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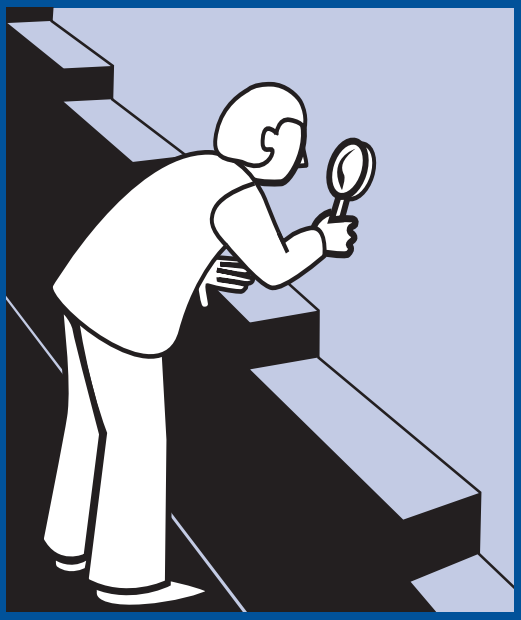

THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

A TOOL FOR ASSESSING SUBSTANCES OF UNKNOWN TOXICITY PRESENT AT LOW LEVELS IN THE DIET

ILSI
International Life Sciences INSTITUTE

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FOREWORD

Man is exposed to thousands of chemicals whether naturally occurring or man-made. The human diet, for example, contains innumerable low molecular weight, organic compounds that could, at some level of intake, represent a risk to human health. Extensive toxicity studies, utilising many animals, are necessary to evaluate the safety of chemicals applied in food or to establish if contaminants to which humans are exposed may cause harm.

The Threshold of Toxicological Concern (TTC) as described in this Monograph is a principle that refers to the establishment of a generic human exposure threshold value for (groups of) chemicals below which there would be no appreciable risk to human health. The concept proposes that such a value can be identified for many chemicals, including those of unknown toxicity when considering their chemical structures. Evidently the establishment of a more widely accepted TTC would benefit consumers, industry and regulators. For example, there is an ongoing concern that humans are exposed to a diverse array of chemicals and there is a demand to evaluate large numbers of chemicals. At the same time there exists a strong pressure to reduce our reliance on animal experimentation and to rely increasingly on *in vitro* and *in silico* data. Use of the TTC principle would eliminate the necessity of extensive toxicity testing and safety evaluations when human intakes of a chemical are below a certain level of concern, would focus limited resources of time, funding, animal use and expertise on the testing and evaluation of substances with greater potential to pose risks to human health and would considerably contribute to a reduction in the use of animals.

In addition, the principle may be applied to the assessment of chemicals in sectors of health risk assessment other than food and could moreover be further developed for environmental risk assessment. For example, application of the TTC principle could also be extended to other categories of chemical use such as cosmetics and consumer products. In this case, of course, appropriate methodologies should be developed to allow for route to route extrapolation and to assess combined multi-route exposure. In addition, the TTC principle can be used to indicate analytical data needs (as, for example, it is used in the USA for indirect food additives), or for setting priorities among chemicals for levels of "inherent concern".

In addition, since the principle is based on safety evaluations relating to daily intake throughout life, the approach may further be used in the assessment of impurities present in compounds, for contaminants at large, and as a science-based approach to indicate potentially acceptable concentrations of chemicals present in nature, which could be utilised in the application of the precautionary principle.

An International Life Sciences Institute (ILSI) – Europe expert group has examined this TTC principle for its applicability to food safety evaluation. This Monograph describes the history and development of the principle and its application to chemicals in food that humans are exposed to at low levels.

Robert Kroes
Utrecht University

INTRODUCTION

What is a threshold of toxicological concern (TTC)?

The threshold of toxicological concern (TTC) is a concept that refers to the establishment of a human exposure threshold value for all chemicals, below which there would be no appreciable risk to health. The story which follows describes how and why this concept was developed, the scientific basis for the human exposure threshold values that have been derived, where the TTC principle is now being applied and its value to the scientific community and to society.

The world of chemicals

As humans, we are exposed to thousands of chemical substances in our daily lives. Over 70,000 chemicals are used commercially and more than 100,000 naturally occurring chemicals have been identified. Exposure can occur at work, from the air we breathe, from consumer products used in the home and garden, from the water we drink or use for bathing, showering or swimming and from the food we eat.

Exposure to chemicals in foods

Some of the chemical substances to which we are exposed come from our diet. The main components of foods themselves, such as fats, carbohydrates, proteins, vitamins and minerals, are all chemicals. These are usually not of concern, unless particular ones are taken in excess or in nutritionally inadequate amounts.

In addition, processed foods may contain chemical additives to preserve, colour, emulsify, sweeten and flavour foods, or to perform some other functional role in the food. Foods may also contain residues of pesticides that are used on crops and traces of veterinary drugs used in food-producing animals. Chemicals used as processing aids, such as machinery lubricants or antibacterial substances in salad washing water, can also leave residues on foods. Chemicals present in food packaging materials and kitchen utensils can migrate into foods during manufacture, transport, storage, heating or cooking of food. Foods may contain contaminants of natural origin, such as toxins from fungi, or metals from natural minerals and soils, and man-made contaminants that find their way into the general environment, such as persistent polychlorinated biphenyls (PCB) and dioxins. Lastly, undesirable chemicals may be generated during cooking or smoking of food, such as acrylamide in fried potatoes and coffee, and polycyclic aromatic hydrocarbons (PAH) from smoking or barbecuing of meat and fish.

What do we know about these chemicals?

For some food chemicals, such as additives, pesticides and veterinary drugs, we have a wealth of information on their chemical and toxicological properties and on what levels of exposure are likely to be safe for humans. Similarly, for vitamins and minerals in food there is information and experience from human consumption about what levels are safe. The situation is different, however, for many other chemicals found in food, such as chemicals migrating from food packaging, flavouring substances, processing aids, unexpected contaminants and substances formed as reaction products or breakdown products during processing, heating and

cooking. For many of these and for many of the non-food chemicals to which humans may be exposed, we often have little or, in some cases, no information on their potential for toxicity. In addition, analytical capabilities for the detection and quantification of chemicals in foods are continuously improving, such that minute traces of a huge array of chemicals can now be identified. Scientists, governments and industry are making concerted efforts to test chemicals to which humans are known to be exposed, according to agreed priorities, but this takes time and considerable resources. It is clearly not feasible to test all known chemicals and probably unnecessary to subject every chemical to extensive testing for toxic effects.

How much is toxic?

Exposure is often used as one of the aspects to be taken into account when setting priorities for testing. This is because the likelihood of adverse or harmful effects is related to the magnitude, frequency and duration of exposure to a chemical. In the laboratory, scientists observe that for most toxic effects, there is an exposure dose, or threshold, below which no adverse effects are seen. If a general threshold, or several thresholds, could be determined for the world of chemicals, below which exposure did not raise safety concerns for humans, then this could be a useful tool, among others, in deciding on the need for toxicity testing. This concept has become known as the Threshold of Toxicological Concern (TTC).

How might a TTC be used?

The TTC concept could be particularly useful, for example, when there is a new discovery of the presence of a contaminant in food, for which there is no toxicological information. It could also be useful in setting priorities for testing among large functionally similar groups of chemicals to which exposure is generally very low, such as flavourings and substances used in food contact materials.

The use of such a tool would have benefits, not only for industry and regulatory authorities, but also for consumers, because it would enable the world's limited resources for toxicity testing and safety evaluation to be focused on chemicals that may pose a real threat to human health. By eliminating the need for unnecessary toxicity tests, it would also reduce the number of animals used in laboratory testing which would be welcomed both by the scientists involved and the general public.

CURRENT APPROACHES TO TOXICITY TESTING AND SAFETY EVALUATION OF CHEMICALS

Deciding on the likely level of concern

The present system for safety evaluation of chemicals is largely based on a case-by-case approach. Scientists first assemble all the information they have on a chemical and make a judgement on the likely level of concern. In the initial stages, the information available may be limited to knowledge of the chemical's structure, where it occurs and what degree of human exposure can be anticipated. For some chemicals there may also be limited toxicity information but this will often be far from complete. At this stage, a decision has to be taken on whether further toxicity or exposure data need to be generated. It is at this point that the TTC concept could be useful (see later).

Deciding on whether there are enough toxicity data

Ideally, for a full assessment of any human safety risks of a chemical in food, results from a range of laboratory toxicity tests are needed (see Box 1). These tests should reveal any adverse effects on the structure and function of the cells, organs, tissues and fluids in the body resulting from short-term exposure or from long-term, daily exposure. Life stages covered in the tests should include not only adulthood including pregnancy, but also infancy and the juvenile period. Testing would normally include not only investigation of any effects on the various organs and systems of the body, but also male and female fertility, reproduction, development of the embryo and foetus and postnatal growth and development. If during these tests effects are revealed on particular systems in the body, such as the immune system or the nervous system, then additional tests focusing on these aspects may be needed. Any knowledge of the effects of the chemical on humans is

BOX 1

Type of laboratory toxicity test	What it can reveal
Sub-chronic toxicity	Adverse effects on structure and function in any part of the body following repeated daily exposure for up to 10 per cent of lifetime
Chronic toxicity	Adverse effects on structure and function in any part of the body following repeated daily exposure over a substantial part of the lifetime
Carcinogenicity	Cancer
Genotoxicity	Damage to the inherited genetic material inside cells (DNA)
Reproductive toxicity	Adverse effects on fertility and reproduction
Developmental toxicity	Adverse effects on the embryo and foetus
Immunotoxicity	Adverse effects on the structure and function of the immune system, or in reaction to immune challenge
Neurotoxicity	Adverse effects on the structure and function of the nervous system and behaviour

particularly valuable, but for many chemicals, no such information is available.

(For more details about toxicity testing methods, see the ILSI Europe Concise Monograph on The Acceptable Daily Intake)

If the data available cover all or most of the above types of tests, then a comprehensive safety evaluation can be conducted. If a non-critical piece of information is not available, those conducting the safety evaluation can use their scientific judgement to make allowances for the missing data. If the missing data are considered to be critical to the safety evaluation, then more tests must be conducted.

Assessment of exposure

At an early stage in safety evaluation, consideration must be given to whether there is enough information on how much of the chemical concerned is present in food, which foods may contain it, how much of the relevant foods are consumed in the daily diet, which sections of the population may be most exposed and at what level. Other, non-dietary routes of exposure to the chemical may also need to be taken into consideration. If the chemical concerned is not detectable in foods, further consideration must be given to whether the analytical limit of detection is sufficiently sensitive to pick up amounts that may still be of toxicological relevance. If the available data are insufficient to enable a good estimate of average and high exposures to be made, it may be necessary to undertake more chemical analyses of foods or to generate more information on food consumption. The generation of such data can be costly. For that reason, exposure assessment should be a stepwise procedure, in which each step contributes to the reduction of uncertainty. The process can be stopped at the point where the estimated exposure is below the level of toxicological concern.

Use of toxicity data to assess risks and safe levels of intake

Effect levels and no effect levels

Toxicological studies in animals are usually conducted using several doses covering a wide range of exposure. For assessment of food chemicals the preferred route of administration is oral. The results of each study will generally, but not always, show some adverse or harmful effects at higher doses and no effects at lower doses. If the substance is toxic, the study will identify the dose (or doses) at which adverse effects are observed, known as an Effect Level (EL). The nature and severity of the effects observed will vary, depending on the type of test, the species of animal and the duration of exposure. The study will also normally identify the maximum dose at which there are no observed effects, and this is called the No Observed Effect Level (NOEL). Thus, from a range of toxicity studies there may be several NOELs and the risk assessment will as a rule focus on the most sensitive relevant study giving the lowest NOEL. Sometimes the term No Observed Adverse Effect Level (NOAEL) is used instead of NOEL to distinguish between an observed effect that is adverse and an effect that is not necessarily adverse. In this Monograph the term NOEL is used and should be interpreted as synonymous with NOAEL.

The results from toxicity studies can be used in two different ways:

1. To predict safe levels of exposure for humans.
2. To predict potentially harmful levels of exposure and the likely nature of the harmful effects.

Setting an Acceptable Daily Intake (ADI)

In the first case, the results from toxicity studies can be used to predict the highest amount of a chemical

ingested on a daily basis by humans that is likely to be safe. For food chemicals this is often expressed as the Acceptable Daily Intake (ADI) or Tolerable Daily Intake (TDI). The term ADI is generally used for substances intentionally added to food, while TDI is generally used for substances appearing in food but not intentionally added. The ADI or TDI is defined as the amount of a chemical, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable risk to health.

An ADI or TDI for a chemical is generally calculated by dividing the lowest NOEL revealed by the toxicity tests by a factor, usually 100, known as a safety factor or uncertainty factor.

$$\text{ADI/TDI} = \frac{\text{NOEL}}{\text{Safety/Uncertainty Factor}}$$

The incorporation of a safety or uncertainty factor gives an additional margin of reassurance to take account of the possibility that humans may be more sensitive than animals and that among humans some may be more sensitive than others. Thus, although it is considered that the toxicity tests conducted in laboratory animals are predictive of likely effects in humans, it is known that there can be variations between species and within species, including humans, in the way a chemical is absorbed, metabolised and excreted in the body (toxicokinetics) and variations in the way a chemical acts on the cells, organs and tissues of the body (toxicodynamics). The NOEL is therefore divided by a safety or uncertainty factor to allow for these possibilities. Thus, an ADI or TDI errs on the safe side, producing a conservative estimate of the intake of a food chemical likely to be without risk for humans.

(For more details of derivation of the ADI and dealing with uncertainty, see the ILSI Europe Concise Monograph on The Acceptable Daily Intake)

Predicting adverse effects

In the second case, the results of the toxicity studies can be used to predict the nature of the adverse effects that may occur in humans (the hazard) and at what level(s) of exposure these adverse effects may occur. Most types of adverse effect for any particular chemical only occur above a particular dose, but the magnitude of that dose may vary depending on species and duration of exposure. As in the previous case of predicting intakes that are likely to be safe, when using laboratory animal data to predict a potentially harmful level of intake for a food chemical in humans, variability between and within species needs to be taken into account.

(For more details of how risks from intakes exceeding the ADI can be assessed, see the ILSI Europe Report on Significance of Excursions of Intake above the Acceptable Daily Intake (ADI))

Do all toxic effects have a threshold?

For most toxic effects caused by a particular chemical there is an exposure threshold below which adverse effects do not occur. At low exposures, the body can usually tolerate some disturbance to its normal biochemical and physiological functions without any overt signs or symptoms of illness. The body also has inbuilt mechanisms for rapidly getting rid of chemicals via metabolism and excretion and for repairing damaged cells and tissues. However, there are some particular types of toxic effect which can be triggered by exposure to very low amounts of a chemical and which can result in long-term illness or permanent (irreversible) damage. This relates to damage to the genetic inherited material in cells (DNA and chromosomes) and cancers caused by damage to the DNA. These are known as genotoxic and carcinogenic effects.

Genotoxic and carcinogenic effects

Genotoxic effects may be detected by *in vitro* tests, such as exposing bacteria to the chemical (e.g. the Ames test) or exposing isolated animal cells or human cells to the chemical. If genotoxic effects are detected *in vitro*, further tests in live animals (*in vivo*) can then be conducted to see if the harmful effects on chromosomes and DNA observed *in vitro* could actually cause damage in the body. Damage to DNA is an everyday event (e.g. from cell division, exposure to the sun's ultraviolet rays or internal exposure to reactive oxygen species) so the body has repair mechanisms to deal with it and every day millions of repairs are successfully carried out. However, studies on genotoxic chemicals offer strong evidence that damage to DNA can occur at very low doses, without an apparent threshold, and that the damage increases steadily with increasing dose. Thus chemicals are described either as "positive" (cause damage) or "negative" (do not cause damage) for genotoxicity. It is at present not possible to define no effect levels for positive genotoxic chemicals. Unrepaired damage to a cell's chromosomes or DNA can have two detrimental consequences; it can cause its growth and division to go out of control (cancer) or, in the case of germ cells (ova and sperm), it can cause mutations that can be passed on to the offspring. However, it is important to note that because of repair mechanisms, damage to DNA does not necessarily result in a mutation or cancer and that ongoing research may eventually allow thresholds for genotoxic substances to be established.

Carcinogenic effects are investigated by exposing animals, usually rats and mice, from a young age throughout and until the end of adult life, to daily doses of a chemical and examining the number and type of tumours that develop. Even though a laboratory animal study on cancer may appear to show a dose at which there is no increase in tumours, if the cause of the cancer can be linked to a genotoxic mechanism of action, on present evidence it is prudent to assume that there is no threshold for the toxic effect. Exposure to any amount, however small or transient, might have a harmful effect in the long-term. This assumption is made because animal experiments cannot, for logistical reasons, utilise sufficiently large numbers of animals to detect small increases in cancers at very low doses and thereby preclude the possibility that they occur.

Thus for chemicals that are shown to be genotoxic, or genotoxic and carcinogenic, when given to animals, it is not possible to set an ADI or TDI using the NOEL/safety factor approach. However, it should be noted that cancer can also be caused by non-genotoxic mechanisms of action for which thresholds can be established. For chemicals acting in this way it is possible to set an ADI or TDI.

Predicting the risk of cancer

For carcinogenic chemicals with genotoxic mechanisms of action, different approaches can be used to assess the risk of cancer at exposures likely to be encountered by humans. Ordinarily, this involves making estimates of risks at low or very low exposures. The approaches taken usually involve use of the dose-response curve obtained in an animal carcinogenicity test. This curve relates incidence of cancer to the various daily doses of the chemical given to the animals over their lifetime. As doses used in experiments are normally high, relative to likely human exposures, an estimate of risk at low exposures is made by extrapolating the dose-response curve downwards to a point below the range of the doses used in the experiment. A variety of mathematical models can be applied to the dose-response curve to make such a low-dose risk estimate. The mathematical models are generally considered to be highly conservative, and so give estimates of risk which not

only err on the side of safety, but may considerably overestimate the likely risk to humans. They can be used to produce an estimate either of the exposure associated with a particular level of risk, or the risk associated with a particular level of exposure. Risk managers can then be given choices about what they would consider to be an acceptable or a "virtually safe dose" (e.g. a dose which results in a predicted incidence of cancer of 1 in a million persons exposed for a lifetime to a particular dose).

Because of inherent limitations in animal carcinogenicity experiments and in the mathematical models used, some risk assessors and risk managers do not view the above approach as an appropriate way to estimate risks for humans. If that view is taken and a chemical is shown to be genotoxic and to cause cancer in animals, risk managers may decide that human exposure to that chemical should be as low as reasonably practicable (ALARP) or as low as reasonable achievable (ALARA). Risk management measures then have to be taken to reduce or eliminate human exposure. It is evident that limits set on this basis may imply different risks for substances of different potencies.

Are genotoxic substances permitted in foods?

In the case of food chemicals, substances are not authorised for deliberate addition to foods (additives), or for use on crops (pesticides) or in food-producing animals (veterinary drugs), if they are shown to be genotoxic, or genotoxic and carcinogenic, when tested *in vivo*. However, many other natural and man-made chemicals can appear in foods as contaminants, and some of these are known to be genotoxic. The TTC may also be useful for assessing these types of substances (see later, step 4 of the decision tree).

THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) CONCEPT: A GENERIC APPROACH

History and evolution of the TTC concept

The TTC concept has evolved from a lengthy history of attempts by scientists over the years, in regulatory authorities and elsewhere, to develop generic approaches to the safety assessment of large groups of chemicals or of individual chemicals of unknown toxicity.

The driving forces behind these efforts have been:

- the continuing improvements in analytical capabilities which allow more and more chemicals to be identified in food at lower and lower concentrations,
- the widely accepted premise that exposure to very low amounts of chemicals is usually without harm,
- the view that the time and attention devoted to a particular chemical should be in proportion to the risk to health,
- the limited toxicological resources worldwide, both in capacity for toxicity testing and for evaluation,
- the desire to minimise the use of animals,
- and the ability to analyse large sets of existing toxicity data to make predictions about the behaviour of other structurally-related chemicals.

Frawley's approach

One of the first efforts was in relation to food packaging materials and was published by Frawley in 1967. Starting from the premise that there must be some uses of food packaging materials that do not involve any hazard to health of the consumer of food, he set about defining a dose which he considered would be without

BOX 2

Frawley's classification of 220 chemicals

Distribution of NOELs (mg/kg in the diet)	Number of chemicals (220)*	Heavy metals and pesticides (88)
<1	5	5
<10	19	19
<100	40	39
<1000	101	72
<10000	151	86

* For 69 chemicals the NOEL was above 10000 mg/kg of diet. 151 + 69 = 220.

harm. He analysed a large data set of 2-year, chronic toxicity studies on 220 different chemicals given via the diet. This represented about 90% of all the available chronic toxicity studies at that time. The chemicals involved were food additives including colours, industrial chemicals, chemicals found in consumer products including cosmetics, chemicals used in food packaging materials, pesticides and heavy metals. Frawley grouped them into 5 categories, according to the dose at which no toxicological effects were observed (NOELs) (see Box 2).

The majority of the chemicals (180/220) had NOELs above 100 mg/kg of diet from chronic exposure. Only 19 had NOELs below 10 mg/kg of diet, all of which were pesticides or heavy metals. The 5 chemicals with NOELs below 1 mg/kg of diet were all pesticides that were known either to accumulate in the body, or to affect the function of the nervous system at low doses. From this analysis, Frawley proposed that for food packaging chemicals (many of which were then untested and of unknown toxicity), the level of 10 mg/kg of diet should be selected, since very few chemicals and only those of a type not likely to be used in food packaging showed toxicity in animals below this level. An additional 100-fold margin of safety should be applied to this level, giving a figure of 0.1 mg/kg of human diet. This was the dietary concentration for any food packaging chemical which he considered could be safely consumed by man. It would equate to an intake of 150 microgrammes/person/day, assuming an intake of 1.5 kg of solid diet.

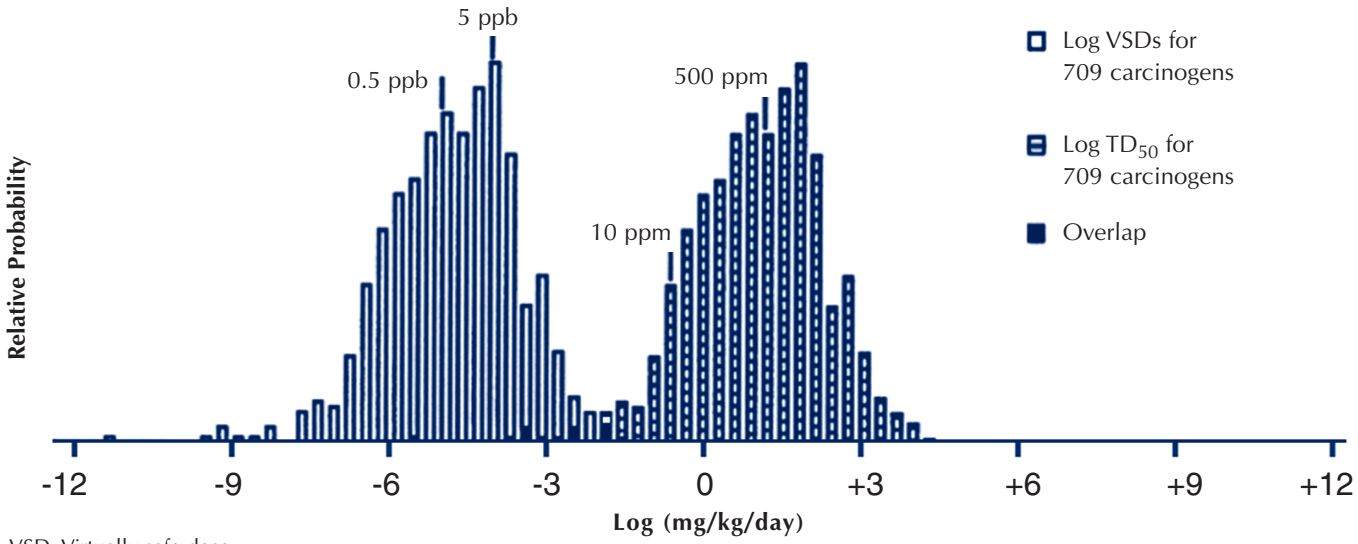
FDA Threshold of Regulation

The next major development was the introduction of a "Threshold of Regulation" policy for food contact materials by the US Food and Drug Administration (FDA) in 1995. The term "Threshold of Regulation" is used in the USA, rather than "threshold of toxicological concern", but the policy is based on the TTC principle. The policy was developed over 10 years, as a consequence of a long-established principle of the law, "*de minimis non curat lex*", which means the law does not concern itself with trifles. For the FDA, this meant that the agency should focus its limited resources on issues of tangible concern rather than trivial ones. Accordingly, the agency developed an approach to set a threshold, intended to protect against all types of toxicity including carcinogenicity, for application in food packaging regulation. If exposure to an individual chemical was below the threshold, consumers would be protected "with reasonable certainty of no harm".

The approach was based on an analysis by Gold and colleagues of nearly 500 chemical carcinogens tested in animals using lifetime exposures, known as the carcinogenic potency database. In the database, the potency of each chemical was expressed in terms of the dose that caused cancer in 50% of the animals (the TD_{50}). The potencies were plotted as a distribution and then, by sliding the curve to the left, transformed into a

FIGURE 1

Distribution of TD_{50}s for chemical carcinogens and extrapolation to a 1 in a million risk



VSD: Virtually safe dose

distribution of exposures calculated to represent an estimated lifetime risk of one in a million of developing cancer or "virtually safe dose" (VSD) (see Figure 1).

Thus, the distribution of carcinogenic potencies could be used to derive an estimate of the dietary concentration of most carcinogens which would give rise to less than a one in a million lifetime risk of cancer, assuming that the risks in animals were representative of those in humans. That concentration was estimated to be 0.5 micrograms/kg of diet. It is this figure which is used as the basis of the Threshold of Regulation policy. From this, a human daily exposure level of 1.5 micrograms/person was derived, by assuming that a person consumes 1500 g of food and 1500 g of fluids daily and that the chemical is distributed evenly throughout the total diet.

Later the carcinogenic potency database was enlarged to over 700 chemicals (Gold and colleagues, 1995), but this did not alter the distribution of the calculated risks. Based on this analysis, should any untested chemical to which the Threshold of Regulation policy is applied turn out to be a carcinogen, the consumer should still be protected. Since toxic effects other than cancer usually occur at much higher exposures, consumers would automatically be protected from those effects too.

It can be seen that the policy contains elements of both scientific and risk management judgements. The

Threshold of Regulation policy means that producers can apply for an exemption from regulation of any chemical originating from food contact materials estimated to be present in the diet at levels not exceeding 0.5 microgrammes/kg. If the FDA is satisfied that the conditions for exemption are met, the chemical does not have to undergo toxicological testing nor the normal pre-market safety evaluation by the agency.

Proposal for generic TTCs according to chemical structure

Analysis of chemical structures

Munro and colleagues in 1996 went on to develop the concept of generic thresholds by analysing toxic, but non-carcinogenic, effects of chemicals, according to their chemical structure. The chemicals were divided into three structural classes, based on a "decision tree" developed earlier by Cramer and colleagues. The three classes are shown in Box 3.

The toxicity database

A reference database was built up using results from oral toxicity tests in rats and rabbits on 613 chemicals with a wide range of structures and uses. The tests included sub-chronic, chronic, reproductive and developmental toxicity studies. From these, the most conservative NOEL for each chemical was selected, based on the most sensitive species, sex and toxic effect. The 613 NOELs were then plotted in three groups, according to structural class (see Figure 2).

Human exposure thresholds

For each of the three distributions of NOELs, a value coinciding with the point on the distribution where 5% of the chemicals had lower NOELs and 95% had higher NOELs was selected (i.e. the fifth percentile NOEL). The lower fifth percentile NOELs were then divided by a factor of 100 to ensure substantial margins of safety. This yielded three values termed "human exposure thresholds", one for each structural class of chemical, shown in Box 4. These human exposure thresholds are also referred to as TTCs.

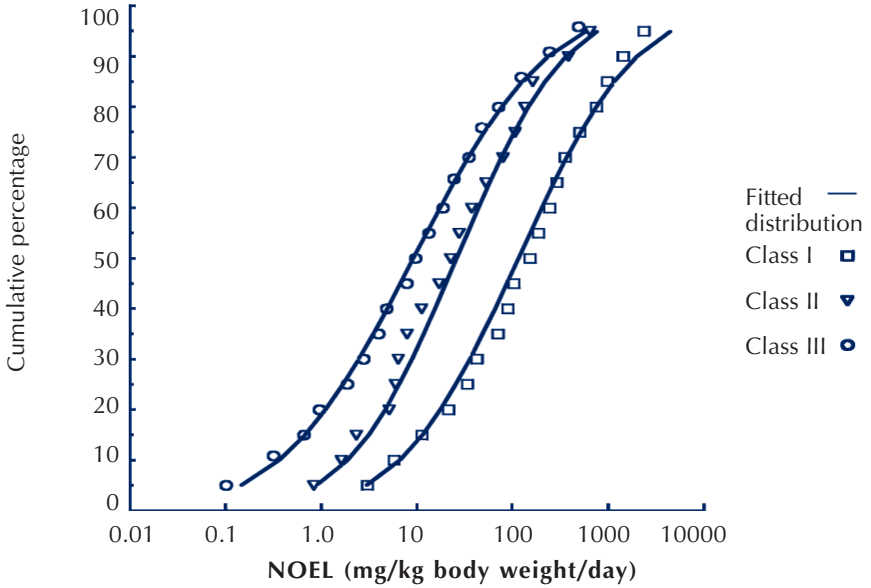
According to this scheme, a threshold can be selected for a chemical of known structure but unknown toxicity: if human exposure is below the relevant threshold of concern for that structural class, it can be assumed with reasonable confidence that the likelihood of any risk to human health is low. Later work increased the number of chemicals in the database from 613 to 900 but this did not alter the cumulative distributions of NOELs, adding further reassurance about the validity of using the database to derive thresholds of toxicological concern (TTC).

BOX 3

Structural classes for chemicals within the TTC concept

Class I	Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.
Class II	Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.
Class III	Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

FIGURE 2



Reprinted from *Food and Chemical Toxicology* Vol 34. Munro IC, Ford RA, Kennepohl E and Sprenger JG; Correlation of a structural class with no-observed-effect levels: a proposal for establishing a threshold of concern, pp 829-867, Copyright 1996, with permission from Elsevier.

BOX 4

Generic TTCs: Derivation of human exposure thresholds from toxicity data

Structural class	Fifth percentile NOEL (mg/kg bw/day)	Human exposure threshold (mg/person/day)*
I	3.0	1.8
II	0.91	0.54
III	0.15	0.09

* The human exposure threshold was calculated by multiplying the fifth percentile NOEL by 60 (assuming an individual weighs 60 kg) and dividing by a safety factor of 100.

Comparison with the Threshold of Regulation

Munro and colleagues emphasised that the human exposure thresholds are intended to apply only to structurally defined chemicals for which there is no evidence of genotoxic carcinogenicity and no structural alerts for genotoxicity. A structural alert is a feature of a chemical structure, such as an epoxide group, which is known to have a predisposition for damaging DNA. Comparing these human exposure thresholds, ranging from 90-1800 microgrammes/day, derived from data on non-carcinogenic effects, with the figure of 1.5 microgrammes/day for the FDA's Threshold of Regulation, based on carcinogenic effects, it can be seen that the thresholds for non-carcinogenic effects are higher by at least an order of magnitude. This is in accordance with what would be expected from our knowledge of the mechanisms of various toxic effects and the doses that induce them, i.e. it is biologically plausible that some carcinogens induce tumours at lower exposures than the exposures needed to induce other toxic effects.

Further validation and refinement of the TTC concept

A tiered approach to Threshold of Regulation

Further work by the FDA has provided support for the use of thresholds higher than 1.5 microgrammes/day for less potent carcinogens. Cheeseman and colleagues used the expanded carcinogenic potency database of over 700 chemicals, together with short-term toxicity data, results of genotoxicity testing and structural alerts, to identify potent and non-potent subsets. This work confirmed the validity of 1.5 microgrammes/day as an appropriate threshold for most carcinogens, but went on to propose that a tiered threshold of regulation could be justified. Examination of the expanded database led them to conclude that a dietary threshold of 4-5 microgrammes/kg could be appropriate for substances without structural alerts and even for substances with structural alerts if they were negative in tests for genotoxicity. The two exceptions to this were N-nitroso and benzidine-like compounds which are more potent carcinogens. If substances had no structural alerts, were negative in tests for genotoxicity and had acute toxicity (LD_{50}) above 1000 mg/kg bw, a dietary threshold of regulation of 10-15 microgrammes/kg could be possible. The tiered approach has not yet been adopted by the FDA.

Cheeseman and colleagues also re-examined the underlying premise of the Threshold of Regulation policy that carcinogenic effects generally occur at lower dietary concentrations than other toxic effects. They analysed information from a database (the Registry of Toxic Effects of Chemical Substances – RTECS) on 3306 substances for which there were oral reproductive toxicity data and on 2542 substances for which there were data from other repeat-dose toxicity tests. For each chemical, they searched for the lowest dose at which a toxic effect was seen. They then divided the lowest effect level for each substance by an uncertainty factor of 1000 to derive a range of "pseudo-acceptable daily intakes" (PADIs). The most likely (median) value for the PADI was 8300-fold above the threshold value derived from the carcinogenic potency database. These results supported the contention that a "virtually safe dose" based on carcinogenicity data would also protect against other toxic effects.

Do human exposure thresholds cover all possible effects?

One issue raised in scientific discussions of the TTC concept proposed by Munro and colleagues was whether potentially sensitive toxicological effects that might occur at low dose levels would be covered by the derived human exposure thresholds (see Box 4). In particular, concerns were raised with regard to whether effects on the nervous system, immune system, endocrine system and development would be absent at

the human exposure threshold values. Although the original database published by Munro and colleagues in 1996 did include some studies measuring these potentially sensitive endpoints, they were insufficient in number to provide a robust answer to the question of potential low-dose effects. An Expert Group was therefore set up by ILSI Europe to examine this question in more detail (Kroes and colleagues, 2000).

Expanded databases were developed for the toxicological endpoints of neurotoxicity (82 substances, comprising 45 with subchronic and chronic neurotoxicity data and 37 with acute neurotoxicity data), immunotoxicity (37 substances), developmental neurotoxicity (52 substances) and developmental toxicity (81 substances). They were analysed to see if these endpoints were more sensitive than those for structural Class III compounds in the original database compiled by Munro and colleagues and to see whether the TTC of 1.5 microgrammes/person/day derived from the carcinogenic potency database adequately covered such endpoints. Once again the distributions for the NOELs were plotted. There was no difference in the cumulative distribution of NOELs for any of the selected endpoints other than neurotoxicity. The cumulative distribution of NOELs for neurotoxicity was not only lower than those of the other selected endpoints, but it was also lower than that for structural Class III compounds. None of the selected non-cancer endpoints were more sensitive than cancer. Moreover, the TTC of 1.5 microgrammes/person/day, based on cancer endpoints, comfortably covered all these effects, including neurotoxicity, being 2-3 orders of magnitude lower than the neurotoxicity NOELs divided by a safety factor of 100.

The ILSI Europe Expert Group concluded that a TTC of 1.5 microgrammes/person/day is conservative and that chemicals present in the diet that are consumed at levels below this threshold pose no appreciable risk. It further concluded that for chemicals which do not possess structural alerts for genotoxicity, further analysis may indicate that a higher TTC may be appropriate.

Exclusion of high potency carcinogens

The TTC of 1.5 microgrammes/person/day used in the Threshold of Regulation policy is designed to protect against the toxicity of most chemicals, including those of unknown toxicity should they turn out to be carcinogens. Nevertheless, the FDA acknowledges that there may be some chemicals with a very high carcinogenic potency that may be unsuitable for the Threshold of Regulation approach. The ILSI Europe Expert Group set out to explore the issue of exceptionally potent chemicals (Kroes and colleagues, 2004).

The carcinogenic potency database used by Cheeseman and colleagues (see earlier) comprising 709 compounds was further expanded to 730 compounds and analysed in order to identify structural alerts that would give the highest calculated risks if present at very low concentrations in the diet. This analysis identified 5 groups of compounds having a significant fraction of their members that may still be of concern at an intake of 0.15 microgrammes/person/day. This is 10-fold below the Threshold of Regulation figure. These 5 structural groups, shown in Box 5, were termed the "Cohort of Concern". Three of the groups are genotoxic (aflatoxin-like-, azoxy- and nitroso-compounds), while two are non-genotoxic (TCDD and steroids). The ILSI Europe Expert Group concluded that compounds with these structural alerts for high potency require compound-specific toxicity data and should be excluded from any TTC approach. The peer review Workshop (see below) recommended using a TTC of 0.15 microgrammes/day for all other substances with structural alerts for genotoxicity which were not part of the cohort of concern.

BOX 5

Cohort of Concern

High potency carcinogens identified by structural alerts and not suitable for the TTC approach

- **Aflatoxin-like compounds**
- **Azoxy-compounds**
- **Nitroso-compounds**
- **2,3,7,8-dibenzo-*p*-dioxin and its analogues (TCDD)**
- **Steroids**

Exclusion for reasons other than carcinogenic potency

In addition to excluding compounds with structural alerts for high potency carcinogenicity, the ILSI Europe Expert Group also made a number of other recommendations for exclusion of particular groups from the TTC approach. It recommended that polyhalogenated -dibenzodioxins, -dibenzofurans and -biphenyls, along with heavy metals, should be excluded on the grounds that they are known to accumulate in the body (see later). Other non-essential metals in elemental, ionic or organic forms should also be excluded because they were not included in the original database of Munro and colleagues. In addition, proteins were not included in the original database and should also be excluded because of their potential for allergenicity (see later) and because some peptides have potent biological activities.

Neurotoxicants

The ILSI Europe Expert Group further explored whether particular neurotoxicants should be considered as a separate class. Using the expanded database from the earlier ILSI Europe work (see above) and locating the most sensitive indicators of effects that they could find, they plotted the NOELs for the most potent neurotoxicants, the organophosphorus compounds (OPs), separately from the other neurotoxicants. They noted that the 5th percentile NOEL for OPs was lower, by around an order of magnitude, than the corresponding NOEL for other neurotoxicants. The other neurotoxicants resulted in a plot comparable to the Class III chemicals, as published by Munro and colleagues. By applying a safety factor of 100 to the 5th percentile NOEL for OPs they derived a human exposure threshold of 18 microgrammes/person/day. The ILSI Europe Expert Group therefore recommended that this figure be used for OPs rather than the value of 90 microgrammes/person/day used for other compounds in structural Class III (see Box 4).

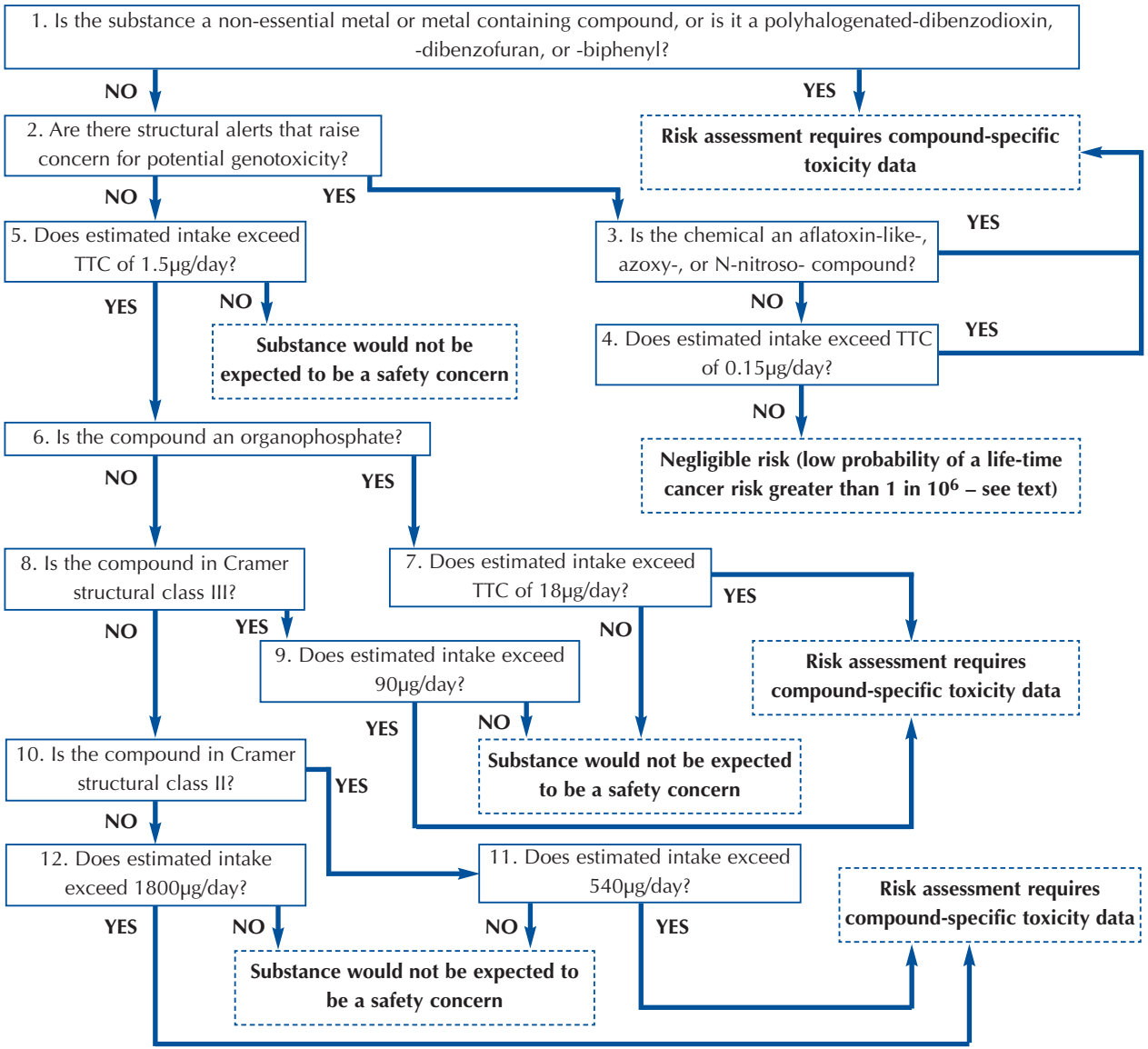
The ILSI decision tree

Development of the decision tree

Following the development of the TTC concept and its subsequent refinements described above, the work of the ILSI Europe Expert Group culminated in the construction of a decision tree, based on a tiered approach, to act as guidance on how and when the TTC principle could be applied as a preliminary step in food safety evaluation. The decision tree was finalised following a peer review Workshop held in March 2003, at which the science behind the various steps in the tiered approach was presented and critically discussed. The decision tree is shown in Box 6.

BOX 6

Decision tree proposed by ILSI Europe to decide whether substances can be assessed by the TTC approach (From Kroes *et al., Food and Chemical Toxicology* 42, p76, 2004)



Use of the decision tree

The decision tree comprises a series of steps, each one framed as a question, to which the answer, either 'Yes' or 'No', will carry the user through to the next step. The questions relate to whether the chemical is suitable for assessing via the TTC concept (see exclusions described earlier), the presence or absence of structural alerts for genotoxicity, and, depending on the chemical's structure, how the level of exposure relates to the relevant human exposure threshold. For any chemical taken through the decision tree process, one of two recommendations will be reached:

either,
the substance would not be expected to be a safety concern,
or,
risk assessment requires compound-specific toxicity data.

The decision tree is only applicable to chemicals of known structure and with low molecular mass as represented in the database. Accordingly, it is not applicable, for example, to polymers. A good estimate of intake or exposure (see later) is critical to the use of the tree, since this determines whether or not the TTC is exceeded. The steps in the tree are described below.

Steps of the decision tree

Step 1. This removes from consideration types of substances and chemical structures that are not adequately represented in the carcinogenicity and toxicity databases used to develop the TTC values.

Step 2. If the substance is not removed at step 1, it can proceed to step 2. This identifies compounds that have the potential for genotoxicity and could be possible genotoxic carcinogens.

Step 3. If the answer at step 2 is YES – it does have structural alerts for genotoxicity – then step 3 identifies those structures that are likely to be the most potent genotoxic carcinogens, i.e. aflatoxin-like, azoxy- and N-nitroso-compounds. These require compound-specific toxicity data and cannot be further assessed by the TTC approach.

Step 4. Substances evaluated at step 4 would all be potential genotoxic carcinogens, but with the most potent structures removed at steps 2 and 3. Step 4 asks if the estimated intake exceeds the TTC of 0.15 microgrammes/day (or 0.0025 microgrammes/kg bw/day). The rationale for this TTC was described earlier. For any substance reaching step 4, with an intake at or below this TTC, the probability that any risk of cancer exceeds 1 in a million is considered to be very low. The inclusion of this step is not designed to allow genotoxic substances to be added deliberately to food, but rather to determine whether there is a safety concern, should they be detected in food, say, as a contaminant.

Step 5. If the answer at step 2 is NO – it does not have structural alerts for genotoxicity – then step 5 asks if the estimated intake exceeds 1.5 microgrammes/day (or 0.025 microgrammes/kg bw/day). This TTC is the one used in the Threshold of Regulation, based on an analysis of carcinogenic compounds, including both genotoxic and non-genotoxic compounds. For any substance reaching step 5, with an intake at or below this TTC, the probability that any risk of cancer exceeds 1 in a million is considered to be very low. As the TTCs for other forms of toxicity are all higher than this value, other forms of toxicity would not be of concern either at intakes at or below 1.5 microgrammes/day.

Step 6. This step identifies organophosphates which have a lower TTC (see earlier) than that for structural Class III compounds in general. This step is not intended to replace the normal regulatory assessments and controls for organophosphates used as pesticides, but can be used to determine if there is any safety concern should a non-approved or unregulated OP be detected in food, for example, as a contaminant.

Step 7. If the substance is identified as an OP at step 6, step 7 asks if the estimated intake exceeds the TTC for OPs of 18 microgrammes/day (or 0.3 microgrammes/kg bw/day). If the answer is NO, the substance would not be expected to be a safety concern. If the answer is YES, the substance requires compound-specific toxicity data and cannot be further assessed by the TTC approach.

Step 8. Having by this stage eliminated potential genotoxic carcinogens and organophosphates, step 8 asks if the chemical falls into Cramer structural class III (see Box 3).

Step 9. If the answer to step 8 is YES – the chemical is in Cramer structural class III – step 9 asks if the estimated intake exceeds the TTC for that class of 90 microgrammes/day (or 1.5 microgrammes/kg bw/day). If the answer is NO, the substance would not be expected to be a safety concern. If the answer is YES, the substance requires compound-specific toxicity data and cannot be further assessed by the TTC approach.

Step 10. If the substance is not in Cramer structural class III, step 10 asks if the chemical falls into Cramer structural class II (see Box 3).

Step 11. If the answer to step 10 is YES – the chemical is in Cramer structural class II – step 11 asks if the estimated intake exceeds the TTC for that class of 540 microgrammes/day (or 9 microgrammes/kg bw/day). If the answer is NO, the substance would not be expected to be a safety concern. If the answer is YES, the substance requires compound-specific toxicity data and cannot be further assessed by the TTC approach.

Step 12. If the substance is not in Cramer structural class II, step 12 assumes that the chemical falls into Cramer structural class I (see Box 3) and asks if the estimated intake exceeds the TTC for that class of 1800 microgrammes/day (or 30 microgrammes/kg bw/day). If the answer is NO, the substance would not be expected to be a safety concern. If the answer is YES, the substance requires compound-specific toxicity data and cannot be further assessed by the TTC approach.

Potential applications of the TTC principle

The ILSI Europe Expert Group has recommended that the TTC principle can be used for substances that are present in food in low concentrations, which lack toxicity data, but for which exposure assessment can provide reliable intake estimates. The decision tree provides a structured approach that allows the consistent application of the TTC principle in a risk assessment context.

Its main applications are anticipated to be in the following situations:

- As a preliminary step in the safety assessment of chemicals present at low concentrations in food.
  Substances expected to have generally low concentrations in food and for which toxicity data are often lacking are flavourings, substances migrating from food contact materials, some natural contaminants, contaminants of environmental origin and substances used at low concentrations in a very limited number of food items which are consumed in very low quantities.

- In the setting of priorities, depending on the level of concern, for more in depth risk assessment.
  Use of the decision tree will help identify those substances for which exposure estimates exceed the relevant TTC and which may therefore require further information for risk assessment.

- In the setting of priorities, depending on the level of concern, for further toxicological testing.
  Substances for which exposure estimates show that they do not exceed the relevant TTC can be considered as low priority for further testing, while substances for which exposure estimates exceed the TTC may require prioritising for further testing,

depending on their structure and the degree to which they exceed the relevant TTC.

- In setting priorities for analytical method development.
  Substances for which present analytical methods do not allow accurate measurement at concentrations that are relevant to their particular structural class TTC, may point to the need for more sensitive analytical methods.

- In setting priorities for more refined intake data.
  Substances for which intake estimates are close to the relevant TTC but contain some uncertainties, may require more refined estimates of intake.

Exposure data needed for application of the TTC principle

A critical aspect of the appropriate application of the TTC principle is the necessity for reliable exposure data. As the TTCs are expressed in terms of microgrammes per person per day, exposure estimates need to be similarly expressed or related to body weight. As use of the TTC approach could mean that consumers are exposed via the diet to substances on which there is little or no toxicity information as long as exposures are below the relevant threshold value, it is important to ensure that exposure estimates are as complete and as accurate as possible, or build in adequate conservatism to account for possible underestimates.

It is necessary to consider not only exposure from food, but also other possible sources of exposure (air, water, consumer products, workplace). In foods, the substance may be widely distributed across many items in the diet or present only in a restricted number and type of food items. Food intake data and analytical data on levels in foods, or information on uses or occurrence in foods, need to be sufficiently robust and comprehensive to enable reliable estimates of intake to be made. Analytical methods used to determine levels in foods need to be sufficiently sensitive to detect low concentrations, relative to the human exposure thresholds, otherwise a large number of 'non-detect' values might give a misleading picture of total exposure. Since particular groups in the population may consume different amounts of specific foods, food intake data may need to be sufficiently detailed to enable these groups to be examined separately, for example, by age, gender or ethnicity. For infants and children in particular, because of their smaller size, food intake expressed on a body weight basis is generally higher than that for adults. Infants and children may also consume greater absolute amounts of some types of foods (e.g. fruits) than adults because of dietary preferences. They may also have a less varied diet than adults (e.g. a high consumption of infant formula or processed baby foods) which has considerable implications for intake estimates.

It is assumed that an adult person may consume 1.5 kg of food and 1.5 kg of beverages per day (for children see "Adjusting TTCs for body weight", page 25). As an example, for a substance that occurs uniformly in the whole diet and is in Cramer structural class I, for which the TTC is 1800 microgrammes/day, the TTC would be reached if there was a concentration of 600 microgrammes/kg in the whole diet. If the substance was only present in beverages, then a concentration of 1200 microgrammes/kg would reach the TTC. If the substance was present in only one food item, consumed in daily amounts of no more than 100 g, then the TTC would be reached by a concentration of 18000 microgrammes/kg. The situation is more complex when a substance is present only in a few food items consumed by a limited number of consumers (e.g. candies consumed by children). In such cases, the per capita assessment should consider the number of consumers exposed as a proportion of the whole population, in order to avoid underestimation of individual intake. Obtaining such data can be resource-intensive and new methodologies, such as post-marketing estimation of the number of consumers could be helpful.

ISSUES AND LIMITATIONS

Allergenicity

Allergic reactions to food are common, sometimes life-threatening and of public concern. Once an individual is sensitised to a particular food or chemical, allergic reactions can occur from exposure to very low amounts. The ILSI Europe Expert Group therefore gave consideration to whether any threshold could be established for allergic reactions. It was concluded that whilst thresholds undoubtedly exist, they have not been established so far, even for common allergens, and are known to vary with each individual and within an individual over time. ILSI Europe's examination of potentially sensitive endpoints, described earlier, included immunotoxicity, but excluded allergic responses, which are a special sub-category of immune reactions. Thus, although the TTC approach does take account of substances causing immunotoxicity other than allergenicity, it cannot be used to assess the concern for allergenicity.

Accumulation

Accumulation describes the process by which the amount of a substance in the body (the 'body burden') increases with repeated exposure. This occurs when the amount ingested exceeds the body's capacity to eliminate it via metabolism and excretion in urine, faeces and expired air. If a substance is not readily metabolised and is also very soluble in fat it will accumulate if exposure is frequent. For such substances there may be considerable differences between species in rates of elimination from the body and the differences may be greater than the safety or uncertainty factor employed in risk assessment to take account of species differences in metabolism and elimination. The TTC principle should not be applied to such substances.

An example is, TCDD (2,3,7,8-tetrachloro-dibenzo-*p*-dioxin, the chemical released in the Seveso disaster), which is eliminated much more rapidly by rodents than by humans. TCDD belongs to a group of chemicals known as the polyhalogenated dibenzo-*para*-dioxins. These are closely related structurally to polyhalogenated dibenzofurans and polyhalogenated biphenyls. Even the low TTC for Cramer structural class III compounds is not appropriate for substances like these which accumulate in the body. Furthermore, such chemicals were not included in the database of Munro and colleagues on which the TTC approach is based. Accordingly, these chemicals are not appropriate for assessment using the TTC approach.

Heavy metals, such as cadmium, can also accumulate in the body, and they were not included in the database of Munro and colleagues. Thus, the TTC approach should not be used for the assessment of metals in elemental, ionic or organic forms. Moreover, for a number of heavy metals it would be unnecessary as there is a vast toxicological literature on the effects of exposure to metals such as lead, cadmium and mercury.

Other compounds present in the diet may also show marked differences between species in their potential to accumulate in the body (e.g. the naturally occurring fungal toxin, ochratoxin A). If this is known then application of the TTC approach is not appropriate.

Endocrine disruption

An important current issue in toxicology is the identification and risk assessment of substances that act to perturb the endocrine system which produces the numerous hormones in the body. Chemicals that directly or indirectly affect either the structure and/or the function of the hormone producing glands or the parts of the brain that control them are known as

'endocrine disrupters'. Exposure during development, either before birth or after, is a particularly vulnerable period for endocrine disruption. The issue of whether endocrine disrupters may be active at very low exposures is an unresolved, ongoing debate among scientists. In view of the uncertainties, it would be premature to include low-dose, endocrine-mediated effects in the TTC approach. Moreover, it is likely that for any chemical already identified as a potential endocrine disrupter, toxicological data will be available which can be used to perform a more comprehensive risk assessment.

Uncertainties, limitations and strengths of the databases

Uncertainties

In any method of risk assessment, there are inherent uncertainties in toxicity, exposure and extrapolation aspects, which risk assessors need to identify and, if possible, quantify. The TTC approach is little different, having its own particular uncertainties, but in this case, any significant uncertainty in exposure estimates would preclude use of the TTC approach. The uncertainties of the TTC approach relate mainly to:

- the validity of assuming the likely toxicity of a known chemical structure, based on toxicity information from similar chemicals falling into one of three broad structural groups;
- the validity of the factor of 100 applied to the 5th percentile NOELs in the database to derive the numerical values for the TTCs;
- whether the database on chemical toxicity used to derive the various TTCs is sufficiently comprehensive to be representative, both of chemical structures and of toxic effects;
- the validity of extrapolating to a 'virtually safe dose' for genotoxic carcinogens, by applying a mathematical model to laboratory animal data.

How the TTC approach addresses the uncertainties

- *the validity of assuming the likely toxicity of a known chemical structure, based on toxicity information from similar chemicals falling into one of three broad structural groups*

The concept that toxic activity and potency bear a relationship to chemical structure has evolved over the years and has been widely studied and broadly confirmed. Some of this work was mentioned earlier in describing the origins of the TTC approach. Three aspects of chemical structure are important - the ease with which particular structures are metabolised (and hence eliminated from the body), whether the structure occurs naturally in the body or is a normal product of intermediary metabolism, and the presence or absence of particular chemical groupings within a structure that are known to cause toxicity.

These three elements were used by Cramer and colleagues to devise their original decision tree in 1978, in which they proposed three main structural classes. The examination of a large number of these three structural classes of chemicals in relation to their NOELs by Munro and colleagues confirmed the expected relative ranking of low, moderate and higher toxicity to structural classes I, II and III, respectively. It is of course recognised that due to the biological complexities of living organisms, including humans, such structure-activity predictions may occasionally turn out to be wrong. It is for this reason that the NOELs used to derive the TTCs are divided by a factor of 100 to provide an extra margin of safety, in case a particular chemical of unknown toxicity does not behave as predicted.

In the case of structural alerts for those substances likely to represent some of the most toxic at low exposures, i.e. those that are potentially genotoxic, this has been the most intensively studied aspect of structure-activity relationships in toxicology and it is widely accepted among scientists that predictions based on these types of alert are robust. Examination of structures for these alerts is incorporated at an early stage in the TTC decision tree.

- *the validity of the factor of 100 applied to the 5th percentile NOELs in the database to derive the numerical values for the TTCs*

As explained above, the factor of 100 was selected to provide an extra margin of safety over and above the 5th percentile NOEL for each structural class. This factor was chosen because historically a factor of 100 has also been used to derive Acceptable Daily Intakes for individual compounds from their compound-specific NOELs (see earlier). Although the original selection of a factor of 100 some fifty years ago was based on scientific judgement rather than detailed evidence, considerable support has emerged in recent years for the use of this figure from studies on human and animal metabolic differences and species differences in adverse responses to chemicals including drugs (toxicokinetics and toxicodynamics). Thus, it is now widely accepted that use of a factor of 100 when extrapolating from NOELs derived from animal studies to predicted safe intakes for humans should generally provide a reasonable margin of safety.

- *whether the database on chemical toxicity used to derive the various TTCs is sufficiently comprehensive to be representative, both of chemical structures and of toxic effects*

This issue has been addressed in several publications of Munro, Cheeseman and their colleagues, and by the ILSI Europe Expert Group, in response to comments by other regulatory and research scientists that some endpoints of toxicity, that might be particularly sensitive, were insufficiently represented within the original database. Accordingly, particular attention has been paid to the endpoints of carcinogenicity, neurotoxicity including developmental neurotoxicity, other developmental toxicity (teratogenicity) and immunotoxicity and evidence provided to show that they are adequately represented within the updated database. For substances which are not represented at all or not well represented within the database, the ILSI Europe Expert Group has recommended that they are not evaluated using the TTC approach. These include high potency carcinogens, metals and polyhalogenated ring-structured compounds. Similarly, substances with particular toxicological features such as endocrine disruption at low doses or potential allergenicity are also excluded.

- *the validity of extrapolating to a 'virtually safe dose' for genotoxic carcinogens, by applying a mathematical model to laboratory animal data.*

This perhaps represents the most contentious area of uncertainty in the TTC approach. As explained earlier, under "Predicting the risk of cancer", not all scientists agree that the application of mathematical modelling to the results from laboratory animal studies on cancer, in order to derive a virtually safe dose, gives an accurate prediction of likely risks for humans. But whatever their viewpoint, they generally agree that the mathematical models used are highly conservative and so are unlikely to underestimate risks to humans. Thus, in utilising this approach to derive a TTC for carcinogens, together with additional decision-tree steps to exclude high potency carcinogens, the TTC approach is very conservative.

Dealing with mixtures

In principle, the TTC approach could be used for dealing with mixtures of substances which have similar toxic mechanisms of action at the biochemical level. If consumers simultaneously ingest a food or foods containing potentially toxic substances that act in the same way (e.g. carrots containing residues of more than one organophosphate pesticide), it would be possible to sum their exposures/intakes and compare the combined exposure/intake with the relevant TTC, provided they were of similar potency or were corrected to a similar potency. If the combined intake was below the TTC, this would indicate that the substances would not be expected to be a safety concern.

If mechanisms of action of the substances in the mixture are known to be dissimilar, then the TTC approach can be followed to assess each individual substance, one by one. Similarly, with a mixture of impurities, some of known structure and some unknown, if the level of the impurity present in the highest concentration is below the human exposure threshold value for structural class III (the class most suspect for toxicity), then it can be assumed that all other impurities, present at lower concentrations, would also be below that threshold.

Application of the TTC approach to subpopulations

Potentially vulnerable subpopulations

An important issue to consider in deciding whether it is appropriate to apply the TTC approach is the nature of the subpopulation(s) predicted to be most at risk because of their exposure. Some subpopulations may be considered vulnerable, not only because of higher exposure, but because of potentially greater sensitivity to toxicity. Such groups might include:

- the elderly due to a reduced capacity for metabolism and excretion of chemicals;
- the very young with immature metabolising capacity for some, but not all, chemicals;
- pregnant women because of vulnerability of the embryo and foetus;
- persons of any age who have a particular genetic makeup (called a 'genetic polymorphism') that impairs or alters the way they handle and respond to potentially toxic substances.

The database used to identify the NOELs for derivation of the numerical TTC values includes toxicity studies on ageing animals, pregnant animals, newborn, very young and juvenile animals. Thus, most of the scenarios identified above are represented in the database and the derived TTCs should cover these subpopulations. In addition, the use of a factor of 100 to derive a TTC from a NOEL takes into account potential metabolic differences between laboratory animals and humans and differences between individual humans.

Exceptional subpopulations which might not be covered are those with certain genetic polymorphisms that have profound effects on metabolic capacity and metabolic pathways. Present knowledge on the nature and prevalence of these polymorphisms in different ethnic groups is far from complete, but already a few are known which would result in certain substances being handled in the body in ways which would considerably erode the 100-fold margin of safety built into the TTC values. At present, it is not possible in most risk assessment situations to identify these potentially vulnerable people. However, this uncertainty applies equally to other, conventional risk assessment approaches.

Adjusting TTCs for body weight

The numerical values for the various TTCs calculated by the ILSI Europe Expert Group are based on a 60kg adult (see Box 4). Where a substance occurs in foods consumed by infants and/or children, users of the TTC approach may wish to consider whether intake estimates should be calculated separately for these groups and compared to the relevant TTC, adjusted for bodyweight. For example, for a substance in Cramer class I, the TTC for a 10 kg, 12-month-old infant would be 300 microgrammes/day instead of 1800 microgrammes/day (i.e. 1800 x 10/60), after adjustment for body weight.

CURRENT APPLICATIONS OF THE TTC CONCEPT

FDA experience

Since the implementation of the Threshold of Regulation in the USA in 1995, applying to food packaging migrants present in the diet at levels below 0.5 ppb, the FDA has dealt with 183 applications under this regulation and issued 78 exemptions using this concept. Applications are considered under an abbreviated review process in order to ascertain whether the dietary concentration that would result from the intended use is at or below the threshold level and that there is no reason to suspect, based on test data or chemical structure, that the substance may be a carcinogen. Although the Threshold of Regulation is designed to protect against all types of toxicity including carcinogenicity, under US law the FDA is not allowed to regulate known carcinogens as food additives (in the USA food contact materials are regulated as indirect food additives). The main reason for rejection of an application has been the submission of inadequate exposure data. The FDA has commented that the Threshold of Regulation has been extremely useful because it is based on sound science and can be applied rationally, consistently and effectively, case by case. It is estimated to have reduced the workload of the agency by around 15%.

JECFA experience

The Joint FAO/WHO Expert Committee on Food Additives, known as JECFA, first considered using a new procedure for the safety evaluation of flavouring agents in 1995. JECFA was tasked with the evaluation of over 2500 flavouring substances in current use. For many

individual flavouring substances, no toxicity or metabolic data existed. The Committee agreed that in view of the large number of substances requiring safety evaluation and the fact that, for the majority of flavouring agents, human intakes are relatively low and self-limiting, a different approach from that normally used for food additives should be followed.

The proposed procedure put forward by Munro and colleagues (1999) was based on the TTC concept, i.e. the three Cramer structural classes and their respective TTCs. It involved integration of per capita intake data in relation to the human exposure thresholds, with information on structure-activity relationships, metabolism and toxicity. As many flavouring substances are closely related structurally, this procedure was considered promising, since it would allow flavourings to be evaluated in chemical groups, not only applying the TTC principle, but also incorporating, where available, any metabolic and toxicity information on any of the flavourings in a group. After applying the proposed procedure to the evaluation of 3 groups of flavouring substances in 1996, JECFA adopted the new procedure for safety evaluation (WHO, 1997). Since then JECFA has evaluated in excess of 1400 flavourings using this scheme. The JECFA recognised the limitation of per capita intake estimates, especially for estimating exposure of individual groups of consumers consuming particular foods, and further methodological developments of this aspect are under discussion in JECFA (WHO, 2001).

Use by other organisations

The TTC principle is also used by the European Medicines Agency (EMEA) to assess genotoxic impurities in pharmaceutical preparations (1). It was moreover used by the former EC Scientific Committee on Food and is now used by the European Food Safety Authority to evaluate flavouring substances (2). The TTC principle has furthermore been endorsed by the WHO International Program on Chemical Safety for the risk assessment of chemicals (3) and also by the EU Scientific Committee on Toxicology, Ecotoxicology and the Environment (4).

(1) The European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Guideline on the limits of genotoxic impurities. CPMP/SWP/5199/02. London, 23 June 2004. Available at: www.emea.eu.int/pdfs/human/swp/519902en.pdf

(2) European Food Safety Authority. Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. Opinion on Flavouring Group FGE.03 Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an orthoester of formic acid, from chemical groups 1 and 2. Opinion expressed on 7 October 2004. Available at: www.efsa.eu.int/science/afc/catindex_en.html

(3) International Program on Chemical Safety, World Health Organization. WHO Food Additive Series 35, WHO, Geneva, Switzerland.

(4) Bridges, J. Strategy for a future chemicals policy. The view of the Scientific Committee on Toxicology, Ecotoxicology and the Environment (CSTEE). Available at: www.eutop.de/chp/Download/BridgesRe.doc

SUMMARY AND CONCLUSIONS

The world of chemicals to which humans may be exposed is very large. Some of these chemicals are closely regulated on the basis of safety evaluations performed by scientists, using extensive information on toxicity. This is the case for chemicals added deliberately to food or known to be present in food, such as food additives, pesticides and veterinary drugs. However, for many other substances also present in the diet, such as contaminants (natural or man-made), flavouring substances and chemicals arising from food processing, including cooking, there may be little or no toxicity information. The threshold of toxicological concern concept has been developed as a tool to enable a preliminary assessment of the likely risk from exposure to a known amount of a substance of known chemical structure, but of unknown toxicity.

The threshold of toxicological concern (TTC) refers to the establishment of human exposure threshold values for chemicals, below which there would be no appreciable risk to health. Extensive exploration over several years of existing data on the relationship between chemical structure and toxic effects has enabled three broad classes of chemical structure to be defined and a numerical value for a TTC for each class to be established.

The ILSI Europe Expert Group on the TTC principle has developed a decision tree which provides a structured approach that allows the consistent application of the TTC principle for risk assessment of substances of unknown toxicity present in the diet for which there are reliable exposure estimates. In the application of the TTC concept to safety evaluation of a chemical present in the diet (and possibly elsewhere), the intake or exposure to the chemical is compared to the relevant TTC for its structural class. If the intake or exposure is below the relevant TTC, this indicates that there is unlikely to be any safety concern. If the intake or exposure exceeds the relevant TTC, this indicates that further information, including chemical-specific toxicity data, may be needed to perform a risk assessment. Thus, the TTC approach offers a tool for risk assessors and risk managers to prioritise chemicals in need of further evaluation or additional safety data.

The ILSI Europe Expert Group has also identified certain types of substances for which the TTC approach should not be used. These include heavy metals, substances that accumulate, such as dioxins, allergens and endocrine disrupters with low-dose effects.

Useful applications of the TTC approach are envisaged to include situations when there is a new discovery of the presence of a contaminant in food, for which there is no toxicological information, and in setting priorities for testing among large functional groups of chemicals to which exposure is generally very low, such as flavouring substances and substances used in food contact materials. The TTC concept is already being applied by organisations such as the US Food and Drug Administration in the regulation of food contact materials and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in evaluations of flavouring substances.

The wider use of such a tool would have benefits for industry, regulatory authorities and consumers because it enables the world's limited resources for toxicity testing and safety evaluation to be focused on exposures to chemicals which may pose a real threat to human health. By eliminating the need for unnecessary toxicity tests, it would also reduce the number of animals used in laboratory testing, and this would be welcomed by both the scientists involved and the general public.

GLOSSARY

Acceptable Daily Intake (ADI): Estimate of the amount of a substance in food or drinking water, expressed on a body mass basis (usually mg/kg body weight), which can be ingested daily over a lifetime by humans without appreciable health risk.

Acute toxicity: Adverse effects occurring within a short time (usually up to 14 days) after administration of a single dose of test substance, or after multiple doses administered within 24 hours.

Adverse effect: Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences.

Allergen: A substance which provokes an allergic response.

Allergy: An inappropriate and exaggerated immune response.

Carcinogen: A substance capable of inducing cancer.

Carcinogenesis: The complex, multistep process of cancer causation.

Chromosome: In the cell, DNA is tightly packaged together with particular proteins into structures called chromosomes. Packaging into chromosomes enables the organised assortment of genes into daughter cells upon cell division, as well as playing a role in controlling gene expression.

Chronic toxicity: Adverse effects following continued exposures over an extended period of time (more than 10 per cent of the lifespan).

Decision tree: A structured approach for making step-by-step decisions about individual chemicals.

Developmental toxicity: Adverse effects on the embryo and/or foetus following exposure during the prenatal period.

Dioxins: A group of environmentally persistent substances with structures containing three connected rings made up of carbon, oxygen and either chlorine, or chlorine and hydrogen. Dioxins may interact with the Ah receptor in the body to produce cancer, reproductive toxicity and immune system effects.

DNA (Deoxyribonucleic acid): A long molecule made up of repeating units (each unit contains deoxyribose, a sugar, a phosphoric acid and one of four bases) joined together in a particular order. Each DNA molecule consists of two strands in the shape of a double helix. Genes are made of DNA, and are responsible for the transfer of genetic information from one cell/generation to the next.

Effect level: The concentration or amount of an agent, found by study or observation, that causes alteration of morphology, functional capacity, growth, development or life-span of the target.

Endocrine disrupter: A substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

Endocrine system: Organs and tissues in the body which produce hormones.

Exposure: Concentration or amount of a particular chemical agent that reaches the target population, organism, organ, tissues or cell, usually expressed in numerical terms of substance concentration, duration and frequency.

Genotoxicity: Ability to cause damage to genetic material. Such damage may lead to mutations and cancer.

Hormone: A chemical substance produced in one part or organ of the body that initiates or regulates the activity of an organ or group of cells in another part of the body.

Human exposure threshold (of toxicological concern): A generic value for human exposure to a chemical falling within a particular structural class, below which there would be no appreciable risk to health.

Immunotoxicity: Adverse effects on the structure and function of the immune system, or in reaction to immune challenge.

***In silico*:** Data generated and analysed using modelling and information technology approaches.

***In vitro*:** Literally "in glass", referring to a study in the laboratory usually involving isolated organ, tissue, cells or cellular fractions.

***In vivo*:** In the living body, referring to a study performed on a living organism.

Long-term toxicity study: A study in which animals are observed during the whole life span (or the major part of the life-span) and in which exposure to the test material takes place over the whole observation time or a substantial part thereof. The term chronic toxicity study is used sometimes as a synonym for "long-term toxicity study".

Margin of safety: The ratio of the no-observed-adverse-effect level (NOAEL) for the critical effect to the theoretical, predicted or estimated exposure dose or concentration.

Mutation: The change in the DNA sequence caused by damage by a mutagen, or by errors in cellular processes that may occur during cell division. Some mutations have no effect on the function of the genes in which they occur, while others inactivate or change the activity of the genes. Some mutations are detrimental to the organism, a few are beneficial. Mutations are a source of variation between individuals, and are a driving force of evolution.

Neurotoxicity: Adverse effects on the structure and function of the nervous system and behaviour.

No observed adverse effect level (NOAEL): The greatest concentration or amount of an agent, found by study or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development or life-span of the target.

No observed effect level (NOEL): The greatest concentration or amount of an agent, found by study or observation, that causes no detectable alteration of morphology, functional capacity, growth, development or life-span of the target.

Polymorphism: A single gene trait that is caused by the presence in the population of pairs of differing but related genes, resulting in more than one phenotype within the population, the less common gene occurring in more than 1% of individuals.

Potency: The extent, relative to dose, to which a chemical is active with respect to a specific particular toxic endpoint.

Pseudo-acceptable daily intake (PADI): An intake for a substance derived by applying a 1000-fold uncertainty factor to the lowest low-effect level for non-carcinogenic endpoints.

Reproductive toxicity: Adverse effects on fertility and reproduction.

Risk: The probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent.

Risk assessment: A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system.

Safety: Practical certainty that adverse effects will not result from exposure to an agent under defined circumstances. It is the reciprocal of risk.

Safety factor: A factor applied to the no-observed-adverse-effect-level to derive an ADI. The value of the safety factor depends on the size and type of population to be protected and the quality of the toxicological information available.

Short-term toxicity study: An animal study (sometimes called a subacute or subchronic study) in which the effects produced by the test material, when administered in repeated doses (or continuously in food or drinking water) over a period of about 90 days (less than 10 per cent of the lifespan), are studied.

Structural alert: A particular chemical grouping within a chemical structure which is known to be associated with a particular type of toxic effect, e.g. genotoxicity.

Threshold: Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur.

Threshold of Regulation: A policy of the US Government allowing regulation of food contact materials present only at very low levels in the diet by an abbreviated procedure.

Threshold of Toxicological Concern (TTC) concept: A concept that proposes human exposure threshold values for groups of chemicals, below which there would be no appreciable risk to health.

Tolerable Daily Intake (TDI): Regulatory value equivalent to the Acceptable Daily Intake, used for food contaminants, i.e. an estimate of the amount of a substance in food or drinking water, expressed on a body mass basis (usually mg/kg body weight), which can be ingested daily over a lifetime by humans without appreciable health risk.

Toxicity: Inherent property of an agent to cause an adverse biological effect.

Toxicodynamics: Description of the interaction between a toxic agent and the target tissue on which it has an adverse effect.

Toxicokinetics: Description of the absorption, distribution, metabolism and excretion of a chemical in the body.

Uncertainty factor: An alternative description of safety factor, which is being used increasingly because it indicates that the factor is to allow for uncertainties in the risk assessment process.

Virtually safe dose (VSD): A human exposure over a lifetime to a carcinogen which has been estimated, using mathematical modelling, to result in a very low incidence of cancer, somewhere between zero and the specified incidence, e.g. 1 cancer in a million people.

FURTHER READING

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