chapter eighteen

Extrapolation of toxicity data in risk assessment

H.J.G.M. Derks, C. Groen, M. Olling, M.J. Zeilmaker

18.1 Introduction

This chapter studies the role of extrapolation in the risk assessment of chemicals occurring in food. The concept of extrapolation is briefly introduced in Section 18.2. Both inter- and intraspecies (interindividual) extrapolation of data on chemicals showing a threshold in their dose–response relationship, and high-to-low dose extrapolation of chemicals which do not, are discussed. Further, some problems, which are in a certain sense specific to food chemicals, are presented.

Sections 18.3.1 to 18.3.4 investigate interspecies extrapolation and its limitations for four food chemicals from various classes. The relevance of extrapolation of toxicity data from one species to another in everyday life is explained in Section 18.3.5, using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as an example.

18.2 Extrapolation

One of the cornerstones of human toxicology is the assumption that toxic effects of chemicals in humans can be predicted from dose–response relationships established in experimental animals. In view of the countless biological and biochemical similarities between species, this assumption seems to be basically sound. Nevertheless, toxicological studies have proved beyond doubt that large interspecies differences in sensitivity to toxic chemicals do occur. In addition, it has been shown that between individuals of an outbred species, like man, similar differences in sensitivity may exist. Toxicology has responded to this problem with the introduction of safety or uncertainty factors which are applied whenever animal toxicity data have to be translated to safe human exposure levels. This process is referred to as *extrapolation* and is considered applicable to all chemicals exhibiting a threshold in their dose–response relationship. If chronic toxicity data have been collected in an experimental animal species, the human acceptable daily intake (ADI) is calculated by dividing the no-observed-adverse-effect level (NOAEL) in the animal by a standard uncertainty factor of 100 (see Chapter 17, Section 17.3.3). Larger extrapolation factors are used when only subchronic toxicity data are available or when the lowest dose tested in the animal still elicits slight toxic effects and repeating of the experiment with yet lower doses is not considered necessary in view of the type and severity of the observed effects. In both cases, the standard uncertainty factor is multiplied by an additional factor varying from 1 to 10. As mentioned above, the standard uncertainty factor is 100, which implies that in practice this additional factor often equals 1. However, extrapolation factors up to 2000 may be applied.

Genotoxic carcinogenic substances are assumed to exhibit no threshold in their dose–response relationship. Therefore, no absolute safe human exposure level can be defined. An important problem a toxicologist is confronted with in connection with this group of substances is that the dose levels needed to establish the dose–response relationship in experimental animals are many orders of magnitude higher than those likely to be encountered in human exposure situations. Simple linear extrapolation from these high doses to find the dose associated with negligible risk is considered to be safe, but rather conservative. Negligible risk is called the Virtually Safe Dose or Risk Specific Dose and is assumed to cause 1 extra tumor in 10^6 subjects after lifetime exposure. More often, mathematical models based on certain assumptions about the mechanism of carcinogenesis are used to fit the high dose data obtained in animals, and to predict effects at low dose levels. An often-used mathematical model is the multi-stage model which assumes carcinogenesis to be a multi-stage process, and tumor incidence to depend on the probabilities of transition of each stage into the next. The number of stages and the transition probabilities are estimated by curve fitting of the experimental data. Other mathematical models have been introduced which may differ in behavior in the low dose region, while they show no differences at high dose levels.

Usually, no adjustments are made to correct for interspecies differences in sensitivity to carcinogenic substances. However, in some cases dose levels have been normalized to body surface area rather than to body weight. Such a normalization may correct for interspecies differences in the pharmacokinetic behavior of xenobiotics.

For the assessment of the toxicological risks due to food chemicals, the same extrapolation methods are used as for other substances. However, chemicals present in food may behave differently from the same chemicals administered either in a pure form or as a solution. The food matrix may influence the extent and rate of gastro-intestinal absorption by several mechanisms. As a result, both the bioavailability and the maximum blood concentration may be affected. Since the route and method of administration of toxic substances to experimental animals are often chosen on the basis of convenience, the validity of animal data for human risk assessment may be compromised. Further, all food

chemicals obviously enter the target animal via the gastrointestinal route and may therefore be affected by species differences in gastrointestinal physiology. Sometimes such problems can be prevented by careful selection of the experimental animal species. Other food-specific problems may arise from micronutrients with a small safety margin which do not allow the use of large extrapolation factors. Some of the above problems are dealt with in more detail in the next section.

18.3 Extrapolation and assessment of toxicological risks due to food chemicals

This section presents a detailed study on the role of extrapolation in estimating the toxicological risks from food chemicals. Examples are chosen from the four categories discussed in this textbook: vitamin A (nutrient), solanine (natural toxin), nitrate and nitrite (contaminants), and BHA and BHT (additives).

18.3.1 Micronutrients: vitamin A

18.3.1.1 Introduction

Vitamin A is required in small amounts in crucial biological processes such as controlling the differentiation and proliferation of epithelial cells, maintaining general growth and visual and reproductive functions. Therapeutically, vitamin A is used in dermatology for curing various skin diseases, and one of the metabolites of retinol, all-trans retinoic acid, is used topically to treat acne. Vitamin A, as retinyl esters, is also taken in various amounts as a food supplement.

The recommended average daily dietary intake of vitamin A was estimated by Sauberlich et al. (1974) at 600 retinol equivalents (RE) per day for adult men. Olsen (1987) estimated a total amount of 625 RE per day on the basis of metabolic turnover data. Adequate levels of vitamin A intake must be such that the concentration in the liver is maintained at 20 RE per g. From these data the regulatory authorities of the US and Canada recommended the following daily intake of vitamin A: male adults and pregnant women: 1000 RE per day, female adults: 800 RE per day, lactating women 1250 RE per day, and children of 1 to 3 yr 400, 4 to 6 yr 500, and 7 to 9 yr 700 RE per day.

18.3.1.2 Assessment of teratogenic risk

One of the most important toxic effects occurring after chronic and/or acute hypervitaminosis A is teratogenicity in early pregnancy. Rosa et al. (1986) described 18 cases of teratogenic effects in humans caused by hypervitaminosis A. Acute and chronic hypervitaminosis A may be caused by consuming vitamin A as a food supplement, or in liver. The vitamin preparations on the Dutch market, for example, contain from 300 RE to 15,000 RE vitamin A per dosing unit. Livers of calves may contain even more, 25,000 RE per 100 g!

From these data it is clear that women who are on a normal or rich diet with respect to the intake of vitamin A run a high risk by consuming liver or vitamin preparations in early pregnancy. Since only few human data are available, especially on the teratogenic effect, risk assessment is based on data obtained in animal studies. To estimate this risk a good interspecies extrapolation model is needed to extrapolate these data to the human situation.

Table 18.1 lists the lowest teratogenic doses of vitamin A in several species. The data clearly show a large interspecies variability in sensitivity to vitamin A.

The differences in route of administration (oral vs. intraperitoneal) and in *kinetics* of vitamin A may cause interspecies variability. Further, the fact that different effects were measured may play a role. There may also be interspecies differences in morphology of the uterus.

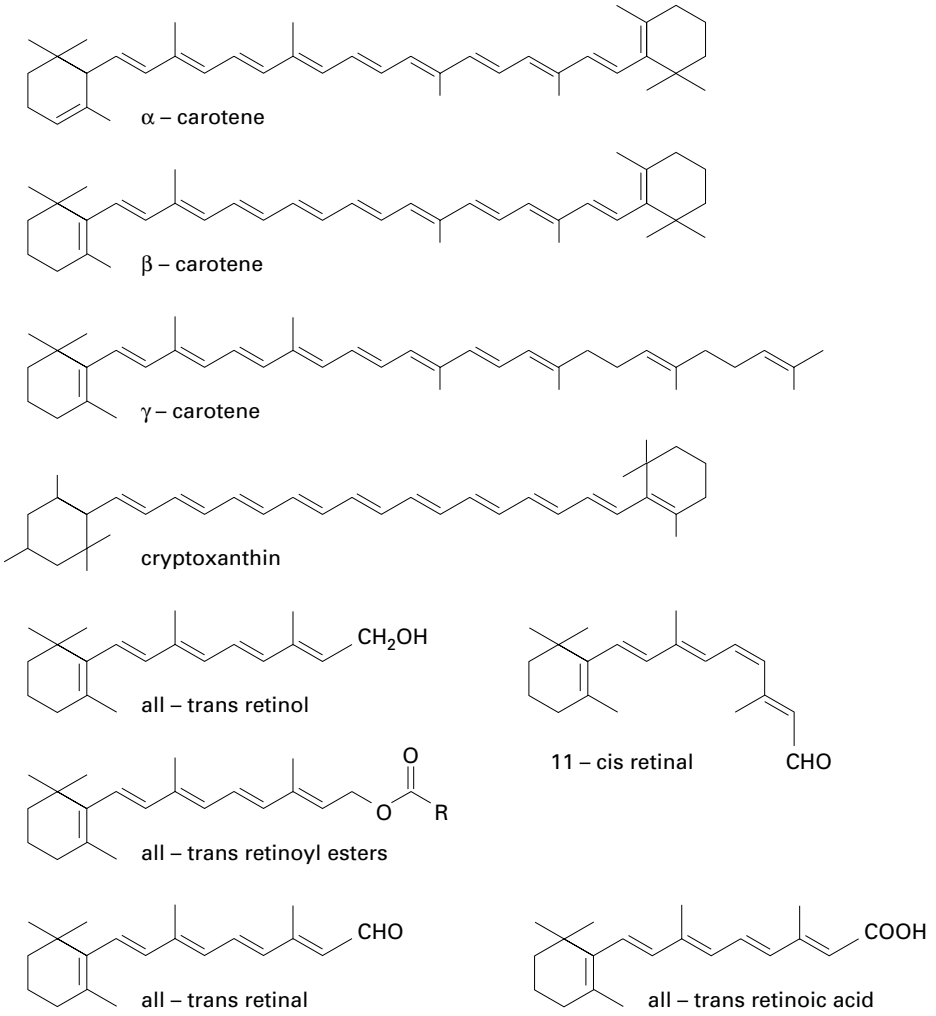
Table 18.1Lowest teratogenic dose of vitamin A in various species after oral (p.o) or intraperitoneal (i.p.) administration

Species (body weight)	Dose (RE/kg/day)	Time after conception (days)	Effect on
Man (60 kg)	120 p.o	14–35	Cranium and face
Mouse (20 g)	3,300 i.p.	9	Cleft palate
Hamster (100 g)	30,000 p.o	8	Exencephalum
Rat (200 g)	50,000 p.o	9–11	Exencephalum

18.3.1.3 Toxicokinetics

As mentioned above, one of the causes of the interspecies' differences shown in Table 18.1 may be found in species' differences in toxicokinetic behavior of vitamin A. Therefore, the toxicokinetics of vitamin A and its precursors are briefly discussed here.

In the lumen of the gastro-intestinal tract, retinyl esters are hydrolyzed and the retinol formed is taken up by the enterocytes by means of passive diffusion. In contrast, carotenoids are taken up as such and converted to retinol in the enterocytes by cleavage.



In the enterocytes, retinol is re-esterified by two specific enzymes and the resulting retinyl esters are incorporated in the chylomicrons, followed by secretion in the lymph and transport to the liver via the thoracic lymph duct and systemic circulation. In the liver 10% of the total amount of the retinyl esters are stored in parenchymal cells and 90% in fat-storing cells. After hydrolysis and binding to specific proteins, retinol and retinoic acid are secreted into the blood and distributed to other organs. If the recommended amount of vitamin A is consumed, the amount in the liver remains constant and the blood concentrations of retinol and retinoic acid remain low. After intake of excessive amounts most processes, such as uptake, esterification, hydrolysis, and binding to proteins may become saturated, leading to an increase in the free retinol concentration and induction of toxic effects.

For the development of an extrapolation model to assess the teratological risk from vitamin A, the following toxicokinetic aspects must be examined in more detail in at least two species: linearity of absorption, bioavailability of retinol after administration of carotenoids or retinyl esters, and capacity of the liver to store retinol and to synthesize the relevant binding proteins. Also, concentration and form of vitamin A that the embryo is exposed to in the case of acute or chronic hypervitaminosis A are of high importance. If these toxicokinetic aspects of vitamin A are elucidated and can be related to physiological and biochemical characteristics, such as lymph flow, blood flow, and enzyme activities of the animals used, extrapolation to humans and an estimate of the risk can be achieved.

18.3.2 Natural toxins: solanine

18.3.2.1 Introduction

Solanine is just one of the countless substances of natural origin that may cause adverse effects in humans. The large number of natural substances known to possess potential toxicity probably only represents a small percentage of those that actually exist. This situation may be attributed to the fact that the available quantities of the substances are too small to use in toxicological experiments, and also because suitable analytical methods are not always available. This section shows the role of extrapolation in the assessment of human health risks due to solanine, and its toxicological evaluation.

18.3.2.2 Toxicological risk assessment

Symptoms of toxicity were recorded during an outbreak of potato poisoning among school children in South-East London in 1969. The peeled potatoes that were consumed contained 330 mg of glycoalkaloids per kg. In other cases, 410 mg and 430 mg of glycoalkaloids per kg potatoes have been reported to cause outbreaks of potato poisoning. From these casuistic data, a lowest-observed-adverse-effect level (LOAEL) (see Section 21.4.4.3) of about 2 mg/kg body weight was calculated. Generally, 200 mg of glycoalkaloids per kg potatoes is accepted as the upper safety limit. This value is based on an average daily intake of 300 g of potatoes by an adult, and includes a safety factor of 2. In a number of countries, this limit has been reduced to 100 mg/kg potatoes, as the safety factor 2 was considered to be inappropriate. Moreover, as compared to the assessment of risks from synthetic chemicals, there is a lack of data concerning long-term repeated intake of relatively small amounts of solanum alkaloids. There are indications that solanine and related substances can accumulate in tissues. This may lead to late toxic effects. Therefore, there is a need for at least semichronic toxicity studies. Summarizing, a more systematic approach is desired to come to a better estimation of the ADI of solanine and other natural toxins.

Table 18.2 Toxicokinetics of solanine in the rat and the hamster after intravenous as well as oral administration

Species	Dose (10^5 dpm[a])		$AUC_{0-\infty}$[b] ($\cdot 10^3$ dpm[a]$\langle$h/ml)		Cl_m[c](ml/h/kg)	F[d](%)
	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
Rat	421 ± 6[e]	1240 ± 170[e]	1390 ± 230[e]	71 ± 33[e]	107 ± 17[e]	1.6
Hamster	364 ± 61	723 ± 8	3250 ± 310	239 ± 91	63 ± 14[f]	3.2

[a] dpm, disintegrations per minute; the solanine was radiolabeled.

[b] $AUC_{0-\infty}$, area under the plasma concentration vs. time curve from time zero to infinity.

[c] Cl_m, metabolic clearance of solanine.

[d] F, mean absolute bioavailability.

[e] Dose, $AUC_{0-\infty}$ and Cl_m are given as mean ±S.D.

[f] $p < 0.05$, compared to iv administration in rats.

18.3.2.3 Toxicokinetics

In the extrapolation of toxicity data from animal to man, interspecies differences in *bioavailability* are a factor to which special attention should be paid. This implies that in the case of solanine, blood levels rather than doses, should be used as a basis for extrapolation.

For most substances, there is a direct relationship between the blood concentration and the concentration at the site of action on the one hand, and between the concentration at the site of action and the intensity of the effect on the other. However, if the dose is used as a basis for extrapolation, the absorption from the site of administration into the general circulation, i.e., the bioavailability, is not accounted for. Lack of information on interspecies differences in bioavailability is an extra source of uncertainty in extrapolation.

Studies on the toxicity of glycoalkaloids have been carried out in different animal species. Severe gastric and intestinal mucosal necrosis was observed in hamsters receiving dried potato sprout material containing high concentrations of glycoalkaloids. Hamsters seem to be more sensitive to glycoalkaloids than rats and mice. However, little information is available on the underlying toxicokinetics.

Recent experiments suggest that the higher systemic toxicity in hamsters (and thus maybe also in man) is due to a higher bioavailability after oral administration. The difference in bioavailability of solanine between rats and hamsters is shown in Table 18.2.

It should always be kept in mind that not only the parent compound but also its metabolites can be toxic. For example, solanine is metabolized via different routes. Its metabolites are not toxic. However, for many other substances, it has been reported that the metabolites induce effects that are different from or stronger than those of the parent compound. In those cases, determination of the bioavailability of the parent compound solves only part of the problem.

Based on the difference in toxicokinetic behavior of solanine between rats and hamsters, and since after oral administration more disorders in the intestinal tract were observed in the hamster than in the rat, the hamster was chosen as a model for subchronic toxicity studies on this glycoalkaloid. The effects on toxicokinetics of factors such as dose level and food matrix have to be elucidated to enable a reliable estimation of the exposure to solanine. Matrix factors deserve special attention, since the public health authorities want to know whether additional requirements should be made for potato products, like starch, present in various types of diets.

18.3.3 Food contaminants: nitrite and nitrate

18.3.3.1 Introduction

As a naturally occurring substance, nitrate (NO_3^-) is a common constituent of the environmental compartments soil and water. From the soil compartment, NO_3^- may be taken up

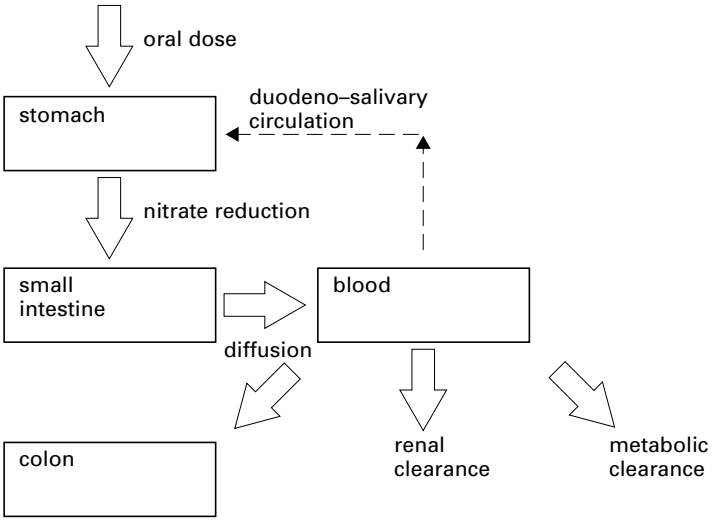


Figure 18.1 Disposal of nitrate following oral administration, including its duodeno-salivary circulation.

in drinking water. Since nitrate is the primary nitrogen source for plants, it enters the mammalian food chain by its ability to accumulate in plant materials. Consequently, the intake of food, especially leafy vegetables and drinking water, is the main route of exposure of humans to NO_3^-. The average intake of NO_3^- via food consumption is estimated at 100 to 150 mg/day and from water at 10 to 20 mg/day. This accounts for more than 99% of the total daily NO_3^- intake.

Nitrite (NO_2^-) is not a natural food component. It is used as a preservative in a number of meat products. In comparison with the daily intake of NO_3^-, the intake of NO_2^- by humans via food consumption is low, i.e., < 0.1 mg/day.

18.3.3.2 Toxicological risk assessment

In order to evaluate the toxicological significance of human exposure to NO_3^- and NO_2^-, the actual intakes of these substances need to be compared with estimated safe exposure levels. Based on a body weight of 70 kg, the actual intake of NO_2^- is <0.001 mg/kg/day, and of NO_3^- 1.4 to 2.5 mg/kg/day. Traditionally, safe exposure levels of humans to chemicals are obtained by extrapolating data from toxicity studies in experimental animals. In rats, the no-observed-adverse-effect level (NOAEL) of sodium nitrate was found to be 500 mg/kg/day and of sodium nitrite 20 mg/kg/day. Application of a safety factor of 100 to these values and correction for the differences in molecular mass between the sodium salts and the ions result in Acceptable Daily Intakes (ADIs) of 0 to 3.64 and 0 to 0.135 mg/kg/day, respectively. The actual intakes of NO_3^- and NO_2^- by the general population amount to 38 to 69% and <0.7% of the ADI, respectively. From this, it can be concluded that on average the actual exposure of humans to NO_3^- and NO_2^- via food and drinking water does not pose toxicological risks. However, mechanistic studies in experimental animals and man have shown that the traditional method of extrapolating data on the toxicities of NO_3^- and NO_2^- from animals to man is inadequate and deserves reconsideration. In order to show the inadequacy of the currently used extrapolation method, the fate of NO_3^- and NO_2^- in man should first be dealt with in more detail.

18.3.3.3 Toxicokinetics of NO_3^- and NO_2^- in man

Following oral administration, NO_3^- is almost completely absorbed (>98%). It is eliminated from the body in three ways (see Figure 18.1). First, NO_3^- is excreted via the kidneys. This

accounts for approximately 60 to 70% of the total nitrate body clearance. Secondly, NO_3^- is metabolized to ammonium, urea and more reduced forms of nitrogen such as nitrous oxide (NO) and NO_2^-. This elimination pathway accounts for approximately 20 to 30% of the total body clearance of nitrate. Thirdly, NO_3^- may be excreted in the sweat which accounts for almost 11% of the total body clearance.

Once absorbed from the gastrointestinal tract, NO_3^- may circulate in the body by entering the so-called duodeno-salivary circulation. This circulation consists of the excretion of NO_3^- by an active transport mechanism from the blood into the saliva, followed by reabsorption of the excreted NO_3^-. Approximately 25% of the orally administered nitrate enters the duodeno-salivary circulation. Once NO_3^- has been excreted into the saliva, it may be reduced to NO_2^- by bacteria present in the oral cavity. In man an estimated 30% of NO_3^- is converted in this way. So, 8% of the orally administered NO_3^- may be converted to NO_2^- by bacterial reduction in the oral cavity. By combining these data with the estimated daily intake of NO_3^- (see Section 18.3.3.1) the daily intake of NO_2^- formed from ingested NO_3^- can be estimated at 7 to 10 mg/day, i.e., 0.09 to 0.14 mg/kg/day which is 0.69 to 1.07 times the current ADI for nitrite! This clearly shows that the setting of safe standards for nitrate and nitrite in food should not be based on the determination of standards for each individual substance but on an integration of knowledge of the disposition of both substances. Ideally, the standard setting should meet the following criteria:

1. standards for dietary nitrate should be based on *expected* derived nitrite toxicity;
2. the accepted intensity of nitrite-induced toxicity in man is equal to the NOAEL of nitrite in experimental animals divided by the product of inter- and intraspecies extrapolation factors. Currently, inter- and intraspecies factors of 10 are used in extrapolating data on nitrite toxicity from experimental animals to man.

If the above criteria are applied to the animal model most widely used in experimental toxicology, the rat, one is faced with the basic problem that the rat probably is not an adequate model for man with regard to nitrite-induced nitrate toxicity. The reason for this is that in literature it has been suggested that the duodeno-salivary circulation of nitrate does not exist in the rat. Hence, in this species nitrite-induced nitrate toxicity cannot be studied adequately. One way to solve this problem is to study nitrate toxicity in an animal species that does have a duodeno-salivary circulation, such as the pig. Alternatively, data obtained in the rat may be used for estimating safe human exposure levels for NO_3^-, i.e., the ADI. The procedure for obtaining the ADI for nitrate is then as follows.

First, a NOAEL for nitrite toxicity is determined in the rat. Dividing this parameter by an extrapolation factor of 100 then gives the ADI for nitrite. A total safe nitrite intake can be calculated by multiplying this value by body weight (BW), say 70 kg. If the direct dietary nitrite intake is negligible, the actual human intake of nitrite is solely determined by the conversion of NO_3^- to NO_2^- by bacteria in the oral cavity. The actual nitrite intake can then be calculated as follows :

$$\text{total safe nitrite intake} = \text{ADI}_{NO_2^-} \times \text{BW} \tag{1}$$

$$\text{total safe nitrate intake} = \text{ADI}_{NO_3^-} \times \text{BW} \tag{2}$$

$$\text{actual nitrite intake} = (\text{total nitrate intake} \times \alpha)/1.35 \tag{3}$$

in which α equals the fraction of NO_3^- converted to NO_2^- by bacterial reduction in the oral cavity and 1.35 (= 62/46) is a multiplication factor for the difference in molecular weight between NO_3^- and NO_2^-. Combination of these equations gives the ADI for nitrate:

$$ADI_{NO_3^-} = \left(ADI_{NO_2^-} \times 1.35 \right) / \alpha \quad (4)$$

With the current $ADI_{NO_2^-}$ of 0.135 mg/kg/day and $\alpha = 0.08$, this would give an $ADI_{NO_3^-}$ of 2.28 mg/kg/day. In comparison with the currently used ADI for nitrate, i.e., 3.64 mg/kg/day, this means a decrease by more than 37%.

Once the ADI for a food contaminant has been calculated, its value may be used to set the dietary standard for that particular food component. This procedure can be easily illustrated by the calculation of safe drinking water levels for NO_3^-. For example, if drinking water accounts for 10% of the daily nitrate intake and the safe nitrate intake is set at a value found by multiplying $ADI_{NO_3^-}$ by BW, this nitrate intake can be estimated at approximately 250 mg/day (70 kg × 3.64 mg/kg/day). Assuming a daily water consumption of 1 l, drinking water is then allowed to contain 0.1×250 per l = 25 mg NO_3^- per l.

This procedure, valid for the *general* population, does not necessarily hold for high-risk groups, i.e., groups with expected high exposure levels and/or increased sensitivity. In the case of nitrate and nitrite, infants are such a group. In infants, the major toxic effect of nitrate and nitrite is nitrite-induced methemoglobinemia. Nitrite entering the blood circulation oxidizes hemoglobin (Fe^{2+}) to methemoglobin (Fe^{3+}), leading to reduced oxygen transport. Neonates are a high-risk group as they are methemoglobin reductase deficient. For the induction of methemoglobinemia, a NOAEL of 100 mg $NaNO_2$ per kg/day has been established in experimental animals. When combining the drinking water consumption of infants with the fractional conversion of NO_3^- to NO_2^- in the gastrointestinal tract and the infant's body weight, the safe NO_3^- concentration of drinking water used for the preparation of infant food can be calculated.

The safe concentration is 30 mg/l which is arrived at as follows. The ADI for sodium nitrite-induced methemoglobinemia can be calculated from the above NOAEL to be 1 mg/kg/day, i.e., for nitrite 0.67 mg/kg/day. If the conversion ofNO_3^- to NO_2^- is taken into account and corrections are made for the difference in molecular mass between nitrate and nitrite, an $ADI_{NO_3^-}$ of 4.52 mg/kg/day can be calculated for infants [$(0.67 \times 1.35)/0.20$] (see Equation 4). In combination with a body weight of 5 kg and a daily water consumption of 0.75 l, this results in a safe drinking water consumption of approximately 30 mg/l. If this concentration is compared to the calculated safe NO_3^- concentration in drinking water for the general population (25 mg/l, see above), it can be concluded that the generally supplied drinking water may be used safely for the preparation of infant food.

18.3.4 Food additives: the antioxidants butylated hydroxyanisole and butylated hydroxytoluene

18.3.4.1 Introduction

To preserve quality and to prevent loss of nutritional value, the addition of antioxidants to food containing fatty acids has a long tradition. Two well-known antioxidant food additives are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (see Figure 18.2).

Although highly lipophilic, BHA and BHT do not accumulate in mammals. The reason for this is the efficient elimination of these chemicals from the body. In the case of BHA and BHT, the discussion on setting the dietary standards has focused on the question whether or not these food additives have to be considered as non-genotoxic carcinogens, and consequently, on whether or not safe human exposure levels for these substances can be established.

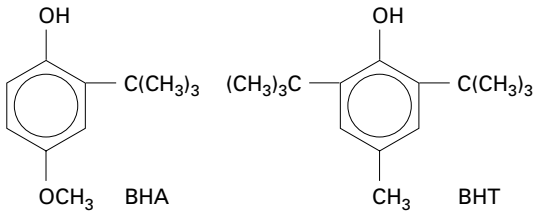


Figure 18.2 Structures of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

18.3.4.2 Toxicological risk assessment of BHA

In the rat, BHA induces epithelial hyperplasia and tumors in the forestomach. Since the forestomach is an organ specific to rodents (rat, mice, hamster) and not found in other animals, the question arises whether this effect can be used as the starting point for setting a dietary standard in man. To answer this question the Scientific Committee on Food of the European Commission Food-Science and Techniques asked in its 1983 evaluation of BHA for additional information on the following subjects:

1. the induction of hyperplasia by BHA in the part of the gastrointestinal tract immediately preceding the stomach, i.e., the esophagus, and the glandular stomach in species without a forestomach, and
2. the genotoxic properties of BHA.

On the basis of additional information from experimental studies, the Committee concluded in its reevaluation of BHA in 1989 that the effect of BHA on the forestomach epithelium is highly specific to rodents and does not occur in non-rodents. Furthermore, epithelial hyperplasia, qualified as a precancerous lesion, was found to be of a reversible nature and showed threshold characteristics, i.e., hyperplasia only occurred above a definite dietary BHA dose level. In species without a forestomach (guinea pig, dog, pig, monkey), BHA did not cause histopathological symptoms in the esophagus and the glandular stomach. All available mutagenicity data are negative, and BHA does not show any genotoxicity at all. Based on these data the Committee concluded that the induction of forestomach hyperplasia and tumors by BHA in rodents is of no significance in the assessment of human health risks from BHA exposure. Further, it was concluded that genotoxicity does not play a role in causing rodent forestomach tumors. Therefore, the Committee classified BHA as a rodent (and not human) carcinogen showing a threshold in the induction of effects. Consequently, the Committee accepted the calculation of an ADI for BHA to be relevant. In order to calculate this ADI, the NOAEL for the induction of hyperplasia in the rat forestomach was used as toxicity parameter for BHA. Experimentally, this NOAEL was found to be 50 mg/kg/day. Applying a standard safety factor of 100, this lead to an ADI of 0 to 0.5 mg BHA/kg/day for the safe chronic exposure level of the human population.

18.3.4.3 Toxicological risk assessment of BHT

As in the case of BHA, dietary standards for BHT were set at an expert meeting of the Scientific Committee on Food of the European Union. In its 1989 meeting, this Committee evaluated all available toxicity data on BHT. The toxicity profile of BHT was summarized as follows. In chronic toxicity studies, BHT induced liver carcinomas and adenomas in the rat at dose levels higher than 100 mg/kg/day. However, BHT was not found to be mutagenic or otherwise genotoxic. Therefore, the Committee considered BHT as a non-genotoxic carcinogen with a threshold in the induction of its carcinogenicity. In

semi-chronic toxicity studies, BHT caused an increase in thyroid weight. In this type of study the lowest dose tested, 500 ppm BHT in the diet, still induced a significant increase in thyroid weight. However, the Committee concluded that "It is reasonable to assume that the likely NOAEL for thyroid weight change will be about 5 times lower than the lowest-observed-adverse effect level, i.e., 500 ppm." In subacute toxicity studies, BHT was found to interfere with blood clotting. The underlying mechanism is a reduction of the activity of vitamin K-dependent blood clotting factors. In the rat, the NOAEL for this effect was found to be 5 mg/kg/day. Taking all toxic effects into consideration, the Committee classified BHT as a non-genotoxic carcinogen in rodents. Likewise, the Committee recommended the determination of an ADI as a safe exposure measure for the human population. Since the NOAEL for the chronic toxicity (neoplasia in the liver) was about 50 times higher than the NOAEL for semi-chronic (increased thyroid weight) and subacute toxicity (hematological disorders), the latter parameter (5 mg/kg/day) was used for the calculation of the ADI of BHT. Applying a standard safety factor of 100, the Committee recommended an ADI of 0 to 0.05 mg/kg/day for BHT.

18.3.5 Extrapolation and standard setting for substances occurring in food

The choice of methods to extrapolate toxicological data from animals to man largely depends on the mechanism underlying the toxicity of the substance under investigation. Traditionally, the extrapolation of toxicity data of substances which give positive results in chronic carcinogenicity studies as well as in genotoxicity studies is carried out by using methods based on the assumption that there is no threshold dose (see Section 18.2). Toxicity data of non-carcinogenic substances are extrapolated by using methods assuming a threshold value mechanism (see also Section 18.2). Although the latter method offers a rather clear-cut possibility to extrapolate toxicity data from one species to another, its application in everyday safety evaluation procedures is often more ambiguous. This will be explained for the food contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

TCDD is the dioxin with the highest toxicity. Dioxins are emitted into the environment by waste incineration and other combustion processes. They may enter the food chain. Chronic toxicity studies in experimental animals showed that TCDD is a liver carcinogen in the female but not in the male rat. Further studies revealed that TCDD was not capable of inducing genotoxic effects in *in vitro*-genotoxicity assays and that its carcinogenicity is probably associated with an altered function of female steroid hormones. These findings were used as a starting point for the evaluation of the toxicological risk from TCDD. In practice, however, different authorities took diverging scientific standpoints for the extrapolation of TCDD toxicity data to man. As a result, quite different estimates of the toxic potential of TCDD were reached. In the Netherlands, for example, an expert panel was of the opinion that the experimental data on the toxicity of TCDD provided sufficient evidence to classify this substance as a non-genotoxic carcinogen in experimental animals. The panel concluded that in the case of TCDD safe exposure levels, i.e., an ADI could be calculated in a valid way. For the calculation of the ADI, liver carcinogenicity (in rats) was chosen as the critical toxic effect. For this effect, a marginal-observed-adverse-effect level (MOAEL) of 1 ng/kg/day was established in a chronic experiment in rats. The MOAEL is the lowest found concentration of a substance which causes a marginal adverse effect. The MOAEL is between the NOAEL and the LOAEL. From this effect level a NOAEL was calculated by applying an extrapolation factor of 2.5. The panel considered this value for the MOAEL–NOAEL extrapolation factor adequate in view of the type of effect observed at the MOAEL. Application of inter- and intraspecies extrapolation factors of 10 then gave an ADI of 4 pg/kg/day. In contrast to the Dutch Health Authorities, the US Environmental Protection Agency (US EPA) concluded that the available information on the toxicity of

TCDD did not give conclusive evidence with regard to its carcinogenicity mechanism. The US EPA decided to consider TCDD as a genotoxic carcinogen and to base its safety evaluation on acceptable rather than safe exposure levels. To calculate the acceptable exposure level 1 extra liver tumor incidence per 10^{-6} after lifelong exposure to TCDD was taken as an acceptable risk level. The calculation of the exposure level was based on a quantitative dose–response relationship between the daily TCDD intake and liver tumor incidence; the relationship was assessed by using a multi-stage carcinogenesis model. This relationship was then used for the calculation of the risk specific dose (RSD, see Section 18.2). This calculation resulted in an acceptable exposure level of 6.4 fg/kg/day.

Whether or not TCDD is considered a genotoxic or a non-genotoxic carcinogen, the extrapolations mentioned above were based on the so-called *external dose concept*. This means that the toxic potential of a substance is proportional to the amount of the substance to which an organism is exposed. The exposure levels were expressed in terms of units of weight of the substance per kg body weight. The external dose concept has been used for the interspecies extrapolation of TCDD toxicity data up to 1991. In 1991, however, this concept was abolished. In that year a World Health Organization Expert Committee decided to use the actual concentration of TCDD in its target organ, i.e., the liver, rather than the ingested amount for the calculation of the safe human exposure levels to TCDD *(internal dose concept)*. The reason to replace the external dose by the internal dose lies in the widely accepted view that the toxicity of a substance is best characterized by the following two factors: the disposition of the substance in the organism (toxicokinetics) and the mechanism underlying its toxicity. In order to assess the disposition of TCDD in mammals as a function of the dose, the Committee used a one-compartment model. Toxicokinetic analyses showed that, at equal exposure levels, TCDD concentrations are expected to be 10-fold higher in the human liver than in rat liver. On the basis of a NOAEL of 1 ng/kg/day for TCDD carcinogenicity in the rat, this analysis predicted a NOAEL of 100 pg/kg/day in man. By dividing this (estimated) NOAEL by a safety factor of 10 (for intraspecies variation) the ADI of TCDD was obtained, i.e., 10 pg/kg/day.

The kinetic extrapolation method used by the WHO Expert Committee is an example of classic toxicokinetic modeling. A limitation of this type of modeling is its inability to give a physiological interpretation of the various compartments forming part of the model. The classic toxicokinetic modeling does not allow organ-specific toxicokinetic and toxicodynamic processes to be taken into account in safety evaluation procedures. To obviate this limitation, alternative kinetic approaches have been developed in the last decade. These so-called physiologically based pharmacokinetic (PBPK) models describe the disposition (absorption, distribution, metabolism, and excretion) of substances in the organism on the basis of blood flows through the organs instead of distribution over compartments. Figure 18.3 gives a diagrammatic representation of a PBPK-model of TCDD disposition in the rat.

A system of five blood flows is shown: blood circulation, and four flows through the liver, fat tissue, slowly perfused organ system (SPO, mainly skin and muscle) and richly perfused organ system (RPO, mainly kidneys, lungs and spleen). After a physiological flow diagram as shown in Figure 18.3 has been defined, absorption and elimination of the substance concerned are included in the model. For TCDD, this refers to absorption, elimination by hepatic metabolism, and biliary excretion of the metabolites formed. The model also includes a toxicodynamic parameter, viz. the induction of hepatic P-450 mixed-function oxidase (MFO), a well-known effect of TCDD and structurally related chlorinated aromatic hydrocarbons. The mechanism underlying this induction has been found to consist of a sequence of events: uptake of TCDD by the liver, binding of TCDD to a cytosolic receptor protein (the aryl hydrocarbon or Ah receptor), and stimulation of the *de novo* P-450 MFO synthesis. The determination of the exposure level of the liver to TCDD

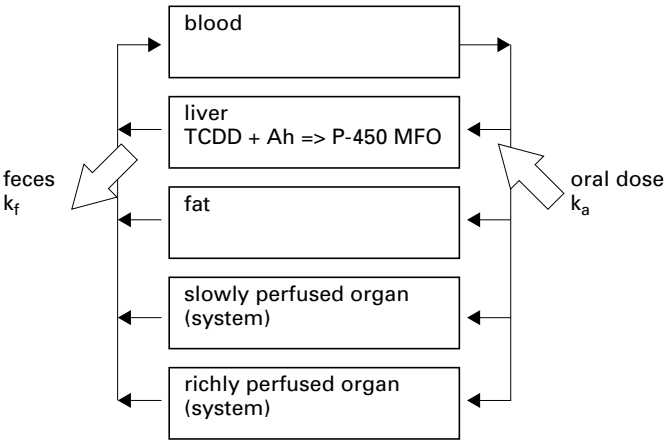


Figure 18.3 Flow diagram for a PBPK model of TCDD disposition in the rat.

is based on this mechanism. The PBPK model has been used to analyze the disposition of TCDD in experimental animals (rat, mouse) and man. The results showed that the disposition of TCDD in these species could be described by *one* PBPK model, irrespective of the dose level (high to low dose extrapolation), the route of administration (route to route extrapolation) and the dosage schedule (acute, semi chronic or chronic exposure conditions). Further, these analyses showed that TCDD-induced *de novo* synthesis of P-450 MFO was the primary factor determining the disposition of TCDD (and thus its toxicological risk) in rodent liver but not in human liver. This underlines the importance of taking interspecies differences in toxicity into account in toxicological safety evaluation. In contrast to classical toxicokinetic modeling, PBPK models can predict the disposition and toxicity of substances in mammals on the basis of a common physiological approach of the organism. PBPK models enable the incorporation of detailed knowledge of toxicity mechanisms as well as variations in the physiological state (growth, pregnancy, sex, disease, age) into toxicological safety evaluation. These possibilities make PBPK models suitable for quantitative and physiologically valid interspecies extrapolation of toxicity data. In this connection, PBPK models are continuously the subject of extensive scientific research.

18.3.6 Concluding remarks

The preceding sections have shown that in principle, extrapolation of data on the toxicity of food chemicals is carried out in the same way as that of chemicals in general. Specific problems may be related to particular subcategories such as micronutrients and using inappropriate methods for the administration of toxic substances to experimental animals. In general, the application of uncertainty factors to establish safe human exposure levels may provide a sufficiently large safety margin to compensate for inter- and intraspecies differences, if in the choice of animal models for toxicity studies, mechanistic aspects are taken into account. In exposure situations above the established safe level, however, the quantitative basis of this methodology is insufficient to allow reliable risk evaluations. Knowledge about the toxicokinetics of a substance in both experimental animal and man reduces the uncertainty in the extrapolation step by enabling the calculation of the quantitative relationships between external and internal dose levels. A more fundamental approach would be the incorporation of toxicokinetic as well as toxicodynamic differences in the extrapolation step. This approach, which is the objective of advanced modeling techniques, may ultimately lead to more adequate quantitative extrapolation methods.

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