomplete. Using the TTC may lead to a decision that for some chemicals makes further work and risk mitigation steps necessary while for others, further work is not necessary. There are many key references on the TTC concept (Kroes and others 2004; Barlow 2005). A brief review of a few foundational papers shows the conservative nature of the TTC and how it can be used to prioritize risks from any low-level chemical detection in food.

Munro (1990) summarized the work by Gold and others (1984) and Rulis (1986) by plotting the distribution of potencies for hundreds of carcinogens (Figure 2). On the left side of the chart is the distribution of doses that produce carcinogenicity in 50% of the tested laboratory animals (designated the TD_{50}). The arrow pointing to the right shifts the log normal curve to the right to extrapolate down to 1×10^{-6}, or 1 in 1,000,000 risk (note the x-axis is on a negative log scale, so lower doses are to the right and higher doses are to the left). Originally, the FDA's Rulis (1986) selected 0.15 μg/person/d as a level that would give less than a 1 in a million lifetime risk of cancer for any of these carcinogens. In a workshop exploring this relationship further, 1.5 μg/person/d was determined to still be very conservative (Munro 1990). In recent years, many more carcinogens have been added to this database, additional papers have been published, and 1.5 μg/person/d has been affirmed as safe.

For toxic endpoints other than cancer, Munro and others (1999) evaluated databases for neurotoxicants, immunotoxicants, and developmental and reproductive toxicants and determined that TTCs for these classes of compounds were even higher than those for cancer. It is therefore highly likely that a TTC that protects against cancer risk will protect against all toxic outcomes. Furthermore, if additional information about a substance is available (for example, it is not carcinogenic or exposure is of brief duration or limited to a small part of the diet), the TTC can safely be set at a level higher than the default (Munro and others 1999). Müller and others (2006) noted that 1.5 μg/d for a lifetime TTC would correspond to a TTC of 120 μg/d for exposures lasting only a month. Similarly, if an exposure was limited to a single food that was rarely consumed, the dietary exposure would be less than 3000 g/d and the TTC could be set at some level higher than 1.5 μg/d. Such information could be useful to risk decision-makers for exposures that are more limited in scope.

Regulatory agencies (such as the FDA and the EFSA) and other organizations (such as the JECFA) have applied the TTC approach to indirect additives, flavors, pharmaceuticals, and personal and household products. Although route of exposure affects toxic responses, the body's metabolic systems do not distinguish among sources of exposure. The TTC has been proposed to apply to any low-level detection of a chemical in food with some prudent exceptions (Barlow 2005; Felter and others 2009). Exceptions include heavy metals, proteins that may be allergenic, and highly toxic carcinogens.

Another consideration is the difficulty in assessing human cancer risk from individual compounds through animal bioassays conducted at high doses. The difficulty arises because of the complexity of the human diet, a variable mixture of naturally occurring and synthetic chemicals, and potential for interactions between components, some of which could be anticarcinogenic (NRC 1996). It is almost certain that the impact of single dietary components on cancer is the sum of numerous effects of a chemical rather than a single biological effect. Moreover,

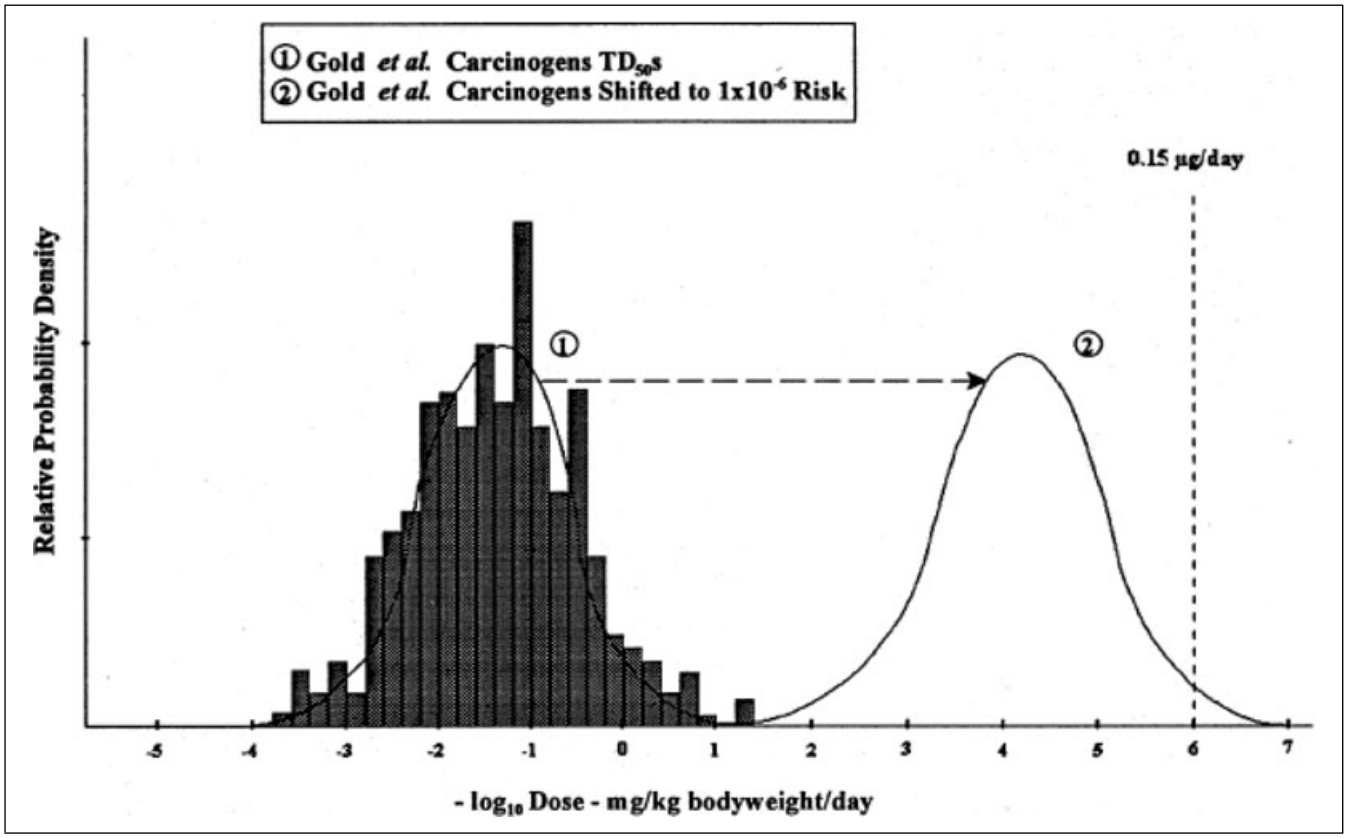

Figure 2—Distribution of potencies for various carcinogens. Source: Munro and others 1999. A procedure for the safety evaluation of flavouring substances. Food Chem Toxicol 37(2–3): 207–32. Used with permission.

carcinogens and anticarcinogens in the diet can interact in a variety of ways that are not fully understood (NRC 1996).

Fundamentally, the potential human health risk posed by a chemical substance is a function of its inherent toxicity and exposure including route, dose, and duration. If there is little or no exposure, then the risk is insignificant. Figure 3 depicts the crux of the TTC concept. The grid shows low-level safe exposures having low priorities as green squares. As exposure and toxic potency increase, so do potential health risks, represented by higher priority yellow and red squares. The human exposure benchmark doses defining low, medium, and high exposures are built upon a body of scientific evidence that has established TTC dose levels representing minimal or insignificant human health risk (Felter and others 2009).

In Figure 3, a semiquantitative estimate of human exposure is combined with a qualitative determination of structural activity/toxicological potency to assist risk decision-making and to set priority for action. Breakpoints for low, medium, and high human exposure are not well defined and vary by class of compound, duration of exposure, and other factors. However, an exposure cutoff of 1.5 μg/d or less is protective for carcinogens and probably all other toxicants. For exposures above a TTC level, the priority can range from low to high, depending on evidence that indicates low-to-high potency based on structure–activity information or other toxicological data about the substance (Cheeseman and others 1999; Kroes and others 2004; Barlow 2005). The breakpoint between medium and high exposures has not been defined but could be some multiple of the TTC. Figure 3 uses tenfold the 1.5 μg/d TTC for carcinogens as a conservative assumption. At high intake levels, priority rises to at least medium or high, requiring typical resource-intensive toxicological approaches used for high exposure situations. Helpful in determining appropriate risk mitigation actions when human exposure and toxicity data are incomplete, the grid's 3 priority rankings are as follows:

- **Green:** The green squares indicate low priorities (little or no safety issue), which correspond to either a low or medium exposure to a substance with a low to medium order of structural activity and acute toxicity. Typically, one would defer to the toxicity profile when available and to the SARs in situations for which only minimal toxicological data are available. Situations occurring within the green squares allow a recommendation of a low priority concern, and little follow-up work is indicated.
- **Yellow:** The yellow squares indicate medium priorities, which correspond to high/low, medium/medium, or low/high exposure/toxicity combinations. Typically, one would defer to the toxicity profile when available and to the SARs in instances of only minimal toxicological data. Because the exposure level and/or toxicity profile is higher in this situation, it is critical that the decision maker have confidence in making sound recommendations to those involved in risk management. Situations involving the yellow squares will often constitute the most difficult decisions that a food safety professional will have to make, based on limited analytical and toxicological data.

Medium priority concerns indicate the need for additional information to allow a risk assessment to occur. Information about potential exposure and toxicological effects is necessary to determine the scope of the issue and source. Toxicological analysis would assure a full and complete understanding of the

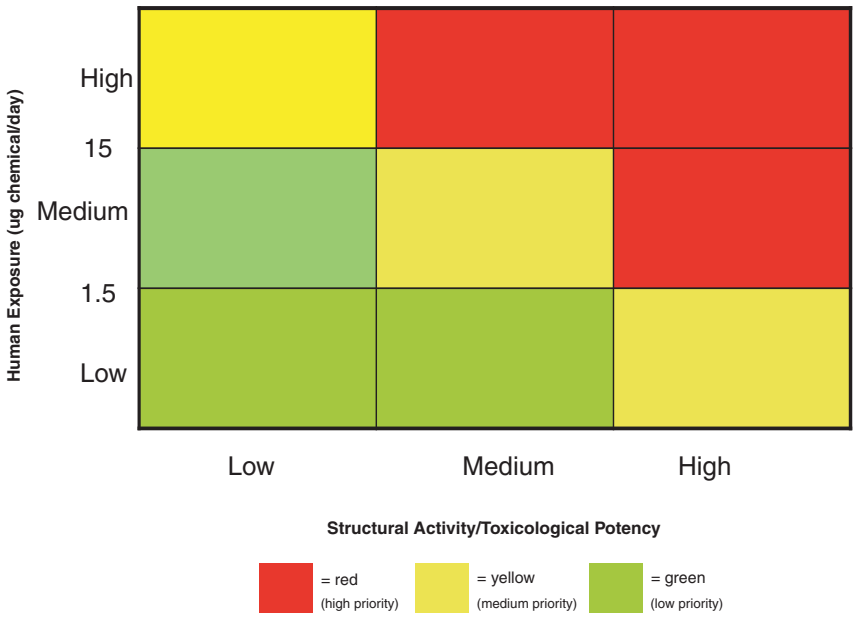

Figure 3—Toxicological priority grid.

existing data set that is available. Consideration of the source of the exposure as per ingredients, commodities, and food products is essential.

- **Red:** The red squares indicate high priorities due to combinations of high or medium exposures with high or medium toxicity. Typically, for high-priority issues, only interim risk management decisions can be made until traditional, full-scale information is available on toxicity and exposure. Because the exposure level and/or toxicity profile is higher in this situation, the decision maker must have complete confidence in making recommendations to senior officials.

Red-square situations often constitute the most clear-cut decisions that a food safety professional has to make, based on limited analytical and toxicological data. High priority concerns receive the highest level of immediate attention and risk management response. As with green and yellow situations, one should consider the source of the exposure as per ingredient, commodities, and whole foods.

TTC approach parameters

A few assumptions must be assured in such an approach to rapidly address potential chemical food safety issues:

- The individual using the TTC approach in making judgment calls must be an experienced scientific and risk management professional with a significant level of education and skill in making critical risk-based decisions. Use of the TTC is intended for skilled professionals with strong scientific training and a background in toxicology or related disciplines.
- On average, adult humans consume 3 kg of foods and beverages (including water) per day. It is not appropriate to sum the 90th percentile estimates of consumption since different individuals consume different foods. The analysis is appropriately conducted one food or food category at a time.
- Primary concern will be for acute toxicants with less of an immediate concern for sub-chronic and chronic toxicants.
- Most chemicals are included for consideration, such as naturally occurring, environmental, and synthetic or man-made compounds. However, because heavy metals (organic and inorganic) and proteins were not part of the databases explored to create a TTC, these are typically excluded. Furthermore, proteins can be allergenic, and there has been concern about potential nonlinearity of the immune system's allergic response. However, much progress has been made in the last few years in the investigation of thresholds for common food allergens; consensus is emerging that thresholds for these are in the mg range, rather than the μg levels for most TTC categories (Taylor and others 2009). For the purposes of this paper, the question on proteins can be left open, pending further research. Other chemical groups that are excluded from this approach are steroids (acting through hormonal mechanisms) and compounds from high potency carcinogen classes (for example, dioxin and dioxin-like, aflatoxin-like, N-nitroso, and azoxy compounds).
- Thresholds exist and can be defined as the level of exposure to a substance below which no significant risk is expected.
- The safety standards imposed by the applicable laws must play a role in the decision-making process.
- Exposure will be limited in duration: either more or less acute (short term). Exposure is supported by real and authentic analytical data.
- There is insignificant risk below 0.5 ppb in the total diet (food and beverage), equal to a total dietary exposure of 1.5 μg/d (Munro and others 1996).
- A low-dose exposure is 1.5 μg/d or less whereas 1.5 to 15 μg/d represents a medium-dose exposure. A case could be made for the use of a 10x factor above the 1.5 μg, which would yield a maximum threshold of 15 μg/d as the upper limit of the medium exposure range. However, a more conservative approach is more prudent, and anything above 4.5 μg/d should be considered a high-dose exposure.
- Toxicological data are available for the chemical under review even if these data are limited in scope.

The following 3 scenarios illustrate how the TTC approach as visualized by the toxicological priority grid can be used for making necessary food safety decisions when available data are limited.

Scenario 1 (green zone or low priority). An environmentally persistent contaminant is found at low ppb levels in several

unrelated foods widely distributed in the food. If it is in the entire diet of 3 kg/d at 2 ppb, then the exposure is calculated to be less than 1.5 μg/d. The toxicological priority grid indicates that this finding is in the green zone. Another example might involve a packaging migrant that is discovered in select food products through advancements in analytical methods. If available toxicological data are inadequate and what is known about the chemical structure of the substance does not raise any alerts and total daily exposure is less than 1.5 μg/d, then this finding would be in the green zone or low priority. No immediate product-related action would be warranted in both examples; however, follow-up investigation would ensue to seek alternative technologies or mitigation strategies.

Scenario 2 (yellow zone or medium priority). Analysis revealed the presence of an unknown analytical peak in a nationally distributed and branded food product. Subsequent investigation determined the structure of the unknown compound and its likely source with exposure limited to similar products at maximum levels of 60 ppb. The worst-case estimate of intake of implicated products determined the overall dietary exposure to be 3 μg/d. Looking at the toxicological priority grid, this finding would fall in the yellow zone or medium priority, meaning that aggressive action would be taken to address the situation, possibly involving discussion with regulatory authorities. However, a decision to recall product might not be taken unless toxicological or other data surfaced to indicate a significant human health risk or noncompliance with applicable legal safety standards.

Scenario 3 (red zone or high priority). Acrylamide, a known carcinogen is present in foods comprising an estimated 40% of the caloric content of the diet. The exposure has been estimated at 0.4 μg/kg body weight, clearly higher than the 1.5 μg/d. Although acrylamide has been under investigation for many years, the degree of human health risk remains unclear. The TTC approach shows this finding is in the red zone in the toxicological priority grid. Further toxicological studies are underway to evaluate the potential health concern for humans and the results of NTP studies are soon to be released. Pending these findings, risk management strategies (may need to be established to lower the human health risk and assure compliance with applicable legal safety standards (Slayne and Lineback 2005).

TTC approach aids food safety managers

Utilizing the TTC approach will assist food safety managers in performing safety and risk assessments of the potential adverse health effects of very low levels of unwanted chemicals in ingredients, commodities, or food products. Scientifically defensible decisions can then be made and senior management and legal counsel advised accordingly. This approach will assist a food safety manager in the overall decision-making process and provide a basis for advising senior management officials and legal counsel on the level and nature of available scientific information, including potential human exposure, structural activity, and toxicological potency and endpoints.

Consumer dietary exposure assessment

When a substance is suspected of being potentially hazardous, a key issue that must be addressed before using TTC or any other decision tool is determining its likely exposure. In the case of substances found in foods, whether their presence is intended or not, the issue can be broken into 2 elements. The first is establishing the level of concentration in various foods, which can be addressed through techniques of chemical analysis. The second element is determining how much of the foods in which the substance is present are consumed. Depending on the known or suspected toxicological parameters of the substance, the issue may be acute exposure, in which case the amount of food consumed per occasion must be determined, or it may be chronic exposure, in which case consumption of the food over a longer period of time must be assessed.

Estimating long-term exposure (for example, exposure during weeks, months, or a lifetime) to substances found in foods is extremely difficult, but a first, highly conservative approach to estimating long-term exposure is estimating exposure during 1 day (24 hours). A worst-case estimate of long-term exposure can then be made based on the simple assumption that foods containing the target substance are consumed every day; thus, the weekly exposure is simply 7 times the 1-day exposure and the yearly exposure is 365 times the daily exposure. Since few foods are actually consumed every day, this approach overestimates long-term exposure, often quite extremely. But it is a conservative assumption in that it cannot underestimate long-term exposure, and at least the directionality of the error is known.

In the United States, estimates of 1-day consumption of all foods in the diet are available through the Natl. Health and Nutrition Examination Survey (NHANES), which is conducted continuously with face-to-face interviews of representative samples of the U.S. population (CDC-NCHS 2008). One element of this survey is a 24-hour dietary recall, in which respondents work with a trained interviewer to recall all of the foods and beverages that they consumed on the previous day, midnight to midnight, either at home or away from home. Additionally, the respondent is asked to estimate the quantity of each food or drink consumed on each occasion during the day, and various memory aids are employed to assist the respondent in making accurate estimates. Despite all efforts, there are numerous sources of potential error, from lack of knowledge of what some foods were as well as memory lapses to incorrect estimations of portion sizes and a desire to make one's diet appear healthier than it is. Nevertheless, 24-hour recalls are believed to be fundamentally valid and, other than direct covert observation, are regarded as the gold standard for estimating 1-day food consumption.

Obtaining precise estimates of 1-day levels of consumption of specific foods requires accessing the database of food-consumption data from the survey. The direct use of the survey is a time-consuming process requiring knowledge of the survey design and statistics to ensure its correct application. These resources are often not available particularly when an immediate estimate is needed. In such cases, it will often be preferable to have a method that can develop an estimate with less precision but one that is certain or almost certain to be conservative—that is, to err in the direction of overestimating consumption of the food and therefore overestimating exposure to the substance in the food.

When a substance of concern is detected in a food, it is useful to consider the source of the substance and the range of foods in which it is likely to be found. In some cases, the substance may have entered the food during or after manufacture and thus may be present only in a single food product. In this case, what is needed is an estimate of the consumption of the single food product as the sole source of the substance of concern. In other cases, the substance of concern may be present in 1 ingredient of a food product and thus may be present in other foods that contain that same ingredient. Thus, if an undesirable substance is found to be present in a commodity such as wheat or oats, it may have entered a large variety of food products—potentially any food product containing wheat or oats as an ingredient. These 2 situations demand different approaches to estimating exposure—1 method for substances of concern that are believed to be present in 1 or a small number of foods and another method for substances believed to be present in commodity ingredients that are components of a large number

of different foods. In the former, what is needed is an estimate of consumption of the food while in the latter an estimate of the total consumption of the commodity from all foods containing it is needed.

Food consumption estimates of the NHANES food categories comprise consumption of 10 broad food categories for high consumers (90th percentile) on a per capita and per user basis (Table 2). The estimates for each category include the identified component as well as other ingredients in the food item from the NHANES 2003–2004 survey. It is also possible to estimate consumption for categories that are defined by the USDA and the Natl. Center for Health Statistics (NCHS). These categories are broad and include the weight of the entire food, including ingredients that are not part of the designated category. For example, the entire weight of a frozen plate meal that is mainly beef would be included in the meat category. Therefore, these estimates are overestimates of the 90th percentile intake of the ingredient but may be useful to estimate the consumption of a food product that contains multiple ingredients on a daily basis. One feature of current food labeling in the United States is helpful in this circumstance. With the Nutrition Labeling and Education Act of 1990 (NLEA), all foods sold directly to consumers are required to have a nutrition facts panel on the label, which includes the serving size of the food. Although in the early days of nutrition labeling the product marketer was responsible for determining the declared serving size, this changed with the NLEA, which required the FDA to establish serving sizes for use by food marketers. In response, the FDA developed the reference amounts customarily consumed (RACC) to serve as the basis for declared serving sizes of all food products. (See 21 CFR § 101.12.)

The FDA determined the RACC by examining food-consumption data from the 1987–1988 USDA Nationwide Food Consumption Survey (NFCS) and the 1989–1990 and 1990–1991 Continuing Surveys of Food Intakes by Individuals (CSFII), the most up-to-date food-consumption survey data available at that time. For each category of food, the FDA calculated the mean and median amount consumed per eating occasion of the food. The agency then used these figures to develop reasonable estimates of the amounts of foods customarily consumed. The FDA then rounded to numbers that could be easily expressed in common household units. For example, most beverages received an RACC of 240 mL, corresponding to 8 fluid ounces.

Table 2—Food consumption by USDA food categories (as consumed).

Food group	90th percentile per capita (g/kg bw/d)	90th percentile per user (g/kg bw/d)
Milk and milk products	18	21
Meat, including beef, pork, turkey, fish, seafood, and mixtures contains these products	7	7
Eggs	2	4
Dry beans, peas, legumes	1	4
Grains including mixtures	13	13
Fruits including fruit juices	10	16
Vegetables	7	7
Fats and oils	1	1
Sweets and sugar	1	2
Nonalcoholic beverages	28	29
Note: figures rounded to nearest g/kg bw/d		

Source: NHANES 2003 to 2004. Categories include the entire food (for example, the ingredients that define the category as well as other components of the recipe); estimates for each individual are the average of their 2-day reported intake.

Since the advent of RACC and declared serving sizes reflect amounts customarily consumed, a reasonable estimate of the average amount of a food that Americans consume can be taken directly from the declared serving size. For example, it is reasonable to assume that the average amount consumed of a beverage labeled as having a serving size of 240 mL is indeed 240 mL. However, this figure represents the amount consumed at 1 consumption occasion. It is quite possible for an individual to have numerous consumption occasions during a day: an individual may have a beverage with breakfast, 1 with lunch, 1 with dinner, and perhaps 1 or more at other times of the day.

For this reason, it is useful to develop a multiplier to estimate 1-day consumption from serving sizes, which represent average 1-occasion amounts. Data on the 24-hour consumption of a number of categories of foods are available from the USDA's Agricultural Research Service (USDA-ARS 1997). These data provide mean daily intakes of a large number of categories of foods consumed by the population aged 2 years and older. The data, however, are provided on a per capita basis and thus offer average amounts consumed by the entire population, encompassing both people who did and did not consume the food. Thus, if 25% of the population consumed a food, and these consumers ate an average of 60 g each, the per capita average intake would be 15 g, representing the 25% of the people who consumed 60 g and the 75% who consumed 0 g. Fortunately, the USDA data also indicate the proportion of the population that reported consumption of each food. Thus, with a per capita average intake of 15 g of a food and the knowledge that 25% of the population consumed the food and 75% did not, the per user average intake is derived by dividing 15 g by 0.25, obtaining 60 g. Once the per-user average consumption of a food is available, that figure can be divided by the RACC to determine the multiplier needed to estimate daily consumption based on RACC (Table 3).

According to Table 3, the mean per capita daily intake of coffee is 259 g; 39.5% of respondents reported consumption of coffee on the survey day. Thus, those 39.5% of respondents consumed a mean of 656 g of coffee. The RACC for coffee is approximately 240 g (actually 240 mL); thus, the 656 g of coffee consumed by users is equivalent to 2.73 RACC. For most foods, mean daily consumption is less than 2 RACC; only frequently consumed beverages such as coffee and soft drinks exceed 2.5 RACC. Thus, a quite conservative estimate of the mean daily consumption of a food can be derived by multiplying the RACC by 2.5.

In risk assessment, it is common to evaluate exposure, not at the mean but at the level reached by heavy consumers of foods containing the target substance. While different regulatory authorities employ different percentiles (for example, 90th, 95th, 97.5th, and 99th), the profile most often used by the FDA is the 90th percentile of intake. The 90th percentile of intake is usually close to double the mean intake (FDA 2006). A conservative estimate of the 90th percentile of intake of a food can thus be derived by reading the serving size in the nutrition facts panel and multiplying by 5.

If the substance of interest is present in a commodity such as wheat, oats, tomatoes, and so on, it is futile to attempt to estimate the consumption of all foods that contain the commodity. Rather, it is necessary to estimate consumption of the commodity itself from all sources. The estimates for the crop group overestimate intake for individual commodities since each crop group contains many foods. Fortunately, although hundreds of commodities exist, only a small number are widely used that constitute a substantial proportion of finished foods and consequently have more than small levels of intake. The EPA has developed a database

Table 3—Daily food intakes expressed in RACC.

Food category	Mean per capita intake (g/d)	Proportion consuming	Mean intake by users (g/d)	RACC (g)	Intake in RACC
Quick breads and pancakes	19	0.227	84	110	0.76
Table fats	4	0.304	13	15	0.88
Yogurt	8	0.040	200	225	0.89
Salad dressings	8	0.293	27	30	0.91
Candy	7	0.154	45	40	1.14
Citrus juice	60	0.204	294	240	1.23
Fried potatoes	24	0.270	89	70	1.27
Chicken	21	0.192	109	85	1.29
Noncitrus juice and nectar	27	0.085	318	240	1.32
Frankfurters, sausages, luncheon meats	21	0.286	73	55	1.34
Beef	24	0.209	115	85	1.35
Ready-to-eat cereal	16	0.285	56	40	1.40
Fluid milk	191	0.556	344	240	1.43
Crackers, popcorn, pretzels, corn chips	12	0.278	43	30	1.44
Rice	23	0.110	209	140	1.49
Bread and rolls	50	0.663	75	50	1.51
Pasta	18	0.074	243	140	1.74
Eggs	18	0.191	94	50	1.88
Mixtures mainly meat/poultry/fish	99	0.362	273	140	1.95
Fruit drinks and other flavored beverages	95	0.197	482	240	2.01
Milk desserts	27	0.174	155	70	2.22
Cheese	16	0.226	71	30	2.36
Coffee	259	0.395	656	240	2.73
Carbonated soft drinks	332	0.504	659	240	2.74

Sources: USDA-ARS (1997) and 21 CFR § 101.12.

Table 4—Consumption of commodities by DEEM™ FCID crop group categories.

	90th percentile per capita (g/kg bw/d)	90th percentile per user (g/kg bw/d)
Dairy products	17	17
Meat (beef, pork, sheep, fish, shellfish, and poultry)	5	5
Root, tuber, and bulb vegetables	4	4
Leafy greens and brassica	2	3
Fruiting and cucurbit vegetables	3	4
Legumes	2	2
Fruits	9	10
Tree nuts	0.005	0.2
Cereal grains	8	8
Oilseeds	0.1	0.1

Source: DEEM – FCID (CSFII 1994 to 1998).

of intakes based on commodities (the Food Commodity Ingredient Database [FCID]), which is publicly available and provides a rapid tool for estimating intakes on a commodity basis. Table 4 shows consumption of commodities by DEEM™ FCID crop group categories.

Inspection of the daily intakes of commodities used as food ingredients reveals that only 43 commodities have 90th percentile daily intakes exceeding about 0.1 g/kg bw/d (Table 5). The highest intake of any single commodity is that of wheat, with a 90th percentile consumption of about 3.4 g/kg bw/d. This level of consumption is so high relative to other commodities that wheat products constitute their own group: A. Wheat is followed by the 6 commodities in group B—apple products, beef products, corn products, oranges and juice, potato products, and tomato products—with 90th percentile daily intakes in the range of about 2 g/kg bw for each commodity (for example, to get a total daily intake you would need to multiply the commodity by its contaminant level).

Risk–Benefit Evaluation

Risks and benefits in the food supply: perception, reality, measurement

Virgil Wodicka (Anonymous 1971) classified the sources of health risks in foods as being, in decreasing order, microbiological, nutritional, natural toxicants, environmental contaminants, food additives, and pesticide residues. Moreover, IFT and 14 other scientific societies concluded that pathogenic microorganisms posed the primary hazard in the American food supply (IFT 1988). With the current epidemic of obesity and the associated risks of serious chronic diseases, the positions today of microbiological and nutritional risks may well be reversed. Furthermore, there are substantial gaps in size between the 2nd and 3rd and the 4th and 5th categories of health risks. Although each of these categories represents important risks to health, those in the 3rd through 6th categories (that is, environmental contaminants, food additives, and pesticide residues) are the focus of this report. Of course, there are other risks, such as misbranding, adulteration, and the promotion of food products on the basis of misconceptions about nutritional value or safety. Although significant, these other risks are not addressed in this report. This section applies only to the more developed areas of the world. In less developed countries all of the aforementioned risks are far greater although the ranking is probably the same.

There is substantial literature addressing the perception of risks (Lowrance 1976; Douglas and Wildavsky 1983; Bandolier 2008). Involuntary risks tend to be overestimated while voluntary risks

are underestimated because they seem controllable. Starr (1969) calculated that voluntary risks are acceptable even when they are approximately 1000 times larger than involuntary risks. Most consumers believe that food should simply be safe and that, therefore, no risks are tolerable. However, many food risks result from personal choice or behavior. During 1998 to 2002, 24% of all traceable cases of foodborne illness were due to mishandling in the home (CDC 2006). Moreover, even safe food is often not consumed in a responsible manner; this helps explain why obesity is far too common and growing (Gordon-Larsen and others 2004; CDC-NCHS 2008). These risks, however, are perceived as voluntary risks as contrasted with the involuntary risks of food additives, novel food processing, and pesticide residues. Obviously many consumers do not control these personal, voluntary risks as well as they should, which explains the high actual ranking of microbiological and nutritional risks.

Table 5—Ninetieth percentile daily intake of commodities by commodity group.

Group	Commodity group	90th percentile exposure (g/kg bw/d)
A	wheat flour/grain/bran/germ	3.40
B	apple, apple juice	2.00
	beef	
	corn (field, syrup)	
	orange, orange juice	
	potato, potato chips	
	tomato, sauce/paste/puree/juice	
C	banana	0.60
	beet (sugar)	
	carrot, carrot juice	
	chicken	
	corn (field, meal, flour)	
	corn (sweet)	
	egg	
	grape, grape juice, wine grape	
	lettuce (head/leaf)	
	onion, all	
	pork	
	rice, rice bran, rice flour	
	soybean oil/flour/milk	
	sugarcane, sugar	
	turkey	
D	barley	0.20
	bean (snap, succulent)	
	broccoli	
	cabbage	
	cantaloupe	
	celery, celery juice	
	coffee	
	cranberry, cranberry juice	
	cucumber	
	grapefruit, grapefruit juice	
	oat, bran flour/groat/folled	
	pea	
	peach, peach juice	
	peanut, peanut butter	
	pear, pear juice	
	pepper, bell	
	pineapple, pineapple juice	
	squash	
	strawberry, strawberry juice	
	vinegar	
	watermelon, watermelon juice	
E	all others	0.04

Source: DEEM- FCID (CSFII 1994 to 1998).

The term "food risk" is almost universally and instantly taken to determine risk to health. In consequence, the term "benefits," is often interpreted as meaning health benefits. That, however, is entirely too narrow a view. Food is not merely a biological necessity; it is also a cultural and ethnic expression, a social activity, a form of self-expression and creativity, and a source of great sensory pleasure. Other benefits lie in cost reduction, availability enhancement, and increases in acceptability, all of which are nonvital benefits.

Flavor preferences for fats and sweets evolved during the hunter-gatherer phase, when high calorie output was necessary to find food needed to survive. What are now largely nonvital benefits—pleasant tasting food, convenience, and satiety—are major factors in consumer food choices. Unfortunately, because of reduced energy output, these now contribute to obesity and chronic diseases such as coronary heart disease (CHD), stroke, and cancer. The bad news is that most consumers will succumb to one of these vital risks. The good news is that, unlike distant ancestors, today's consumers will live long enough to get chronic diseases because of the advances made in the quality and safety of our food supply. Vital risks and benefits, including health benefits, are measurable in medical and epidemiological terms: disease rates, life expectancy, and medical status. They are not easily or persuasively measured in dollars. In contrast, nonvital risks and benefits are usually and easily measured in dollars by what people are prepared to pay to avoid or acquire them. In spite of the fact that health risks and nonvital benefits are not measurable in commensurable terms, we make these trade-offs every day, especially when they involve nonvital benefits and voluntary vital risks.

Disease prevention compared with health promotion

Beyond simply enough food, the health benefits associated with food that are most clearly demonstrated are in reduction of the 6 risk categories. The largest and most spectacular of those benefits probably lie in the past. Enrichment of milk with vitamin D banished rickets, which until early in the 20th century was a common form of malnutrition. Iodization of salt banished goiter, and flour enrichment decreased beriberi and pellagra. Preservation technologies, antioxidants, and improved processing substantially reduced the risks of foodborne illness and of food loss. Many of these contributions were large, unmistakable, and measurable in the amount of illness prevented and increased life expectancy. In contrast, health benefits are harder won, less clearly demonstrated, and more difficult to quantify.

In developed countries, where deficiency diseases are a thing of the past, attention has increasingly focused on finding the health-promotion effects of foods. This indicates a shift away from focusing on disease caused by a lack of dietary nutrients to focusing on the role that diet plays in contributing to chronic diseases. The multi-factorial nature of these diseases obscures the specific role of each factor. As a result, dietary guidelines for reducing chronic disease risk tend to be general, such as food pyramids and include broad dietary advice, such as eating a diet high in fresh fruits and vegetables. Evidence suggests that a diet high in fruits and vegetables is associated with a reduced risk of cancer (NRC 1989, 1996; Ames and Gold 2000). It is also clear that eating too much food (obesity) is associated with increased risk of certain cancers, adult-onset diabetes, CHD, and stroke (CDC 2005). Beyond these broad statements, the relationship of specific foods and specific food constituents to reduce risk of disease becomes less clear. The role of supplements of essential nutrients and other dietary

supplements and their interaction with other lifestyle factors is now less certain.

Bruno and others (2006) demonstrated that 1000 mg of vitamin C per day (approximately 17 times the recommended dietary allowance [RDA]) inhibits the depletion of vitamin E in smokers. Clearly such depletion is disadvantageous, but how much this affects the risk of lung cancer or CHD is far from clear, especially given the multi-factorial nature of these diseases. Kirsch and others (2006) found, in a survey of the diets and supplement use of 29361 men, that supplementation with vitamin E and beta-carotene was associated with reduced risk of prostate cancer for smokers, not for nonsmokers. This agrees with the results of the alpha-tocopherol, beta-carotene cancer prevention (ATBCCP) trial (ATBC 1994). In more than 29000 male Finnish smokers, those who received 50 μg/d of vitamin E had a lower incidence of prostate cancer than the control (nonsupplemented) group. However, the same ATBCCP trial showed that the male Finnish smokers who took beta-carotene or both beta-carotene and alpha-tocopherol had a 17% increased incidence of lung cancer, and an 8% increased overall mortality after 6 years. Further, the vitamin E treatment was associated with a significant increase in hemorrhagic stroke. Fortunately, both the favorable effects from vitamin E and the adverse effects from beta-carotene and vitamin E largely disappeared during the 8-year follow-up study after discontinuation of the interventions (ATBC 2003). Obviously the importance of smoking cessation far exceeds the utility of such supplements. In contrast, the Heart Outcomes Prevention Evaluation trial, using 400 IU (approximately 30 times the RDA), found no effect on cancer of the prostate or any other site (Lonn and others 2005). A trial of vitamin E supplementation in nearly 40000 American women, half taking the supplement, half not, appeared to show that the vitamin did not prevent cancer or heart disease, having shown only that vitamin E reduces the risk of death from heart attack in women 65 years of age and older, and without any effect on overall mortality (Lee and others 2005). The researchers concluded that the data do not support recommending vitamin E supplementation for cardiovascular disease or cancer prevention among health women.

It seems reasonable to suggest that these and other contradictory results stem from incomplete knowledge of the mechanisms involved in the interplay of the multiple risk factors and the ultimate causes of chronic diseases. We also lack biomarkers—early stage, predisease warning signs—that might provide information early enough to alter lifestyles, diets, and other risk factors. Metabolomics or metabonomics may fill some of these crippling gaps in our understanding. We already know that there are essential nutrient interactions: for example, vitamins C and E, iron and vitamin C (NAS 2000a), and molybdenum and copper (NAS 2000b). Thus, it is reasonable to assume that the benefits of whole foods are by no means fully captured in supplementation with specific constituents. Other constituents in the food matrix that may play a decisive modulating role are as yet unknown.

Pursuing the potential carcinogenicity and anticarcinogenicity of substances through high dose animal feeding studies, which are dissimilar from human exposures involving a large complex of chemicals, is far from human reality (NRC 1996). Similarly, the tools for pursuing interactions of food constituents with each other and with genetic and environmental factors are still very crude. Epidemiology can be helpful in identifying risk factors and potential benefits, but it can be unhelpful as well, often being insufficiently sensitive—especially when dealing with common disease outcomes. Thus, persuasive knowledge linking specific dietary constituents to reduced risk of chronic disease is lacking. Therefore, the more general recommendations that have evolved over the years are useful for choosing dietary patterns intended to promote health and reduce the risks of diet- and obesity-related disease. The U.S. Dept. of Health and Human Services and the USDA offer such advice. "Dietary Advice for Americans 2005" presents much information, intended primarily for health professionals, based on consensus judgments of current science. MyPyramid.gov presents an updated version of the food pyramid and provides very general advice intended for consumers, taking into account age, height, weight, and exercise habits. These publications emphasize consumption of diets that contain the following:

- abundant fruit and vegetables, especially dark green and orange vegetables,
- whole grain foods as a major source of carbohydrate,
- fat-free or low-fat milk and milk products,
- moderate consumption of lean meats, poultry, fish, eggs, and nuts,
- low intakes of saturated and trans-fats and caloric sweeteners (for example, sugar, corn syrup, honey) with total fats accounting for no more than 25% to 35% of all calories,
- foods high in nutrient content (nutrient-dense), thereby leaving more room for discretionary calories, and
- low intake of sodium, equivalent to 1 teaspoon of salt per day from all food sources.

In addition, these guidelines stress the need to maintain body weight within a healthy range by balancing calorie intake with calories expended. To that end, they urge at least 30 min/day of active exercise for adults (more for children and adolescents). Beyond this, only some encouraging, hopeful, but limited, indirect and sometimes conflicting evidence is available.

Whole food perspective: benefits as well as risks

With respect to food, as information emanating from research into the qualities of various food components reaches the public's attention through journalists' reporting, nutrition nonsense and food faddism, often arises and leads to nations of avoiders of specific foods and ingredients such as salt, fat, meat, carbohydrates, and coffee. One of the key reasons for this problem is that the assessment of risk to human health of food ingredients and food contaminants has historically been conducted independently of possible health benefits of specific foods containing certain ingredients or contaminants. It was not until passage of the Nutrition Labeling and Education Act in 1990 that communication of the benefits of foods in reducing risk of disease was actively encouraged in the United States. In addition, different scientific approaches have been used to estimate health risks and benefits of food ingredients and contaminants. While the assessment of risks has been advanced over the past 30 years both qualitatively and quantitatively, similar advances have not been achieved in either the qualitative or quantitative assessment of benefits. For example, in addressing the carcinogenicity of individual food chemicals, the U.S. Natl. Academy of Sciences/Natl. Research Council (NRC 1996) set forth several key qualitative conclusions about the nature of chemical substances comprising foods:

- "The great majority of individual naturally occurring and synthetic chemicals in the diet appears to be present at levels below which any significant adverse biologic effect is likely, and so low that they are unlikely to pose an appreciable cancer risk."
- "The varied and balanced diet needed for good nutrition also provides significant protection from natural toxicants."
- "Current evidence suggests that the contribution of excess macronutrients and excess calories to cancer causation in the United States outweighs that of individual food microchemicals, both natural and synthetic."

- "Most naturally occurring minor dietary constituents occur at levels so low that any biologic effect, positive or negative, is unlikely" (NRC 1996).

While there was sufficient consideration in this report of the qualitative health benefits of foods that contain trace levels of synthetic or naturally occurring chemicals, there were little or no quantitative assessment techniques available to assess the benefits of specific foods, and unfortunately, such techniques still remain untested and not validated. However, there is new benefit-risk analysis activity currently underway in Europe and financially supported by the European Commission, Directorate General Research under the 6th Framework Programme (BRAFO 2008). Coordinated by the Intl. Life Sciences Inst. (ILSI) Europe, this project (BRAFO) is anticipated to be completed in December 2010. Through this activity, a framework is being developed for quantitatively comparing human health risks and benefits of foods. The project will consider the quality of data and strength of effects, initially using 3 categories of food and food ingredients as test cases: natural foods (such as oily fish and soy), macronutrient replacement agents (such as sweeteners and fat substitutes), and the impact of heat processing on foods. The methods developed will hopefully inform future approaches to quantify benefit-risk analysis related to foods globally.

When considering the complexities of developing quantitative benefit-risk assessment methodologies, it is instructive to consider the scope of the problem and the project goals as expressed by the BRAFO project team:

"The risk assessment of compounds in food is a mature process that follows a well-developed scientific approach; the strategy followed is the result of a substantial amount of thought and experience. Such a risk assessment has served society well to the extent that it has protected consumers from the potentially harmful effects of chemicals to which they might otherwise have been exposed through food consumption. For chemicals used to secure the integrity of food that require prior approval, such as pesticides or packaging materials, this works reasonably well, although it is difficult to weigh the indirect benefit against residual risk. For chemicals with putative direct health benefits such as vitamins or phytoestrogens, the situation is more complex. It is necessary to evaluate both risks, manifest as negative impacts on health, and those benefits that produce a positive impact on health" (BRAFO 2008).

Some initial progress has been made and reported on this effort by the European Food Safety Authority, which sponsored a scientific colloquium in July 2006 to begin addressing these issues (EFSA 2007). The objectives of the EFSA colloquium were as follows:

- to have an open debate on scientific approaches and methods available and tools and data needed for conducting a risk-benefit analysis of foods and food components,
- to explore opportunities and limitations for defining a common scale of measurement to compare risks and benefits quantitatively, and
- to define further research needs.

Participants of the EFSA colloquium were asked the following questions:

- What human health risks and human health benefits should be considered?
- What human health risks and human health benefits can be quantified?
- What tools/data do we currently have to quantify the human health risks and human health benefits?
- What tools/data would be needed to quantify the human health risks and human health benefits?
- What type of risk-benefit analys is needed (systematic qualitative assessment, semiquantitative assessment, fully quantitative assessment)?
- Do we need risk-benefit analysis for different population groups?
- When is it useful to carry out a risk-benefit analysis?
- What could be a common scale of measurement to compare human health risks and benefits?
- Where is the borderline between risk-benefit assessment and risk management?

It will be important for the United States and other countries to closely follow the groundbreaking developments being forged by the Europeans in the science, methodologies, and policy considerations of risk-benefit assessment.

Seafood: methyl mercury risks compared with nutritional benefits

The consideration of the nutritional benefits of seafood consumption in conjunction with the adverse effects of methylmercury is an example of deliberations involving a considerable amount of valuable data. Seafood consumption has long been viewed as promoting health because it is a good source of protein and omega-3 fatty acids (particularly eicosapentaenoic acid and docosahexaenoic acid) and contains micronutrient constituents such as selenium). Nonetheless, consumers have become concerned about the safety of seafood for a number of reasons, some stemming from concern about the pollution of water resources and others from the long-standing problems of microbiological contamination. Seafood can be contaminated with organic pollutants, microbial pathogens, and heavy metals, particularly methylmercury. The Inst. of Medicine (IOM) recently assessed the health risks and benefits of seafood consumption, focusing on the safety of seafood consumption by different population groups (IOM 2007). The IOM concluded that although the particular constituents providing health benefits are not fully known and data on benefits are largely from associations with consumption of individual ingredients, consumers must balance the potentially growing risks against the known health benefits of seafood consumption. More specifically, the IOM emphasized that all groups are likely to benefit from consuming seafood and that certain groups, such as those at risk for heart disease, are most likely to benefit.

The IOM reported complex relationships between risks and benefits for seafood consumption. Methylmercury-contaminated fish from rivers, lakes, and bays have been associated with many documented cases of human illness and deaths. Methyl mercury accumulates in the large muscles of predatory fish such as swordfish, king mackerel, and tilefish. The higher a fish is in the predatory hierarchy, the greater its potential for methylmercury contamination. For this reason, the FDA established an action level of 1 ppm methylmercury in the edible portions of fish, shellfish, crustaceans, and other aquatic animals (fresh, frozen, or processed).

Using available information on consumption patterns, occurrence of detrimental and beneficial seafood components, and susceptible populations, the IOM developed recommendations for seafood intake. In short, children up to 12 years of age and pregnant women or women at risk of pregnancy should consume seafood somewhat more moderately than others and avoid large predatory fish. The IOM emphasized the need for improvements in the data on contaminants and public reports regarding local conditions that may impact seafood contamination (IOM 2007). More recently, the FDA issued a draft quantitative risk and benefit assessment of consumption of commercial fish on fetal neurodevelopment effects and coronary heart disease and stroke in the general population (FDA 2009). The results were generally

consistent with research reported in recent years in the scientific literature.

The Maillard browning reaction in products: risks and benefits

Considering the benefits associated with consumption of a given food while also assessing the risk of individual components is especially controversial when it relates to substances shown to be carcinogens formed during the processing and heating of foods and beverages (Jagerstad and Skog 2005; Somoza 2005). Carcinogens from heated foods, produced by the Maillard browning reaction (MBR) between carbohydrates and amino acids and proteins, have been a health concern since the 1970s. Trace levels of animal carcinogenic and mutagenic PAHs have been found in barbecued steaks, coffee, and other heated foods, and N-nitrosamines have been discovered in fried bacon and beer. Polycyclic heterocyclic amines, many of which were eventually shown to be fairly potent mutagens and animal carcinogens, have been detected in overheated meats. More recently, both acrylamide and furan have gained widespread attention and concern because each has been proven carcinogenic to animals. Assessing individual heat-induced food chemicals and their safety in humans requires determining why such intense interest in trace amounts of carcinogens exists when there is little evidence linking MBR reactions to disease in humans.

An important toxicological consideration that has received little attention to date is that many MBR products are beneficial to health because many contain antioxidants, antimutagens, and anticarcinogens (Manzocco and others 2001; Lee and Shibamoto 2002; Jagerstad and Skog 2005; Somoza 2005). In addition, some MBR products induce the formation of carcinogen-detoxifying enzymes such as glutathione transferase (Lindenmeier and others 2002). For example, furan as well as other volatile heterocyclic flavor compounds have been shown to be a good antioxidants (Fuster and others 2000; Lee and Shibamoto 2002; Yanagimoto and others 2002, 2004). Melanoidins, chemically complex brown Maillard polymers, have been shown also to have significant antioxidative properties (Borelli and others 2002; Delgado-Andrade and Morales 2005; Delgado-Andrade and others 2005; Daglia and others 2008).

Furan is a simple flavor compound produced by heating many foods and beverages, and it has been known for decades to occur at trace levels (ng/g or ppb) in some processed foods (for example, coffee and other browned foods) (Maga 1979; Hasnip and others 2006; HEATOX 2007). Furan has also been found to be an animal carcinogen (IARC 1995). Whether furan is a human carcinogen is not yet known. In its role as an antioxidant, it may be able to protect foods from producing health-damaging oxidants during heating, processing and storage, and furan intake may also be adding a beneficial antioxidant to the human diet. Such considerations of comparing risks and benefits are critical in determining whether the consumption of a particular food or beverage should be safe. Recent improvements in analytical methodology and scientific instrumentation have made it possible for the FDA to accurately measure the amount of furan in a wide variety of foods (Morehouse and others 2008). Results from analysis of more than 300 processed foods indicated that furan can be found at levels ranging from nondetectable to more than 100 ng/g. Exposure estimates for several adult food types were calculated, and brewed coffee emerged as the major source of furan in the adult diet (0.15 μg/kg bw/d). For consumers 2 years of age and older, furan intake was estimated to be about 0.2 μg/kg bw/d or about 14 μg/d for an adult weighing 70 kg.

In 2002, Swedish researchers detected acrylamide in many fried and baked foods, especially potato chips and French fries, at levels from 30 to 2300 ppb (Dybing and others 2005; JECFA 2006; Mucci and Wilson 2008). The researchers attributed the production of acrylamide to the higher temperatures required for production of the desirable color, flavor, aroma, and texture of foods. Because acrylamide is a neurotoxicant and rodent carcinogen, academic, government, and industry research groups initiated major research programs to determine the level of dietary intake of acrylamide in various countries, ways to mitigate acrylamide formation, and whether acrylamide occurrence in food is a significant public health risk. The consensus today is that currently available information on acrylamide in foods is not sufficient to draw firm conclusions about its cancer risk to humans (JECFA 2005). A recent European review considered for the first time the value of performing a detailed risk–benefit evaluation of mitigation measures on the acrylamide content of foods, taking into account the nutritional value and beneficial health impact of consuming the food and beverage products containing acrylamide (Seal and others 2008). In the meantime, international public health and regulatory authorities have stated there is no indication at this time that consumers need to change their eating or drinking habits.

The main challenge of the presence of furan, acrylamide, and other cooked-food carcinogens is that these compounds also make food palatable and desirable (that is, appealing aroma, color, texture, and flavor) as sources of nutritive value. Many researchers have concluded that compounds such as melanoidins and furan may help to prevent *in vivo* oxidative damage such as lipid peroxidation, which is associated with cancer, diabetes, atherosclerosis, inflammation, arthritis, immune deficiencies, and even aging. Although living organisms are known to be protected from active damaging oxidants by enzymatic systems, natural antioxidants (α-tocopherol, carotenoids, and ascorbic acid in fruits and vegetables) are also known to protect cells from oxidation and humans from various diseases. Thus, it is critical to consider any beneficial health-protective effects oin evaluating the safety of acrylamide, furan and other heat-induced animal carcinogens formed during the MBR process.

Although it is important to evaluate the toxicological risks of heat-induced chemicals in foods, it is equally important to fully evaluate the safety of whole foods and beverages using modern toxicologic and epidemiologic techniques. It is becoming increasingly important to recognize that health-benefitting food chemicals occur naturally as do other health-protective compounds during heat processing and cooking. It is thus critical to evaluate the beneficial health effects of heated foods and beverages and then to undertake a thorough risk–benefit evaluation of the whole food or beverage. Such an evaluation must carefully consider how best to interpret animal toxicology results for individual chemicals as well as any information indicating that a food or beverage may actually be cancer protective when evaluated as a whole. Although regulatory and health authorities assessing food safety are obliged to focus great attention on the occurrence and potential risk of suspected toxicants and carcinogens in foods, they should also be encouraged to systematically investigate the health benefits of whole foods. In the United States, the FDA is precluded from considering the potential benefit of most food ingredients when evaluating the safety of the ingredient. The EPA has broader authority; in considering the tolerance for pesticide residue, it can assess whether the value of the pesticide increases a food crop's availability.

Consequently, we must encourage health and regulatory authorities around the world to make 2 important changes in their historic approach to food safety assessment: (1) carefully assess the risks and benefits of whole foods and beverages using a risk–benefit approach rather than assessing only the well-studied, individual toxic chemicals in these products; and (2) give much

greater public health consideration to the potential health benefits of heated foods and beverages.

Case study: coffee

Although the health risks of coffee and its key ingredients, particularly caffeine, have been the focus of thousands of published scientific/medical studies addressing almost all known animal toxicity endpoints and human disease outcomes, more recent research has focused on the possible health benefits of coffee consumption. Many negative notions about coffee drinking and study conclusions about caffeine have been transformed into myths. Health benefits have been discovered and validated as a result of more recent mechanistic and epidemiologic research studies (van Dam 2008). Much of what led to the demonstration of caffeine's adverse effects in the past 30 years is now known to have resulted from methodological weaknesses occurring in the animal toxicology and human epidemiologic research.

Today, there is a well-established database on the benefits of human consumption of coffee and caffeine in improving both physical, energy-requiring performance and cognitive mental performance in humans. Further, a growing body of literature appears to conclude that moderate coffee consumption (3 cups to 5 cups/day) is not only safe for human consumption but also associated with the reduced risk of a number of diseases like cancer (Dórea and da Costa 2005; Higdon and Frei 2006; Lopez-Garcia and others 2006; van Dam 2006, 2008; Clemens and Coughlin 2007; La Vecchia and Tavani 2007; Nkondjock 2009). In fact, 2 Harvard prospective cohort studies of more than 41,700 men and 86,200 women showed that coffee was not associated with any increased risk of cancer death and was not associated with an increased mortality rate in either men or women (Lopez-Garcia and others 2008). These researchers also noted the possibility of a modest benefit of coffee consumption on CHD mortality.

It is well known that coffee contains trace levels of up to 40 identified animal carcinogens, including acrylamide, furan, caffeic acid, various aldehydes, PAHs, and ochratoxin A (Gold and others 1984; Aeschbacher and others 1989; Lee and Shibamoto 2002). A few of these chemicals (such as caffeic acid and ochratoxin A) occur naturally in green coffee beans, but others are formed at trace levels during the coffee roasting process (Somoza 2005; Monteiro and others 2007). However, the compounds in coffee that may contribute to the observed reduction in risk for some cancers have now been identified. Consequently, the dietary cancer risk of coffee consumption cannot properly be assessed by examining just the concentrations and potencies of the individual animal carcinogens contained in the product. Ironically, coffee contains natural (chlorogenic acid, polyphenolic antioxidants, kahweol, and cafestrol palmitates) and heat-induced protective chemicals that may help to reduce the potential carcinogenic risk of other coffee chemicals reported to be animal carcinogens (Huber and others 2002; Somoza 2005; Clifford and others 2006; Monteiro and others 2007). It may well be that the anticarcinogenic effects of these components outweigh any risk from the known carcinogens, leading to overall cancer protection at the organ sites that have shown reduced cancer risk among coffee drinkers.

In concert with these favorable developments about coffee consumption are ongoing investigations in many laboratories throughout the world to determine the biochemical mechanisms by which these beneficial health effects may operate. One of the most exciting areas of mechanistic research is the possible cancer-protective role of coffee's naturally occurring polyphenolic antioxidants (chlorogenic acid derivatives) and heat-produced antioxidants (MBR products, including volatile heterocyclic compounds and brown melanoidin polymers) (Lee and Shibamoto 2002; Somoza 2005; Clifford and others 2006; Fujioka and Shibamoto 2006; van Dam 2008; Sacchetti and others 2009). Studies in many countries have recently shown that coffee is the major individual source of dietary antioxidant potential in humans, exceeding wine, tea, chocolate, fruits, and vegetables (Pellegrini and others 2003; Svilaas and others 2004; Mattila and others 2006). Laboratory studies have also shown that some coffee constituents can induce the formation of phase II carcinogen-detoxifying enzymes (Faist and Erbersdobler 2001; Somoza and others 2003).

Thus, coffee demonstrates the fact that dietary cancer risk cannot properly be assessed by examining only the trace concentrations and carcinogenic potencies of individual animal carcinogens contained in a product; rather, a risk-benefit assessment from a whole food perspective is needed. Coffee and other foods should be evaluated using an approach that takes into account the beneficial effects of any natural, cancer-protective chemical compounds that are simultaneously present.

Importance of the Food Matrix

Efforts have been directed to determine the real contribution of whole foods as sources of specific nutrients (for example, carotenoids, folate) or nonnutrients (for example, isoflavones and ferulic acid), and establish conditions (before or after processing) that maximize their purported benefits (Van het Hof and others 1999; Castenmiller and others 2000; Adam and others 2002; de Pascual-Teresa and others 2006). The food matrix effect is defined as the overall role of single or multiple dietary compounds assembled in various physical states on the disposition of specific chemicals that may or may not be beneficial in foods. The role of the food matrix on the bioavailability and disposition of nutrients and nonnutrients is a complex, critical topic under continuous study as the types of foods and the way in which they are consumed evolves. The bioavailability, efficacy, and potency of many chemicals and products derived from their digestion and metabolism have not been adequately addressed.

Parada and Aguilera (2007) more clearly define food microstructure as the spatial arrangement of particular elements in a food and their interactions at levels below 100 μm. Either nature can impart food microstructure, such as in raw foods, or processing can, such as in baked, fried, confectionary, or heated foods. Natural food structures can be divided in 4 broad categories: (1) fibrous structures assembled from macromolecules into tissues with functions (such as muscles); (2) fleshy materials from plants bonded together at the cell walls (such as tubers, fruits, and vegetables); (3) encapsulated embryos of plants that contain a dispersion of starches, proteins, and fats assembled in order (such as in grains and pulses); and (4) complex fluid dispersions (such as nectars, honey, and milk). Specific nutrients and nonnutrients could be embedded and interact with themselves or with the components of the food matrix within a larger, more complex continuous medium from natural or artificial origin (Parada and Aguilera 2007).

The different processes that take place during the disruption of the food matrix and release of chemical substances within the whole food are vital. During mastication, the initial step of the eating process, food mixes with saliva in the mouth and forms a heterogeneous bolus. Chewing disrupts the matrix into small pieces, reducing particle size and enlarging surface area for enzymatic digestion. This process promotes the efficiency of digestion and gastrointestinal absorption of many chemicals and their interaction with other chemicals. Further digestion of macromolecules such as protein and starch occur in the stomach and intestine, resulting in the release of most trapped chemicals. This allows for maximum exposure and interaction of nutrients and nonnutrients for further absorption.

Metabolism of food constituents can be impacted by other dietary constituents. For example, grapefruit juice is well known for its impact on the metabolism of other dietary constituents. In some cases grapefruit juice can augment bioavailability of functional components. The potential mechanisms of action by furanocoumarins and flavonoids in grapefruit juice include the inhibition of the intestinal first-pass metabolic enzyme (cytochrome P4503A4) and brush border pump (p-glycoprotein) (Bressler 2006). Consequently, concomitant consumption of grapefruit juice could increase the plasma concentration of various drugs, especially those with high first-pass metabolism, which involves felodipine and atorvastatin in humans (Bailey and Dresser 2004). In other cases, grapefruit juice could decrease absorption of a few specific drugs (such as fexofenadine, digoxin) by inhibiting the organic anion transporting polypeptide (Dresser and others 2005; Bailey and others 2007). Thus, in this instance the food matrix carries free components that directly affect the bioavailability of widely used drugs potentially leading to adverse clinical effects.

Factors in the food matrix that alter the bioavailability of chemicals of interest can be grouped into those that are related to its microstructure (muscle fibers, cell walls, and so on) and those related to its inherited composition (lipids, proteins, or single compounds). These sets of factors also could affect intrinsic chemicals (parts of the raw or processed food) and extrinsic chemicals (ingested with foods). Factors related to the microstructure of the food matrix occur naturally or are created artificially and condition the chemicals of interest, affecting their release or solubilization during digestion. The formation of gluten during baking and dissolution of fat micelles in emulsions are examples of the impact of natural macrostructures.

Composition factors in the food matrix relate to interactions between chemicals present in the food matrix with important structural or chemical factors responsible for the proper digestion, absorption, and metabolism of nutrients and nonnutrients in the body. These interactions directly or indirectly alter the disposition of the interested chemicals in the body. For example, consumption of food matrices high in fats promotes the release of bile salts that then facilitate the solubility of nonpolar chemicals in the intestinal tract, thus promoting their rapid absorption (Zhi and others 1995). In other instances, chemicals within the food matrix lead to increased or decreased absorption of extrinsic. Another example is the potential role of dietary fiber or prebiotics in the promotion of gut microflora that could then promote the bioavailability, synthesis, or modification of specific chemicals of interest (Cashman 2003).

Bioavailability involves both the absorption and metabolism of a dietary chemical. In considering health-promoting dietary constituents, the most relevant to bioavailability is the amount and persistence of the ingested dietary constituent that reaches the targets for the health benefit. Bioavailability is more commonly assessed by measuring the amount of the ingested chemical that gets into systemic circulation since in most cases the precise targets, the form of the dietary constituent that is required at the targets, and how long the constituent must remain at the target for a health effect are unknown. Using the systemic circulation as a measure of bioavailability is complicated by the metabolism of dietary chemicals. Thus, although bioavailability is critical in assessing the potential benefits and risks of intake, it generally can only be estimated or studied on a comparative basis. There are many examples in which bioaccesibility of nutrients could be hindered because of their position within the natural microstructure.

Carotenoids, a group of nonpolar plant pigments, provide the red to yellow tones in plants and function as photo-protective or photo-accumulative agents (Demming-Adams and others 1996). Their chemistry, absorption, and role in health and disease have been documented (Castenmiller and West 1998). Although more than 600 carotenoids have been identified, only a few are well-characterized: alpha-carotene, beta-carotene, cryptoxanthin, astaxanthin, lutein, and lycopene. In general, bioavailability of carotenoids is dictated by their bioaccesibility and fat solubility. Carotenoids are released from plant food matrices after the disruption of cells and other microstructures during mastication and food processing (Castenmiller and West 1998; Van het Hof and others 1999; Zaripheh and Erdman 2002). Thus, it is important to understand the combined role of microstructure and composition in the absorption of fat soluble chemicals.

The bioavailability of water-soluble vitamins is also subject to food matrix effects. For example, the bioavailability of folic acid depends on the food microstructure. The function of folic acid in cells is well known (Stanger 2002). Folates are bound to macrostructures such as proteins in plants (Hossain and others 2004). Van het Hof and others (1999) reported that disruption of the food matrix could help in the release of folate and directly increase its absorption.

As a determinant of the food matrix, food processing can alter the availability of nutrient and nonnutrients by influencing release, transformation, and subsequent absorption of chemicals in the digestive tract. Heating in the production of tomato paste can facilitate the conversion of the all-trans form of lycopene, which may reduce the risk of prostate cancer (Hadley and others 2002), to the more bioavailable cis form (Unlu and others 2007). In fermentation of calcium-fortified soy milk, the bioavailability of calcium, which is less bioavailable from vegetable sources, was increased and formation of the isoflavone aglycone was increased (Tang and others 2007). Food processing can also change the chemical composition of foods, removing or accumulating factors that could predispose the absorption of chemicals. For example, soy protein isolate from soy flour has more bioaccesible isoflavones than the flour but lacks the fermentable fiber that could be used by gut bacteria in the production of equol.

In summary, classification of the effects of food matrix on risks and benefits associated with various chemical substances in food is complex. Furthermore, it is essential to add to consideration of these effects information about critical variables that are intrinsic to the individual, such as gender, age, health status, alcohol consumption, and customary food consumption. Given the complexity of the food matrix and its role in altering how dietary chemicals are handled in the body, extrapolating observations to the general population is difficult.

Case study: soy isoflavones and breast cancer

Isoflavones are a subclass of phenolic compounds that naturally occur in soy plants in many forms: aglycones, beta-glucosides, and malonyl, and acetylglucosides. Isoflavones in unprocessed soy beans and raw products (such as flour, concentrates, isolates, and so on) are present predominantly as glucosides (Cornwell and others 2004). Further food processing alters the initial composition and ratio of glucosides present, and fermentation leads to formation of aglycones in final products (Cassidy and others 2006; de Pascual-Teresa and others 2006). After consumption, most isoflavones are converted to their aglycone forms before absorption (Setchell and others 2002).

The reproductive toxic effects of phytoestrogens, isoflavones, lignans, and other nonsteroidal chemicals found in plants and plant products were first described by Bennetts and others (1946) after observing that sheep consuming subterranean red clover demonstrated symptoms of hyper-estrogenization such as prolapse of the uterus, milk secretion from virgin ewes, castrated males, and reproductive failure. The specific phytoestrogens responsible for these estrogenic effects were isoflavones and coumestrol present in the subterranean clover. Similar effects

were reproduced in laboratory animals after oral administration of either a soy-based diet or a diet supplemented with genistein (Carter and others 1955; Matrone and others 1956). Phytoestrogens accumulate in many plant species at various concentrations. Phytoestrogens elicit different estrogenic potencies; they bind to estrogen receptors and are thought to mediate estrogenic effects through mechanisms similar to that of estradiol.

Environmental estrogens clearly present a risk to humans; however, the effects of long-term exposure to less potent phytoestrogens, which may be consumed in high quantities up to hundreds of milligrams per day, are largely unknown. It is now clear that phytoestrogens at levels present in plant foods can induce toxic lesions in the reproductive tract during development in laboratory animals. Huge gaps exist in our understanding of the biological/toxic effects of chronic exposure to these ubiquitous low affinity estrogen receptor ligands.

For example, consumption of soybeans and their isoflavone extracts has been widely publicized as a natural, alternative therapy for a plethora of hormone-related conditions, including breast and prostate cancers, menopausal symptoms, cardiovascular disease, and osteoporosis (Stephens 1999; Brewer 2000; Arjmandi 2001; Kleijn and others 2002). Soybeans and isoflavone extracts have been extensively investigated for their purported beneficial role in breast cancer prevention. Genistein, an isoflavone present in soybeans, has been investigated for its biological activity related to breast cancer, and its estrogenic activity is well-documented (Wang and others 1996; This and others 2001). Genistein is effective in reducing growth of cultured cancer cells at high concentrations above 10 μM, potentially through the inhibition of tyrosine phosphorylation and cell cycle progression at G2/M. Moreover, genistein prevents breast cancer in laboratory animals when administered to prepubertal Sprague–Dawley rats either by intraperitoneal injection or orally, reducing the number of dimethylbenzanthracene-induced mammary tumors later in life (Lamartiniere and others 1998). These effects are likely mediated via an estrogenic mechanism, as estradiol and progestins and diethylstilbestrol have also been shown to be protective in the same chemically induced mammary cancer model (Petrakis and others 1996). These chemo-preventive effects are potentially mediated through an estrogenic effect to accelerate mammary gland differentiation (Lamartiniere 2002). A more differentiated mammary gland proliferates less and reduces DNA damage from chemical carcinogens.

Although genistein demonstrates chemo-preventive abilities, it also promotes growth of preexisting tumors. Allred and others (2001a, 2001b, 2004) demonstrated that either genistein or its aglycone given alone or as part of a soy product of different processing levels stimulated the growth of human breast cancer cells in a preclinical post menopausal breast cancer model. Lu and others (1996) demonstrated that consumption of soy milk for a month prolonged the menstrual cycle. Intake of a soy protein beverage (42 mg genistein, 27 mg daidzein) increased nipple aspirate fluid in premenopausal but not postmenopausal women (Petrakis and others 1996). McMichael-Phillips and others (1998) reported that short-term dietary soy administration stimulates breast cell proliferation, suggesting an enhanced breast cancer risk. In another study, women fed soy protein (60 g texturized soy protein, 45 mg isoflavones) demonstrated an increase in the E-responsive gene pS2, suggesting an estrogenic effect on the breast (Hargreaves and others 1999). Overall, the results from human and preclinical animal studies clearly demonstrate that soy foods and their isoflavones are estrogenic and their effects depend on many factors such as the time of exposure and the ultimate bioactive concentration.

Although prevention is a critical component of cancer control, there is the perception that diets effective during prevention may also be effective during cancer treatment. However, the assumption that the beneficial effects of consuming wholesome soy foods could be extrapolated to their single constituents may not be correct. The potential interactions between bioactive phytoestrogens and other components add to the complexity of the food matrix, enhancing or reduce the ultimate effect on health.

Faughnan and others studied the effects of age, gender, and food matrix on the urinary kinetics of isoflavones (0.44 mg/kg) in a small human cohort after it had consumed soy foods, textured vegetable protein, and tempeh, a fermented soy food (Faughnan and others 2004). They reported that the food matrix influenced the recovery of genistein in the urine of female participants but not males. Similar pharmacokinetic studies later complemented these observations (Cassidy and others 2006). Greater changes in the area under the curve were observed in the participants consuming isoflavones present in a liquid matrix. Nevertheless, the form in which the isoflavones were present in foods—glucosides and aglycones—was a stronger indicator of bioavailability because the area-under-the-curve values for tempeh, which has more isoflavone aglycones, were not different from soy milk containing mostly isoflavone glucosides. There was no gender effect on the pharmacokinetic outcomes measured. These observations clearly describe the complexity of designing experiments to isolate potential variables (that is, gender and food matrix), which could impact the bioavailability of multiple chemicals.

Allred and others (2004, 2005) found that consumption of soy flour, not soy protein isolate, that contained equivalent amounts of genistein did not promote tumor growth in an athymic mouse model although plasma concentrations of genistein were similar. They concluded that the presence of specific compounds, perhaps carbohydrates, in soy flour may have counteracted the effect of genistein. De Pascual-Teresa and others (2006) used *in vitro* and *in vivo* techniques to determine bioaccesibility and bioavailability of extrinsic isoflavones (approximately 53 mg isoflavones per serving) added to 3 different foods: cookies, fruit juice, and chocolate bars. Isoflavones were present predominantly as glucosides in all foods. Cookies had a higher proportion of aglycones (twice as much as the others) likely due to their exposure to higher baking temperatures (150 °C). After simulated *in vitro* digestion, the recovery of isoflavones was approximately 22% for the cookies compared to more than 90% for the other foods. The researchers explained that these observations were the result of complex factors such as starch and gluten microstructure in the cookie matrix that could have reduced the complete release of isoflavones as has been reported in the past (Sanz and Luyten 2006). These observations were not replicated *in vivo*. Thus, use of *in vitro* simulation techniques to assess bioaccesibility of chemicals has limited application and must be followed and confirmed after thorough *in vivo* assessment.

Other studies have evaluated the effects of the food matrix on the bioactivity of isoflavones. Using a preclinical model of postmenopause, Allred and others (2001a) demonstrated that soy protein isolates containing increasing concentrations of genistein (15 ppm, 150 ppm, and 300 ppm) stimulated the growth of estrogen-dependent breast cancer cells in a dose-dependent manner. Interestingly, the diet containing soy flour did not promote tumor growth or tumor regression even when the amounts of genistein in the serum were similar in all groups. Using a similar model, Power and others (2006) demonstrated that adding flaxseed, a rich source of lignans, to a diet containing soy protein isolate or genistein caused tumor regression similar to that in response to a basal diet without phytoestrogens. Apparently, flaxseed inhibited the tumor-stimulatory effects of genistein or soy protein isolate with genistein but did not inhibit their beneficial effects on bone. It is possible that a combination of soy and lignan-rich foods is a

more feasible way to enjoy soy products without the potential of inducing adverse effects in women with breast cancer.

Since the popularity of soy-based food products and supplements has steadily grown over the past 2 decades, human exposure to phytoestrogens has increased, particularly among women seeking relief of menopausal symptoms. As breast cancer risk increases with age and lifetime exposure to estrogen, genistein may therefore adversely impact human health through stimulation of breast cancer tumor growth in postmenopausal women. Concern exists over the fact that a significant proportion of the population that is at high risk of breast cancer and those with breast cancer or a history of the disease follow some form of complementary and alternative medicine (CAM) therapy that includes high soy and purified isoflavone dietary regimes. Alternative therapies allegedly ameliorate chemotherapy distresses, postmenopausal symptoms, sexual discomfort, or any other age-related symptoms (Eisenberg and others 1998; VandeCreek and others 1999; Boon and others 2007). Very little research has been conducted to determine which diets are appropriate for older women who are at high risk or undergoing breast cancer therapy for have a history of the disease. In the future, these products may be determined to be safe for some subpopulations and harmful for others. As more bioactive food components are identified and provided in enriched forms, this will continue to be a significant safety issue facing regulatory agencies.

Current scientific evidence is insufficient to identify dietary supplements and dietary sources of phytoestrogens as either harmful or beneficial. As scientists have shown, the behavior of phytoestrogens in the life cycle may depend on factors such as timing of exposure; digestion, absorption, and individual metabolism; hormonal status; previous and current health conditions; consumption of prescribed and nonprescribed therapies; amount and profile of phytoestrogens in foods or supplements; other foods consumed and previous processing; and individual genetics (Helferich and others 2008).

Methodological assessment of bioavailability

Bioavailability of functional ingredients can be assessed *in vivo* in humans or animals and *in vitro* using model systems and cultured cells. *In vivo* studies in humans can use pharmacological approaches comparing the area under the concentration curve of an oral dose with an intravenous dose. This approach requires that a pure constituent be used and that it is known that it is safe when administered intravenously. More commonly, dietary bioavailability is assessed by determining the amount of the constituent of interest in the food administered over a time course following intake measuring blood, urine, and fecal levels to assess disposition after a test meal. The study of lycopene by Unlu and others (2007) is an example. Area-under-the-concentration-curve studies in humans are generally considered the gold standard for bioavailability of a potential health promoting chemical because of their use of human research subjects and capability of assessing food forms of an ingredient. Such studies are limited by the fact that only a few human subjects are included, variability between subjects is often great, the chemicals assessed in the blood may not be the most bioactive form, and the studies do not assess the agent at its target.

A wide variety of *in vitro* models include incubations that are designed to recapitulate the conditions in the gastrointestinal tract, such as studies recently reported by Granado-Lorencio and others (2007), in which sequential phases of the digestive tract, such as salivation and duodenal digestion, are mimicked using enzyme incubations. Inverted gut studies are also common. In such studies, a portion of the gut is excised and turned inside out, the agent of interest is injected into the inverted sac, and the transportation of the agent across the intestine is assessed by measuring accumulation in the media in which the inverted sac is incubated. These *in vitro* studies may allow more complete assessment of metabolism in the portion of the intestine involved and uptake across an intestinal membrane, but they are limited because they are designed to mimic only one specific aspect of the absorption process.

A common cell culture model for assessing bioavailability is the Caco-2 model of colon cellular uptake, which has been widely used to assess oral drug uptake (Huebert and others 2004). This model was used by Boyer and others (2004) to assess flavonoid uptake. Although it is an excellent model for screening a large number of compounds, the Caco-2 model is a colon cancer cell that may not mirror human colon cells in all aspects. This model reflects only interactions of the chemical of interest with colonocytes when clearly other cells/tissues in the intestinal tract may be important in bioavailability. Furthermore, cell culture studies do not recognize the complex interaction of epithelial cells with stroma and other cells in the intestine.

Risk communication

In assessing the risks of a newly discovered and undesired substance in food, its interaction with other food components must be considered with care. Evaluated risks must be compared with the benefits of the whole food in which the substance was found. Finally, it is necessary to address how those risks, and any accompanying benefits of the foods in which they occur, should be communicated to the public. Risk communication is the process of making risk assessment and risk management information comprehensible to lawyers, politicians, and judges and business, environmental, and community groups. These groups and individuals often want to know if something is safe, not whether its risks are uncertain and complicated. Some difficulties in risk communication are conveying the key message in an appropriately concise yet trust engendering manner, addressing unrealistic expectations, being perceived as credible, suitably timing communication of the key message, and addressing differences in public perceptions of risks (for example, differences in how voluntary against involuntary risks are perceived) (Slovic 1987; NAS 1989; Sandman 1989). Good risk communication includes recognizing that risk perception is valid, giving the facts, not hiding the ugly truth or biasing the message, admitting the unknown, acknowledging the role of judgment, and comparing risks only if they are comparable and comparing such risks against the benefits of the foods in which they are found to occur. Additionally, risk communication must involve all stakeholders and give positive action steps when possible, must not patronize, and must be timely.

Conclusions

All food companies face new issues necessitating evaluation. The mere presence of a chemical in a food does not mean that the substance necessarily poses a risk to health. A new approach is needed for quickly determining the scope of the issue and the appropriate type of response. It is possible to evaluate new issues that potentially impact food safety by assessing the available toxicity data and information on potential dietary intakes. An approach is needed that will allow food safety managers to use all available data in conducting risk-based hazard evaluation, which would consider the toxicity of low levels of a hazard, which may have always been an inherent, undetected part of the food supply and potential exposure to the hazard.

Experienced scientific and risk management professionals who have a significant level of education and skill in making critical risk-based decisions can use a variety of tools to determine any new issue. A systematic approach that allows the use of

screening will facilitate the process. Screening criteria are appropriate to identify potential substances that are of critical concern and need immediate attention. A TTC evaluation can be an efficient screening and prioritizing tool for the decision-making process, particularly when data are incomplete. Using the TTC may lead to a decision that, for some chemicals, further research and risk mitigation steps are necessary while for others, further research is not necessary and a decision about risk is possible. The concept of specifying human exposure thresholds relies on knowledge of the range of toxicological risks for structurally related substances and on knowledge regarding the toxicological potency of relevant classes of chemicals for which good toxicity data exist. Structure activity can allow an evaluation using information about a similar chemical.

In general, scientific research supports that an exposure of less than 0.5 ppb in the total diet (food and beverage), equivalent to a total dietary exposure of 1.5 μg/d, poses insignificant risk. The TTC provides an acceptably high probability of health protection as well as practicality. A toxicological priority grid based on toxicological potency, thresholds of toxicological concern, and potential consumer exposures illustrates the TTC concept. The grid's 3-category priority rankings provide a rapid and effective screening tool for determining appropriate risk mitigation actions when human exposure and toxicity data are incomplete. The approach also incorporates 2 sets of food consumption data that will allow rapid determination of the range of exposure: (1) a set for substances of concern that are believed to be present in only 1 or a small number of foods, and (2) another set for substances believed to be present in commodity ingredients that are components of a large number of different foods.

Using the TTC approach will assist food safety managers in performing safety and risk assessments of the potential adverse health effects of very low levels of unwanted chemicals in ingredients, commodities, or food products. Scientifically defensible decisions can then be made and senior management and legal counsel advised accordingly. However, all regulatory requirements must be met in all situations. The applicable standard of safety imposed by law must be identified and integrated into the decision-making process.

In reality, a perfect data set on which to base definitive conclusions on chemical concerns in food is nonexistent. There will always have to be limits on how far to pursue different options to manage assessed risks. The more scientific substantiation that can be provided, the better, and where key science gaps occur, collaborative cross-sector investigation is a valuable tool to clarify that issues are being taken seriously.

Risk management options are most effectively developed in consultation with stakeholders, taking account of wider practicalities, implications, and available science while being sensitive to uncertainties. If information is not forthcoming the risk is higher for disproportionate action, particularly for issues of greater public concern.

Sometimes public perceptions may differ or deviate from the available science on uncertain chemical risks. Effective risk communication and education help provide balanced perspectives, particularly when beneficial food qualities may be compromised by overly cautious or nonscience-based risk management pressures. Whether or not there are scientific uncertainties, good production and manufacturing practices should never be compromised and food safety must remain a priority.

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