Risk assessment of chemicals in European traditional foods

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The principles of risk assessment of chemicals in food are briefly presented and summaries of assessments of representative chemicals found in the European traditional foods are given. In addition, new developments in various areas of the risk assessment are presented, such as subdivision of the safety factor, susceptibility of infants and children to chemicals, risk assessment of compounds that are both genotoxic and carcinogenic, the concept of threshold of toxicological concern (TTC) now being used in the assessment of flavouring substances, and some remarks on excursions above the acceptable daily intake (ADI).

Introduction

Risk assessments of chemicals in traditional foods have predominantly focused on the potential adverse health effects arising from the presence of single components or groups of closely related chemicals in the foods. Today ‘traditional foods’ are no longer entirely produced in private homes or by small family enterprises. Raw materials for cooking, such as meat, cereals, vegetables, and fruits are now produced on large, industrial farms using chemical production aids, such as veterinary drugs for farm animals and pesticides for crops. At the same time more pre-prepared and refined traditional foods and local specialities are offered by industry. The industrialisation has necessitated the use of a number of chemicals in the food production, e.g. in order to preserve the food or retain colours and flavours during transport and storage, or to prepare ready-to-eat traditional foods.

In the 1950–1960s, the awareness of the potential impact of chemicals in food on human health was primarily centred on compounds deliberately used in the production, such as food additives and pesticides. However, with the emerging skills of analytical chemists to detect more and more compounds at lower and lower levels in food, it has become obvious that a number of unwanted contaminants could also be present in food. These contaminants may originate from environmental pollution or be formed during the production or cooking of food. In addition, a number of naturally occurring toxic substances are found in food, particularly in plant foods.

Now is there an international agreement within the World Trade Organization (WTO) on the principles used in the safety evaluation of food additives and other chemicals in commercial use in the food production. These principles were agreed upon at the Uruguay Round and are laid down in ‘Agreement on the Application of Sanitary and Phytosanitary Measures’ (the SPS Agreement). This agreement requires health and safety measures to be based on sound scientific risk assessment and WTO recognises FAO/WHO Codex Alimentarius Commission (CAC or ‘Codex’) standards as a reference point for the safety of foodstuffs traded internationally (WTO, 1994). These standards are for instance established in the Codex Committee on Food Additives and Contaminants (CCFAC), which uses the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as an advisory committee with regard to the safety evaluation of food additives and contaminants. In the EU until 2003, the European Commission’s Scientific Committee for Food (SCF) performed safety evaluations of food additives and contaminants. This task has now been taken over by the European Food Safety Authority (EFSA).

In the following the principles of risk assessments of chemicals in food are briefly presented and summaries of assessments of several representatives of the above-mentioned chemicals in the European traditional foods are given. In addition, new developments in some areas of the risk assessment are presented, such as subdivision of the safety factor, susceptibility of infants and children to chemicals, risk assessment of compounds that are both genotoxic and carcinogenic, the concept of threshold of toxicological concern (TTC) being used in the assessment of...
flavouring substances, and some remarks on excursions above the acceptable daily intake (ADI).

**The risk assessment as part of the risk analysis**

The risk assessment is a part of the risk analysis concept, which, as described by Codex, includes risk assessment, risk evaluation, and risk communication. These three elements are separate tasks, performed by different players, but should be part of an interactive process.

The risk assessment of chemicals in food is a purely scientific process that requires expertise in toxicology and nutrition (for the intake assessment). It contains the following steps:

In the *hazard evaluation* the adverse effects of the chemical is described. Human data are seldom available from epidemiological studies and the risk assessor has to rely on results from animal experiments and *in vitro* studies. For the compounds that are deliberately used in the food production a large number of studies are required for their regulation. These include studies on absorption, distribution, metabolism and excretion (toxicokinetics), acute, short-term (3 months in the rat), and long-term, chronic (2 years in the rat) toxicity, and short-term and *in vitro* tests for mutagenicity, clastogenicity, and genotoxicity. Studies on carcinogenicity, often combined with the long-term studies, as well as studies on reproductive and developmental toxicity are also required. In addition, special *in vitro* and *in vivo* studies on effects on the nervous system, the immune system, and endocrine systems, as well as mechanisms of action are often also available.

The *hazard characterisation* describes and evaluates dose–response relations for the most sensitive adverse health effects reported in the available studies. In cases where the compound exerts toxicity by a mechanism that has a threshold the hazard characterisation often results in the establishment of an acceptable daily intake (ADI) or tolerable daily intake (TDI), see later. This applies also to carcinogenic compounds that act via a non-genotoxic or an indirect genotoxic mechanism. However, when a compound is a directly acting genotoxic and carcinogenic substance a threshold for the effect cannot be easily defined *per se*, and an ADI or TDI will not be established and the evaluation may be carried out using either a risk extrapolation or a margin of exposure approach, see later.

The third step is the *exposure assessment*. Here, the intake of the compound from food is estimated at the time of consumption. The estimates should embrace both average, medium, and maximum intake figures from regular food, special foods, and all foods (regular and special foods) and should concern the whole population, segments of the population, and individuals.

Finally, the *risk characterisation* combines the hazard characterisation and the exposure assessment and evaluates the qualitative or quantitative probability for a health risk in a given population as well as the seriousness of any health risk.

The risk management includes an identification of the food safety problem and an evaluation of its magnitude and seriousness, and consequently how to handle it. In the evaluation, the risk manager may include cost–benefit considerations before deciding how to manage the case (ban the compound, introduce limitations, or accept status quo). Finally, it must be controlled whether the implemented approach had the desired effect.

The risk analysis should also include a clear and interactive risk communication with consumers, industry, and other stakeholders.

**Risk assessment of compounds used deliberately in the production of food**

The use of food additives, pesticides, and veterinary drugs is regulated on the basis of the acceptable daily intake (ADI). The regulation should ensure that the amounts permitted in various foods of a given compound would not result in the consumer having a daily intake higher than the ADI. The ADI concept was originally developed in the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and defined as ‘an estimate of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk’ (IPCS, 1987). The ADI is derived from the above-mentioned studies in humans, experimental animals and *in vitro*. Compound that shows both genotoxicity and carcinogenicity will not be accepted for deliberate use in food and the evaluation of for instance a food additive will normally be related to toxicity induced by a mechanism for which there is a threshold. The ‘no observed adverse effect level’ (NOAEL) is then determined from the most sensitive study in the most sensitive species tested. The ADI is established from the NOAEL by dividing it with a safety factor of 100.

<table>
<thead>
<tr>
<th>Table 1. Derivation of the acceptable daily intake (ADI)</th>
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</thead>
<tbody>
<tr>
<td>Pivotal toxicological study</td>
</tr>
<tr>
<td>Critical effect in the pivotal study</td>
</tr>
<tr>
<td>No observed adverse effect level NOAEL (mg/kg bodyweight per day)</td>
</tr>
<tr>
<td>Default safety factor of 100</td>
</tr>
<tr>
<td>ADI (mg/kg bodyweight per day)</td>
</tr>
</tbody>
</table>
Risk assessments of contaminants in food

Contamination of food with toxic chemicals may originate from the environmental pollution with chemicals released from or formed during a wide number of industrial and anthropogenic activities. Examples of well-known contaminants that enter food due to widespread environmental pollution are polycyclic aromatic hydrocarbons (PAH), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD, PCDF; ‘dioxins’), polychlorinated biphenyls (PCB), a number of other persistent halogenated organic compounds, and toxic trace metals such as cadmium, lead and mercury. Contaminants may also be formed during production processes or during cooking of traditional food. One class of contaminants that may be associated with traditional foods are mycotoxins, produced by moulds either in crops during unfortunate weather conditions or during storage of crops under humid conditions. Well-known examples are aflatoxins, ochratoxins, trichotheneces, and fumonisins. Groups of contaminants that have been especially associated with traditional food production and cooking procedures comprise a number of compounds that have been associated with genotoxic and carcinogenic effects in experimental animals. Examples are acrylamide being formed in appreciable amount during frying or baking of carbohydrate-rich food, such as potatoes, cereals, and coffee, diethyl carbamate formed during fermentation processes, and the heterocyclic aromatic amines (‘cooked food mutagens’) formed during frying, baking and grilling of fish and meat. Also PAH may be formed in appreciable amount during smoking and barbecuing of food. Another group of well-known potent carcinogens are the N-nitroso compounds that are formed during the reaction of nitrite with secondary amines and amides, in particular during pickling, smoking and frying of nitrite/nitrate treated meat and fish products.

The procedures used in the risk assessment of contaminants are essentially the same as those used for food additives. However, in this case a tolerable daily intake (TDI) is established instead of an ADI. For compounds that have very long half-lives in the human body, such as dioxins, lead, mercury, and cadmium, it has been found more appropriate to express the tolerable intake on a longer-term basis. Therefore, for such compounds tolerable weekly intakes (TWI) are normally established.

Recent developments in risk assessments

Subdivision of the safety factor

The safety factor is used to extrapolate from a group of test animals to average humans and from average humans to potentially sensitive human sub-populations. The default 100-fold factor is considered to comprise a factor of 10 to allow for differences between test animals and humans (inter-species differences) and a factor of 10 to allow for human variability (inter-individual differences) (IPCS, 1987). In the early 1990s Renwick (1991, 1993) proposed a scheme to further subdivide these two components of the safety factor. Each factor of 10 was subdivided in order to allow for the two areas of uncertainty, i.e. differences in toxicokinetics (aspects such as absorption, distribution,
Susceptibility of infants and children

Susceptibility of infants and children to food additives and contaminants has been a dominating subject in the discussion of food chemical safety. It has been argued that infants and children in general are more susceptible to chemical insults than are adults due to the premature developmental state of their biochemical and physiological processes. They also have a higher food intake than adults, on a per kilogram body weight basis, and they have different dietary habits and food preferences.

The following questions were discussed at an ILSI Europe Workshop in 1997 (Larsen & Pascal, 1998):

- How big are the differences between infants or children and adults from a susceptibility point of view?
- Do testing methods cover these differences adequately?
- Are differences in food intake (between infants and children and adults) a point of concern?
- Are special safety factors or regulatory principles required for infants and children?

In considering infants and children it is important to remember that the ADI does not apply to neonates and infants before the age of 12 weeks. This is because maturation of the xenobiotic metabolising systems and elimination processes are not finalised until 3–6 months after birth and the protocols for toxicity testing do not mimic feeding of neonates and young infants on other diets than mothers milk. Therefore, food additives to be used in infant formulae require a special evaluation.

By examining the above-mentioned components of the safety factor, it was found that the elimination/clearance of chemicals is either similar or in many cases higher in children above the age of 3–6 months than in adults. Therefore, it was concluded that an increased safety factor was not required for differences in toxicodynamics. As regards differences in toxicodynamics, it is not possible to make general statements about age-related differences. For some chemicals immature animals are more sensitive than adults while in other cases they are less sensitive, depending on the compound and its effects. Overall, it was concluded that no numerical figures can be established that reflect age-related differences in susceptibility to chemicals, and that this issue should be addressed on a case-by-case basis. The toxicological database should adequately cover the most sensitive effects and the most sensitive age groups. If there is scientific evidence that infants and children are most sensitive to a particular food additive, that evidence must drive the derivation of the ADI.

As regards the currently used test methods, there are no major systematic differences in toxicokinetic parameters between neonatal and young experimental animals and their human counterparts. In fact, xenobiotic-metabolising enzymes are more developed in the human foetus and neonate than in the foetal and neonatal rat. Reproduction studies cover different developmental periods up to weaning, whereas the 2-year toxicity/carcinogenicity studies cover only the late period of juvenile growth. The ADI derived from these studies thus cover older infants and children as well as the foetus during pregnancy and the neonatal and young infant during the nursing period. However, concern was expressed that that the currently used methods do not adequately reveal delayed functional toxicity. This is related to the fact that apparent sub-toxic doses of certain compounds given to the developing foetus, neonate or infant during a developmental period of high susceptibility might produce functional deficits of the central nervous, endocrine, reproductive and immune systems that become manifest in adult life. Well-known examples of such compounds are dioxins, lead, and mercury. Although, delayed functional toxicity is less likely to occur with food additives than with, e.g. pesticides and that obvious signs of such functional deficits are in fact revealed by the current methods, it was agreed that more attention should be paid to parameters that address the function of the nervous, reproductive, endocrine, and immune systems.

The workshop considered that an optimal test protocol would be a two-generation study, covering in utero exposure, the suckling period, ‘creep feeding’, weaning and rapid juvenile growth, and where the F1-generation was used for evaluation of chronic toxicity/carcinogenicity. Adequate parameters to examine delayed functional toxicity should be included.
Based on results from comprehensive surveys it was concluded that on a body weight basis infants and children potentially would have a high daily intake of those food additives that are used in the types of foods and drinks they consume and prefer. However, the fact that infants and children have a higher intake of some food items than adults is not part of the ADI. The regulator should consider this when the ADI is used to establish the use levels of food additives in such foods.

Use of the threshold of toxicological concern approach in the safety assessment of flavouring substances

Until 1995 JECFA had only evaluated about 70 of the approximately 3000 flavouring agents used in foods. In order to speed up the evaluation of flavouring agents JECFA from 1995 to 1998 developed and adopted a new Procedure for the Safety Evaluation of flavouring Agents (IPCS, 1996; 1998; Munro, Ford, Kennepohl, & Sprenger, 1996; WHO, 1995, 1997). Using this procedure JECFA has now evaluated more than 1500 flavouring substances.

The flavouring substances are divided into three structural classes based on increasing structural complexity and structural alerts (class I, II, and III) according to Cramer, Ford, and Hall (1978). Class I substances have simple chemical structures and are efficiently metabolised by high-capacity pathways. Class II substances are ‘intermediate’ substances with less innocuous structures but without structural features suggestive of toxicity. Class III substances have chemical structures that do not permit presumption of safety or even suggest toxicity or reactivity.

Munro and co-workers (Kroes et al., 2000; Munro, 1990; Munro, Kennepohl, & Kroes, 1999; Munro et al., 1996) have established a comprehensive database containing conservative NOAELs for a large number of different chemicals and toxicological end-points. This database was used to establish the fifth percentile NOAELs for each structural class. By applying the conventional default safety factor of 100 on the fifth percentile NOAELs the following human intake thresholds were obtained: For structural class I: 1.8 mg/person per day, class II: 0.54 mg/person per day, and class III: 0.090 mg/person per day. The procedure finally includes the acceptance of a threshold of toxicological concern (TTC) of 1.5 μg/person per day. This latter threshold was based on an evaluation of a large number of long-term toxicity/carcinogenicity studies. Thresholds are also given for compounds in the database that have shown developmental toxicity or neurotoxicity (organophosphorus pesticides) (Table 3). The concept of threshold of toxicological concern has been further refined by the suggestion to include also specific TTC for groups of particular chemical carcinogens (Kroes et al., 2004) taking advantage of the work done by Cheeseman, Machuga, and Bailey (1999).

In the absence of other suitable and better methods JECFA based its intake estimates on comprehensive surveys on annual poundage data from the USA (data from the US National Academy of Sciences/National Research Council in 1987 and 1995) and Europe (data from International Organization of the Flavour Industry (IOFI) in 1995), respectively. The estimates were based on the assumption that the surveys accounted for only 60% of the production and that the entire amount produced was consumed by only 10% of the population (‘eaters only’). JECFA has recommended that information

### Table 3. Comparison of various NOAELs and threshold values

<table>
<thead>
<tr>
<th>Category</th>
<th>5th Percentile NOAEL (mg/kg bw per day)</th>
<th>Human exposure threshold (μg/person per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural class I</td>
<td>3.0</td>
<td>1800</td>
</tr>
<tr>
<td>Structural class II</td>
<td>0.91</td>
<td>540</td>
</tr>
<tr>
<td>Structural class III</td>
<td>0.15</td>
<td>90</td>
</tr>
<tr>
<td>Developmental toxicity</td>
<td>3.46</td>
<td>2076</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>0.03</td>
<td>18</td>
</tr>
<tr>
<td>Threshold of regulation</td>
<td>0.15</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Table 4. Safety evaluation procedure for flavouring agents

1. What is the structural class of the compound?
2. Can the compound be expected to be metabolised to innocuous products?

Yes | No
---|---
A3. Intake greater than threshold of concern? | B3. Intake greater than threshold of concern?
| If yes: data must be available on substance or a closely related substance |
| If no: |
| If yes: no safety concern expected |
| If no: |
| A4. Is the substance or metabolites endogenous? |
| If yes: no safety concern expected |
| If no: |
| A5. Does a NOEL exist for substance or closely related substance that provides an adequate margin of safety? |
| If yes: no safety concern expected |
| If no: additional data required |
| B4. Does a NOEL exist for substance or closely related substance that provides an adequate margin of safety? |
| If yes: no safety concern expected |
| If no: |
| B5. Intake greater than 1.5 μg/person per day? |
| If yes: additional data required |
| If no: |

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on intake be periodically updated to ensure the validity of the evaluations.

The procedure for the safety evaluation of flavouring agents proceeds through a number of steps in which several questions have to be answered (Table 4).

The EFSA scientific panel of food additives, flavouring agents, production aids, and food contact materials (AFC Panel) also use this approach for assessment of flavouring substances. However, the AFC Panel has not accepted the TTC of 1.5 mg/person per day, and additional intake estimates are being made using a modified theoretically anticipated maximum daily intake (M-TAMDI) approach.

Excursions above the ADI

Another frequently asked question is: what are the health implications of exceeding the ADI? This has been dealt with at an ILSI Europe Workshop on the Significance of Excursions of Intake above the Acceptable Daily Intake (ADI) in 1998 (Barlow, Pascal, Larsen & Richold, 1999; Larsen, & Richold, 1999). The following questions were asked:

- What methods should be used to estimate intakes?
- For how long can excursions above the ADI be tolerated and by how much can it be exceeded?
- Do the same principles apply to contaminants that have tolerable daily intake (TDI) or Provisional Tolerable Weekly Intake (PTWI) values?

The workshop realised that due to the complexity and expense of the studies, it is not realistic to expect that comprehensive and precise intake data will be available for the vast majority of substances. It was advised to use a step-by-step procedure to identify compounds for which excursions of intakes above the ADI could possibly occur (occasional or regularly):

- First step: use a conservative, theoretical/hypothetical approach, such as the Budget method.
- If there are no problems in relation to exceeding the ADI/TDI: do not waste more money.
- If problems are indicated: do an initial risk assessment on worst case scenario.
- If still problems: a more refined intake estimate is needed taking into account a number of compound related issues.
- A compromise could be a 3 day dietary study supplemented with a food frequency questionnaire (% consumers). Minimum population size: 200 persons.

There was general agreement that the ADI and its derivation is an appropriate and scientifically credible basis for the safety assurance of food additives that show thresholded toxicity. The ADI for food additives relates to lifetime exposure and provides a large margin of safety. Although toxicologists in general have no serious health concerns about occasional excursions of intake above the ADI/TDI, it was considered that such excursions were generally undesirable, in particular for a prolonged period.

The meeting concluded that no general guidance could be given on how long and by how much excursions above the ADI could be tolerated, and that a case by case evaluation was required. However, a number of examples were provided that could form the basis for such evaluations. Key issues in the evaluation would be: consumption patterns, the nature of the pivotal study (long- or short-term), reversibility of the toxicity, relevance for special age groups, and toxicokinetic (general rules for steady-state levels) and toxicodynamic parameters (no general rules).

As regards the evaluation of contaminants the TDI or PTWI for food contaminants is based on the same principles as the ADI, and therefore, excursions above the TDI/PTWI should be considered in the same way as excursions above the ADI. It was, however, noted that for contaminants having very long half-lives in humans, short-term excursions above the PTWI would have no great impact on body burdens.

It was also noted that estimates of human exposures to chemicals not were precise and it was stressed that the precision of the estimates should not exceed the precision of the methodology. Therefore, interpretation of intake data in relation to the ADI should recognise the limitations of the estimates (Larsen & Richold, 1999).

Margin of safety for non-genotoxic chemicals in foods

In the following comparisons are made between a number of non-genotoxic chemicals in food based on a margin of safety (MOS) approach. The margin of safety is the ratio between the NOAEL used for the derivation of the ADI/TDI and the estimated human intake of the compound. The intake estimates and toxicological descriptions for most of the compounds were taken from the Danish Food Monitoring Program (DVFA, 2005) (Table 5).

Cadmium accumulates in the body, primarily in kidneys and liver, and has a biological half-life of several decades. The toxic effect occurs primarily in the kidneys and the most sensitive effect is proteinuria. A provisional TWI (PTWI) value has been established by JECFA at 7 µg/kg body weight. The mean and 95th percentile intakes of cadmium in Denmark have been estimated at 10 and 16 µg/day for 1998–2003. This is equivalent to 14 and 22% of the PTWI value, respectively. The food groups that contribute the most to the intake are bread and cereals followed by vegetables (DVFA, 2005).

Ingested lead accumulates in the body, and the most sensitive adverse effect is associated with the development of the central nervous system in the foetus and newborn child. A possible association between increased lead concentration in blood and lower intelligence quotient has been substantiated, and a lower threshold value could not be determined. A PTWI value has been established by JECFA at 25 µg/kg body weight. The mean and 95th percentile
intakes of lead in Denmark have been estimated at 17 and 30 μg/day for 1998–2003. This is equivalent to 7 and 11% of the PTWI value, respectively. The food groups that contribute most to the intake are beverages followed by vegetables, bread and cereals, fruit and sugars (DVFA, 2005).

Mercury also accumulates in the body, and the most toxic species is methyl mercury, which occurs in fish. The adverse effect of inorganic mercury is first apparent in the kidneys, while methyl mercury primarily affects the developing central nervous system. A provisional TWI value has been established by JECFA at 5 μg/kg body weight per week for mercury in general and at 1.6 μg/kg body weight per week specifically for methyl mercury. The mean and 95th percentile dietary intakes of mercury in Denmark have been estimated at 1.9 and 4.1 μg/day for 1998–2003, respectively. About 60% of the mean mercury intake (1.1 μg/day) originates from fish, and it is assumed that all mercury contained in fish is present as the more toxic methyl mercury, whereas the mercury in all other foods occurs as inorganic mercury. The methyl mercury fraction of the total mercury intakes corresponds to 12% of the PTWI for the mean intake and to 41% of the PTWI for the 95th percentile intake. The food groups that contribute most to the mercury intake are fish, followed by bread and cereals, fruit, beverages, and vegetables (DVFA, 2005).

### Table 5. Estimated margins of safety (MOS) and margins of exposure (MOE) for chemicals in food

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Intake</th>
<th>ADI/TDI/TWI</th>
<th>Safety factor</th>
<th>MOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food additives</td>
<td>Below ADI</td>
<td>Various</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pesticide residues</td>
<td>Below ADI</td>
<td>Various</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Veterinary drug residues</td>
<td>Below ADI</td>
<td>Various</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td><strong>Persistent environmental contaminants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compounds</td>
<td>Intake&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ADI/TDI/TWI&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Safety factor&lt;sup&gt;d&lt;/sup&gt;</td>
<td>MOS</td>
</tr>
<tr>
<td>Lead</td>
<td>19 μg/day</td>
<td>25 μg/kg per week</td>
<td>17 (human data)</td>
<td>~10–15</td>
</tr>
<tr>
<td>Mercury</td>
<td>1.9 μg/day</td>
<td>1.6 μg/kg per week</td>
<td>17 (human data)</td>
<td>~5–10</td>
</tr>
<tr>
<td>Cadmium</td>
<td>10 μg/day</td>
<td>7 μg/kg per week</td>
<td>17 (human data)</td>
<td>~5–10</td>
</tr>
<tr>
<td>Dioxins + dioxin-like PCB</td>
<td>1 pg TEQ/kg per day</td>
<td>14 pg/kg per week</td>
<td>37 (human data)</td>
<td>~5–10</td>
</tr>
<tr>
<td>PCB</td>
<td>0.9 μg/day</td>
<td>0.03 μg/kg per day&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1000?</td>
<td>~2000 (20)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΣChlordane</td>
<td>0.11 μg/day</td>
<td>0.5 μg/kg per day</td>
<td>100</td>
<td>32,000 (320)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΣDDT</td>
<td>0.27 μg/day</td>
<td>0.5 μg/kg per day</td>
<td>100</td>
<td>13,000 (130)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.13 μg/day</td>
<td>0.05 μg/kg per day</td>
<td>100</td>
<td>2,700 (27)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Endosulfan A</td>
<td>0.03 μg/day</td>
<td>6 μg/kg per day</td>
<td>100</td>
<td>1,400,000 (14,000)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCB</td>
<td>0.09 μg/day</td>
<td>0.16 μg/kg per day</td>
<td>300</td>
<td>12,500 (125)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΣHeptachlor</td>
<td>0.05 μg/day</td>
<td>0.1 μg/kg per day</td>
<td>100</td>
<td>14,000 (140)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.06 μg/day</td>
<td>1 μg/kg per day</td>
<td>500</td>
<td>600,000 (6000)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>126 ng/day</td>
<td>100 ng/kg per week</td>
<td>500 (LOAEL)</td>
<td>4000 (40)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

| Compounds | Intake<sup>g</sup> | BMDL10<sup>g</sup> | MOE<sup>h</sup> |
| PAH | 4 ng/kg per day | 0.1 mg/kg per day | 25,000 |
| Acrylamide | 0.001 mg/kg per day | 0.3 mg/kg per day | 300 |
| Ethyl carbamate | 80 ng/kg per day | 0.3 mg/kg per day | 3800 |

<sup>a</sup> The margin of safety (MOS) is the margin between the estimated human intake of a compound and the intake that did not produce adverse effects in the most sensitive study in experimental animals or humans (the NOAEL).

<sup>b</sup> Intake data taken from DVFA (2005).

<sup>c</sup> Taken from DFVA 2005.

<sup>d</sup> Taken from the IPCS INCHEM database that contains all evaluations performed by FAO/WHO expert groups.

<sup>e</sup> PCB in, which case this author has estimated a TDI.

<sup>f</sup> Figures in brackets are adjusted on a body burden basis, assuming a factor of 100 between the elimination half-lives in humans and the half-lives in experimental animals.

<sup>g</sup> Taken from WHO (2005).

<sup>h</sup> The margin of exposure (MOE) is the ratio between a defined point on the dose–response curve (reference point, BMDL10) for the adverse effect of the compound in the animal carcinogenicity study and the estimated human intake of the compound.
endosulfan) is that the liver is one of the most sensitive organ systems in experimental animals. Following high daily doses, mice and rats develop liver cancer. None of the substances are genotoxic, and it is assumed that the carcinogenic effect shows a threshold. Some of these organochlorine compounds have also shown a potential to affect hormone systems in vitro and to affect reproduction and developmental neurotoxicity in vivo, but these effects are seen at higher doses than those producing liver toxicity. The tolerable daily intake (TDI) or acceptable daily intake (ADI) values that have been established for these organochlorine compounds are given in Table 5. The mean intake estimates in Denmark were estimated at lower than 5% of their respective ADI/TDI values (DVFA, 2005).

Several risk assessments have been carried out internationally on ochratoxin A. JECFA has established a PTWI of 100 ng/kg bw based on nephrotoxicity in pigs, the most sensitive species, and a safety factor of 500. Ochratoxin A has produced renal tumours in rats, but JECFA was of the opinion that these tumours were proceeded by the renal toxicity. The intake estimates showed that the average intake was below the PTWI. This was also true for persons having a high intake of ochratoxin A (the 0.95 quantile). The major food sources for ochratoxin A are cereals (DVFA, 2005).

The polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and dioxin-like polychlorinated biphenyls (dioxin-like PCB) are ubiquitous in food of animal origin and accumulate in fatty tissues of animals and humans. The most toxic and best-studied congener is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and reproductive and developmental toxicity. Toxic equivalency factors (TEF) have been established for the other PCDD, PCDF and dioxin-like PCB, relative to TCDD and the combined toxicity of a sample can then be expressed as toxic equivalent (WHO–TEQ). The EC Scientific Committee for Food evaluated PCDD, PCDF, and dioxin-like PCB in food in 2001. The assessment used the most sensitive adverse toxicological end-points of TCDD in experimental animals. These were developmental and reproductive effects in the male offspring of rats administered TCDD during pregnancy. Because of the large difference between rats and humans in the biological half-life of TCDD the assessment used a body burden approach to compare across species and derived a tolerable intake of 2 pg TCDD/kg bw per day. As TCDD has a very long half-life in the human body, it was found more appropriate to express the tolerable intake of TCDD on a weekly basis. Finally, the tolerable intake of TCDD was extended to include all the 2,3,7,8-substituted PCDD and PCDF, and the dioxin-like PCB, and expressed as a group tolerable weekly intake of 14 pg WHO–TEQ/kg bw (SCF, 2000, 2001). For dioxins and dioxin-like PCB the average estimated intake for adults in Denmark constitutes approximately 50% of the TWI. Persons with high dietary intake of dioxin and dioxin-like PCB (e.g. the 0.95 quantile) are close to or exceed the TWI, depending on the origin and hence the contamination level in especially the fatty fish they consume (DVFA, 2005).

The safety assessment of the non-dioxin-like PCB is particularly complicated, involving mixtures of congeners having different toxicological properties and effects. Most toxicological studies were carried out on the original, commercial products that are not representative of the mixtures that are concentrated in the food chains. There are also a number of other uncertainties in the existing toxicological studies concerning PCB. The existing database on PCB does not allow the establishment of a TDI. However, for this comparison of contaminants in food a tolerable daily intake of 30 ng/kg bw was assumed based on effects on liver, thyroids, and neurobehavioral development in experimental animals (ATSDR, 2000).

The mean and 95th percentile intake of sum-PCB in Denmark, which have been estimated at 0.9 and 1.4 μg/day for 1998–2003, are equivalent to 43 and 67% of that value, respectively.

The major contributor to the intake of dioxins and PCB are milk or milk products, eggs, meat, and fish (DVFA, 2005).

For these compounds the margins of exposures have been estimated (Table 5). From this exercise it seems clear that the compound having the lowest margins of safety (MOS) are the toxic trace metals and the dioxins and PCB. At first glance the persistent organochlorine contaminants seems to have high MOS value when compared on a daily intake basis. However, when adjusted for the large differences in half-lives between humans and animals the margins became more comparable to the margins for the regulated chemicals.

Risk assessment of contaminants that are both genotoxic and carcinogenic. Margin of exposure approach

For compounds with both direct DNA-acting genotoxic properties and carcinogenic properties it has generally been assumed that there is no dose without a potential effect (no threshold) and, in theory, there will always be a risk associated with exposure even to the lowest dose levels. In contrast, threshold-based mechanisms are conceivable for genotoxic agents, which do not react with DNA, but indirectly cause DNA damage, for instance through oxidative stress. As adequate human data are usually not available, data from rodent carcinogenicity studies have to be used in the risk assessment. In these studies, the animals are exposed to the compound for the major part of their lifetime at high

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dose levels, so that a detectable and statistically significant tumour incidence can be produced. These data must then be extrapolated to the usually much lower human exposure levels.

In the EU, risk assessors have until now evaluated such carcinogens in the diet on a case-by-case basis using a ‘weight of evidence’ approach, and have applied the ALARA principle (level in food should be as low as reasonable achievable). However, the ALARA principle does not provide risk managers with much of a basis for setting priorities related to the potency of the compound and the extent of human exposure.

In the USA, a quantitative hazard characterisation has been performed by low-dose extrapolation of animal data using a wide range of models to calculate a dose for an acceptable risk (often set at 1 extra cancer per million exposed persons). However, the slope of the dose–response curve and the mathematical model used are the decisive factors that determine the risk estimates when extrapolating over 3–4 orders of magnitude. The model most often used is a linear extrapolation from the observable range, and the apparent precision of the calculations does not reflect the uncertainty in the risk estimate. The results are therefore open to misinterpretation because the numerical estimates may be regarded as quantification of the actual risk.

The concept of a linear relationship down to zero dose originates from studies on covalent binding to DNA of compounds that are both genotoxic and carcinogenic. DNA binding normally shows a linear dose–response relationship in the low-dose range, with no indication of a threshold. This has therefore been the argument to suggest a linear decrease of mutagenicity, and eventually of cancer risk, at low doses. However, a DNA adduct does not in itself have genetic consequences, but needs to be fixed into a mutation through DNA replication. The probability for a DNA adduct to be fixed is dependent on the rates of DNA repair and cell proliferation, which are influenced by dose. There is now an emerging scientific database on cell 'household' mechanisms, like DNA repair, that indicate non-linear relationships. In addition, endogenous physiological processes normally produce a relatively high level of DNA damage that is efficiently repaired. This suggests that the contribution of very low doses of compounds that are both genotoxic and carcinogenic to background damage may be negligible. As the high doses applied in carcinogenicity bioassays usually elicit significant toxicity with regenerative cell proliferation in target organs, simple linear extrapolation from experimental data to effects at low doses may lead to a considerable overestimation of the true incidence.

In order to avoid any quantitative estimate, but still be able to prioritise among compounds, JECFA and EFSA have recommended to use a margin of exposure approach (MOE) in the assessment of compounds that are both genotoxic and carcinogenic (EFSA, 2005; WHO, 2005). MOE is the ratio between a defined point on the dose–response curve (reference point) for the adverse effect of the compound in the animal carcinogenicity study and the estimated human intake of the compound. JECFA and EFSA recommend using the benchmark dose (BMD) approach to estimate the reference point. The benchmark dose approach is based on mathematical modelling being fitted to the experimental tumour data within the observed range and estimates the dose that causes a low but measurable response. The use of BMDL10 (benchmark dose lower limit) representing the lower bound of a 95% confidence interval on the benchmark dose corresponding to a 10% tumour incidence (BMD10) was recommended as a reference point on the dose–response curve. A number of routinely used models are included in the US EPA BMD software program, which is freely available on the Internet: (http://cfpub2.epa.gov/ncea/cfm/recordisplay.cfm?deid=20167).

Different exposure scenarios should be provided, e.g., for the whole population and for specific groups of the population depending on the compound considered and its presence in the diet. Risk managers can use the magnitude of an MOE for priority setting since the MOEs, calculated for different compounds and intake scenarios, can vary broadly; a small MOE represents a higher risk than a larger one.

The selection of a MOE that would be considered acceptable is a societal judgement and primarily the responsibility of risk managers, but EFSA proposed that a MOE of 10,000 or higher, based on the BMDL10 would be of low health concern and might be viewed as low priority for risk management actions, although a MOE of that magnitude should not preclude the application of risk management measures to reduce human exposure. In this MOE of 10,000 was embedded a factor of 100 for inter-species differences (differences between animals and humans) and intra-species differences (differences between human individuals). This is the conventional approach for evaluation of threshold toxicities and is also relevant for genotoxic and carcinogenic compounds. An additional factor of 100 was suggested for the nature of the carcinogenic process and the use of a reference point on the dose–response curve. The mode of action includes irreversible steps and the probability of genetic alterations at critical targets may be dependent on the efficiency of repair of DNA damage and cell cycle control. Candidate genes, which may influence individual cancer risk by counteracting fixation of DNA-lesions into mutations, include DNA repair genes, immune function genes, and 'gatekeeper' genes controlling cell cycle and apoptosis. The reference point is not equivalent to a NOAEL and effects can occur at lower doses. The dose effect relationship below the reference point, and
the dose level below, which cancer incidence is not increased are unknown, representing additional uncertainties.

JECFA used this approach to evaluate the carcinogenicity of polycyclic aromatic hydrocarbons (PAH), acrylamide, and ethyl carbamate in food (WHO, 2005).

The carcinogenic PAH induce tumours in different tissues depending on the site of administration. After oral administration to mice and rats tumours have been found in the gastro-intestinal tract, skin, auditory canal, liver, lung, blood cells, mammary gland, kidney, and pituitary gland. JECFA used studies in mice fed either benzo[a]-pyrene (BaP) or coal tars (containing known amounts of BaP and other carcinogenic PAH) in their diets for 2 years. BaP produced increased incidences of papillomas and carcinomas in forestomach, oesophagus and tongue, whereas the coal tars produced increased incidences of tumours in lung, forestomach, liver, small intestine, and blood vessels. Tumours of the forestomach could be accounted for by the BaP content of the coal tars, but, the overall carcinogenic potencies of the complex coal tar mixtures were 2–5 times higher than that of the BaP content alone.

JECFA used BaP as a marker for the carcinogenic PAH in food. As reference point for comparison with the estimated human intake of PAH, JECFA used the BMDL10 for BaP from the mouse studies with coal tars. Based on eight different statistical models a BMDL10 of 0.1 mg BaP/kg bw per day for all tumours combined was chosen for BaP as a marker for the carcinogenic PAH in food. JECFA concluded that a representative mean intake of 4 ng BaP/kg bw per day and a high-level intake of 10 ng BaP/kg bw per day could be used in its evaluation. Comparison with the BMDL10 indicated MOEs of 25,000 and 10,000, respectively. Based on these MOEs, JECFA concluded that the estimated intake of PAHs from food were of low concern for human health.

Acrylamide is formed during heating of carbohydrate-rich food, and is genotoxic and carcinogenic. In the rat, it have produced follicular adenomas in the thyroid gland, peritesticular mesotheliomas, pheochromocytomas in the adrenal gland, mammary tumours, glial tumours in the central nervous system, squamous papillomas in the oral cavity, adenocarcinomas in uterus, adenomas in the clitoral gland, and pituitary adenomas. In the dose–response analysis, JECFA used eight different statistical models that were fitted to the experimental data. This resulted in a range of BMD10 and BMDL10 values for each endpoint considered. Choosing alveolar and bronchiolar neoplasms in male and female mice as the critical end-point, the values for BMDLs ranged from 0.3 to 0.5 mg/kg bw per day. JECFA decided to use the more conservative lower end of this range for the evaluation. The estimated average intake of ethyl carbamate in foods was 15 ng/kg bw per day. Compared with the BMDL10 value (0.3 mg/kg bw per day), the resulting MOE was 20,000. However, with the inclusion of alcoholic beverages in the estimated intake (80 ng/kg bw per day), the resulting MOE was 3800. On the basis of these considerations, JECFA concluded that intake of ethyl carbamate from foods excluding alcoholic beverages would be of low concern, but the MOE for all intakes, food and alcoholic beverages combined, is of concern and therefore mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages should be continued.

Conclusions

The use of food additives, pesticides, and veterinary drugs in the production of European traditional foods has successfully been regulated on the basis of the acceptable daily intake (ADI). This regulation has ensured that the amounts permitted in foods of a given compound would not result in the consumer having a daily intake higher than the ADI. A similar approach using the term tolerable...
daily intake (TDI) has been applied for unintended contaminants in foods showing threshold effects. However, in the case of contaminants they cannot just be eliminated from foods by banning their presence, as can be done with, e.g. food additives. Therefore, when evaluated on a margin of safety basis, some contaminants, such as dioxins, PCB, lead, cadmium, and mercury seems to be of major concern as regards the safety of traditional foods. Recent developments in various areas of the risk assessment, such as new approaches for subdivision of the safety factor, a new method for risk assessment of compounds that are both genotoxic and carcinogenic, and the introduction of the concept of threshold of toxicological concern (TTC), now used in the assessment of flavouring substances, are expected to considerably improve future risk assessments of European traditional foods.

References


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