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Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements

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Abstract

There is a growing interest by both consumers and industry for the development of food products with 'functional' properties, or health benefits. These products may take the form of dietary supplements or of foods. The health benefits are given by particular ingredients, and in many cases these are derived from botanicals. The variety of plants providing these functions is large, ranging from staple food sources such as cereals, fruits and vegetables, to herbals as used in traditional medicine. The food or ingredient conferring health properties may consist of the plants themselves, extracts thereof, or more purified components. The scientific literature is abundant with articles not only on the beneficial properties, but also on possible adverse health effects of plants and their components. The present report discusses the data required to determine the safe use of these types of ingredients, and provides advice on the development of risk assessment strategies consistent with due diligence under existing food regulations. Product specifications, composition and characterisation of standardised and authentic materials, documented history of use and comparison to existing products (taking into account the effect of industrial processing), description of the intended use and consequent exposure are highlighted as key background information on which to base a risk evaluation. The extent of experimental investigation required, such as in vitro, animal, and/or human studies, depends on the adequacy of this information. A decision tree is presented as an aid to determine the extent of data requirements based on product comparison. The ultimate safety in use depends on the establishment of an adequate safety margin between expected exposure and identified potential hazards. Health hazards may arise from inherent toxicities or contaminants of the plant materials, including the mechanism of the intended beneficial effect. A lower safety margin may therefore be expected than for food ingredients or additives where no physiological effects are intended. In rare cases, post launch monitoring programmes may be envisaged to confirm expected exposures and adequacy of the safety margin. This guidance document was elaborated by an expert group of the Natural Toxin Task Force of the European Branch of the International Life Sciences Institute—ILSI Europe and discussed with a wider audience of scientists at a workshop held on 13–15 May 2002 in Marseille, France.

Keywords: Botanical preparations; Health benefits; Risk assessment; Margin of safety

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1. Introduction

There is an increasing number of products with perceived and real health benefits available on the food market. These products include foods, fortified foods (with minerals, vitamins and/or botanical extracts) and dietary supplements. The market for these health products is growing rapidly. In the USA, sales increased by more than 40% between 1992 and 1996 to reach a global value of more than 14 billion US dollars in 1996 (Greger, 2001). Similar trends were observed in Japan and Western European countries (Greger, 2001).

The development of this market has been linked to several drivers (Greger, 2001; NNB, 2002). Firstly, consumers are increasingly taking charge of their health and, therefore, there is a public demand for health-related products. Secondly, the scientific understanding of the beneficial health impact of bioactive components of foods and dietary supplements has improved significantly and has been extensively communicated to the public. Thirdly, many food sectors, especially agricultural production and processing, are suffering economic difficulties; for many regions of the world the development of added-value crops and agricultural ingredients with health applications is thought to be one of the solutions to respond to this economic pressure.

Botanical products are an important part of the health food market. Functional botanical products cover a broad range of materials, and various types of products have been developed and promoted (NNB, 2002). They include whole foods with benefits beyond basic nutrition, such as cranberry against urinary tract infections or whole grain oats for cholesterol-lowering. Others are dietary supplements, usually marketed in pharmaceutical-like unit dose form such as tablets, capsules, powders or drops. These products may be derived from plants used as primary food sources such as fruits, vegetables, cereals or legumes. Typical examples of such products would be soy extracts for estrogenic activity of isoflavones and tomato extracts containing the antioxidant lycopene. Functional botanical ingredients may also be derived from plants used as secondary food sources such as spices, tea, coffee or cocoa. Typical products in this category would include rosemary, green tea and garlic extracts. Other botanical products consist of, or may be derived from, plants traditionally applied for health maintenance purposes such as herbal teas. In addition, there is an increasing interest amongst consumers and product developers regarding the use of traditional medicinal products from all around the world (New Nutrition Business, 2002; GAO, 2000). These plants are traditionally used in specific cases of either reduced health or disease conditions. Some of them have been considered for food applications (e.g. *Ginkgo biloba*, ginseng extract in beverages or cereal bars, St. John's wort or *Echinacea*). Materials with no history of human exposure have also been used as a source of functional ingredients. For example, stanols from paper industry by-products have been applied in cholesterol-lowering products (Turnbull, 1999). Although functional ingredients are intended to provide health benefits for consumers, their increasing use in dietary supplements and food products has triggered significant concerns amongst scientific and regulatory communities. These products often fall into a grey/undefined regulatory area between food and medicines (Kwak and Jukes, 2001a,b). Furthermore, there is debate regarding the information required to document beneficial effects and how to communicate such benefits through claims (Clydesdale, 1998; Roberfroid, 1999). There have been a number of recorded cases of intoxications with botanical products raising the issue of their safety (Burkhard et al., 1999; Haller and Benowitz, 2000; Nortier et al., 2000; Ernst, 2002). However, there have been few initiatives regarding the scientific data necessary to establish the safety of functional botanical products when used in food and dietary supplements.

Several cases of intoxication reported with botanical products have been shown to result from contamination with other plant species or through misidentification of species (e.g. see Nortier et al., 2000; De Smet, in press). This indicates that species identification is a critical factor, especially for botanicals where related and more toxic species/genera are known. The part of the plant used as a source material for the product has to be defined. This is of particular importance if toxic constituents are present in higher concentrations in a part of the plant that is not normally consumed. For example, the roots of the comfrey plant (used in some herbal preparations) contain much higher concentration of pyrrolizidine alkaloids than the leaves (used to prepare infusions) (Betz et al., 1994). The safety assessment of plant preparations is complicated by important sources of variability in composition, such as the natural biological variances in plant chemicals and the impact of production processes. Various types of extraction can be applied to raw materials resulting in significant changes in the quantities and proportions of key components affecting safety and benefit. In addition, the component(s) responsible for the claimed benefit may not be the same as that (those) giving a risk of adverse effects, and each may be under different metabolic regulation within the plant, and be affected differently by environmental influences. Consequently, the safety of the preparation may vary independently of the benefit. The effects of combinations of different alkaloids cannot be reliably predicted from the data for single alkaloids (Couet et al., 1996). The problem of the compositional variability of botanical preparations was recently highlighted in the case of ginseng dietary supplements (Harkey et al., 2001); differences in concentrations of specific key chemical markers of up to 200-fold were

observed among 25 products obtained in health food stores.

The biological activity of functional botanical products gives rise to other important safety considerations. There are internationally accepted standard approaches for the safety evaluation of food constituents such as food additives and pesticide residues, which are generally based on a series of in vitro and in vivo studies in animals (WHO, 1987). This leads to the determination of health-based guidance values indicating a safe level of exposure to compounds in foods (e.g. the Acceptable Daily Intake or ADI). The derivation of these values incorporates the use of large uncertainty factors, and provides a wide margin of safety. However, a similar approach may not be appropriate in the case of functional foods, because the margin between adverse physiological effects, and the desired beneficial effect may be lower than the usual safety margin and for whole foods it may not be feasible to feed animals at levels high enough to derive large safety factors as is possible with purified material. Other aspects that need consideration include the fact that a product with a documented benefit for a specific part of the population may not necessarily be safe for another part of the population, raising the issue of how to control exposure to minimise the risk for other non-targeted population groups. Also there have been many reports documenting clinically significant interactions between botanical products and medicines.

Whatever the regulatory status or claim, products must be safe for their intended use. Compared with other food-related materials, the safety assessment of functional botanical products raises a series of specific issues. The aim of this document is to provide a general framework in which to establish the safety of functional botanical products for human consumption. It is not intended to address claim substantiation or regulatory issues.

The present guidance focuses both on botanical preparations as ingredients and on the actual food application. The safety of botanicals bred with the assistance of genetic engineering technology, or which would be classed as Novel Foods are addressed by existing guidelines. However, aspects of the present document would be relevant when assessing the safety of such products with physiological (or functional) health benefits.

Substances that are intentionally added to food must be demonstrated to be safe. The general paradigm of risk assessment, namely (a) hazard identification, (b) hazard characterisation, (c) exposure (intake) assessment, and (d) risk characterisation has received wide acceptance in the food area (Food Safety in Europe, 2002, Renwick et al., in press) and is considered a good basis for the safety evaluation of botanical products applied to food and dietary supplements. Most of the botanical preparations (raw materials and sometimes finished products) falling under the scope of the present guidelines are likely to have a certain history of human exposure either from foods or from other sources, such as health-related products (e.g. herbal teas, herbal medicines). It is proposed that the assessment of a botanical preparation should exploit all existing knowledge and should compare the material under study with one or several adequate comparators with a history of human use. Such an approach is considered as a good starting point to identify potential hazards and to determine the need for further information such as experimental toxicological data.

Comparison requires detailed background and compositional information on the new product and comparators, an assessment of the history of use of the traditional products and a description of the intended use and consequent exposure of the new products. This approach served as a framework to design the present guidelines. The guidance paper was developed by an expert group of the Natural Toxin Task Force of the European Branch of the International Life Sciences Institute—ILSI Europe and discussed with a wider audience at a workshop held on 13–15 May 2002 in Marseille, France.

2. Product specification/characterisation

The safety evaluation of food ingredients and additives requires data on the material of commerce, i.e. the material that will be used in the final food product or food supplement for human consumption. It is essential that the material should be well identified and characterised. There are three aspects to the production of a consistent material:

1. Identification of starting material
2. Process applied to starting material
3. Standardisation and nature of final product

The extent of information necessary for an appropriate characterisation of the plant material should be decided on a case-by-case basis, taking into account all available relevant knowledge. The history of use of the raw material, the history of food application, the manufacturing process, the expected exposure to the new product and the anticipated levels of toxicants in the finished product as consumed are key information requirements to prioritise the type and extent of data necessary.

The biological properties of botanical preparations should be reproducible from batch to batch and, therefore, product standardisation is necessary. The composition of botanical preparations may need to be adjusted with appropriate ingredients to comply with existing standards. Standardisation may be based on a level of one or several active constituents, on a phytochemical marker that might not be responsible for the biological

activity or on a fixed plant/extract ratio (PER) (indicating that the botanical preparation itself is regarded as the active constituent). Constituents known to be responsible for adverse effects should be included as criteria for standardisation. Markers that are not related to the biological activity, either beneficial or adverse, should be characteristic and typical for the species. In cases where appropriate marker compounds or active constituents are not known, a chemical fingerprint of the material would be required with limits on the range of variability. A variety of analytical 'fingerprinting' approaches may be used to characterise a botanical preparation. For chemically complex botanicals, several chemical techniques are normally used to adequately characterise the material. While some techniques, such as HPLC can yield profiles of characteristic components, such techniques are unlikely to be sufficient alone. For example, ginseng which was selected for toxicological evaluation by the US National Toxicology Program (NTP) was characterised by a battery of analytical techniques including: water/volatile content, concentrations of organophosphate insecticides, nitrosamines, total and individual amino acids, selected anions, total and ethanol-soluble carbohydrate, simple sugars and disaccharides, molecular weight distribution of the glycans, the identity of all possible UV-detectable components by HPLC–MS, ginsenoside profile by HPLC–UV, the volatile organic profile by GC–FID and the identity of all possible components by GC–MS (Graves et al., 2003). Fingerprinting should be applied in conjunction with historical product data that define the normal range of variability of the authentic material. Appropriate fingerprint methods can be used to confirm authenticity and to detect adulteration (see Technical Annex A).

2.1. Recommendations

Information appropriate to characterise adequately the botanical preparation and end product is provided in Table 1. Data should be obtained on a sufficient number of batches to allow an evaluation of the natural variability and the establishment of appropriate specifications. Sampling plans should be designed with appropriate consideration for analytical and inter-batch variability in the botanical preparation and end-product.

3. History of use

The history of use (food or other) of the material under consideration (raw material and finished product) may provide valuable indications of safety. In fact the EU Novel Foods Regulation defines a novel food as a food without a history of use in the EU (EC, 1997b). The traditional and/or established use of the botanical preparation as a medicinal product is also an important factor to consider. In general, history of use includes information on period, nature and objective of use. For herbal products, two terms are distinguished: traditional use for which limited scientific documentation are available and established use, which are supported by scientific publications (Bast et al., in press).

Most of the foods commonly eaten have not been investigated systematically with respect to their impact on consumer health. Generally, they are presumed safe unless a significant risk has been identified in humans. However, it should be emphasised that the absence of evidence of toxicity is not the same as evidence of the absence of toxicity. Without specific investigations, only acute and severe adverse effects are likely to be identified. To demonstrate the safety of a food requires specific intake information together with data on health impact. Although epidemiological observations, clinical and other case reports and/or experimental human studies may exist for a restricted number of foods e.g. coffee (Schilter et al., 2001), health information is lacking for most food plants.

For botanicals used for health purposes, information obtained from historical or ethnographical sources related to traditional applications usually refers to beneficial and therapeutic effects. Information can be obtained either directly from observation using ethnological approaches or indirectly through the review of traditional documents and specific monographs. This information must be used with caution and requires taking into account how health and disease are perceived and handled in the culture where the history of use is documented. The information obtained may indicate potential pharmacological activities, which would need to be taken into account as part of the safety assessment. In addition, information on adverse health effects such as poisoning may be provided in many traditional documents and specific monographs as well as in ethnopharmacology and ethnotoxicology literature. Often such information is limited to acute toxicity, since acute adverse effects can be more easily related to the recent use of the product. Because of the complexity of associating the development of delayed toxic effects with the chronic use of a product it is, in general, very difficult to identify chronic toxicity using historical sources. For some botanical products, epidemiological and clinical human studies addressing both efficacy and safety are available (Di Carlo et al., 2001; Ernst, 2002).

3.1. Recommendations

The history of use and the information on health effects should be reviewed for the product under consideration, for relevant raw materials and for similar products. Important data to be searched for are listed in Table 2. Such information may allow identification of potential hazards that may require further toxicological investigation.

Table 1
Information relevant to ensure product identification, characterisation and standardisation

Botanical source:
Identity
Scientific name (plant family, genus, species with name of authority, and if relevant, variety, and chemotype), common names
Part(s) of the plant used
Geographic origin (continent, country, region)

Growth conditions:
Wild or cultivated
Good agricultural practice
Site of collection, time of harvest, stage of growth
Drying, fermentation
Storage conditions
Pre- and post harvest phytosanitary treatments (e.g. use of pesticides)

Raw material (e.g. dried plant material):
Specifications according to standard reference (e.g. Pharmacopoeias) including:
  identity tests (e.g. macroscopic examination, microscopy, FT-IR, TLC, HPLC, GC)
  quantitative tests to determine
    (i) constituents relevant for the beneficial effects
    (ii) constituents of toxicological relevance

Process applied to starting material:
Steps of preparation (e.g. extraction process, solvents)
Methods used
Specific precautions (light, temperature sensitivity)

Botanical preparation:
Standardisation criteria (markers: toxic or physiologically active constituents, other relevant constituents; plant-extract ratio)
Specifications (level/range for markers)
Physico-chemical properties of the relevant constituents (stability)
Purity criteria (e.g. microbiological, mycotoxins, pesticides, heavy metals, residual solvents, other contaminants) either by chain control or analysis
Level and nature of excipients
Formulation methodology
Storage conditions

End product (food or supplement containing the botanical preparation):
Fate in food or formulated product (e.g. stability)
Industrial food processing
Preparation for consumption

4. Product comparison

Comparisons between the product under study and adequate comparators are important in defining further data requirements. Several scenarios may result from such comparisons. For example, in the case of a traditional culinary herb or spice used as a supplement, consideration would need to be given to the likely intakes from the proposed new dietary application, since a significant increase in consumption might still trigger safety questions even from a traditional source. However, a situation where no increased exposure is expected would usually preclude the need for further safety data.

Another example would be if an extract or fraction of a traditional culinary herb or spice or other food is to be used as a food supplement. In this case, the fraction or extract may be considered not to contain any component that was not already present in the original food and the safety evaluation could focus on the effects of the fractionation process e.g. the qualitative nature and concentrations of the components in the extract relative to the traditional product. Again, the likely resultant intakes and the bioavailability of the components may need to be considered since extraction of a component of a food from the plant matrix may have significant effects on absorption from the gastrointestinal tract.

In the case where there is no history of food use for a botanical, evidence of prior human exposure and possible beneficial (efficacious) or adverse effects in relation to herbal medicinal use may still provide valuable information in evaluating safe levels of intake/exposure. Such data may be useful in providing a rationale for special studies.

4.1. Recommendations

Information on processing, chemical composition, product quality, product-mediated physiological effects and history of use and exposure should be used to compare the

Table 2
Important data to be searched to evaluate the history of use and exposure from food and food supplements

Information and characterisation of the product(s) for which there is a history of use

Traditional usage:
- Serving size/Daily intake/Doses
- Frequency
- Preparation
- Duration
- Purposes
- Target population(s)
- Known warnings (precautions, contraindications, adverse effects.)

Potential reported pharmacological activity (or 'Indications for use')

Estimated dietary exposure of the active ingredient(s) via product and habitual diet taking into account geographical/cultural variation

Period of use

Information on health/adverse effects:

- Epidemiological data
- Clinical and other case reports
- Experimental human studies

Evaluate the reliability and strength of data

product under investigation with adequate comparator(s). Table 3 provides guidance on how product comparison may be used to define safety data requirements.

5. Intended use, dietary exposure, dietary consequences

Botanicals and botanical preparations may be used as such (e.g. in the form of capsules, tablets or softgel form) or in food products (e.g. beverages, cereal bars). They may be intended to be consumed over a long period of time (e.g. phytosterol ester-containing fat spread for cholesterol control), or it may be advised that consumption should be of short duration (e.g. products for weight control, stimulation of the immune system in wintertime). The recommended intakes associated with the intended beneficial effect should be established based on tests in humans or based on history of use. Some products will be designed for specific populations. These different product concepts may lead to highly specific patterns of exposure, which have to be considered as part of the safety assessment.

Botanicals or botanical preparations may be present in different, not mutually exclusive, food products. For example phytosterol esters may be added not only to fat spreads, but also to other products such as sausages, salad dressings or milk products. In addition, different botanicals or botanical products may have similar mechanisms of action (e.g. phytosterol esters and phytostanols) or may act on the same physiological target (e.g. reduction of blood cholesterol) through different modes of action. In these cases, the aggregate exposure to the bioactive constituents should be taken into account.

New applications of a botanical or preparation with a history of use in the diet may result in a 'significant increase in intake', and this would trigger an evaluation into the safety relevance of this increase. However, the determination of what constitutes a 'significant increase' needs further discussion.

A number of approaches already applied to chemical substances deliberately added to the diet (e.g. food additives, flavouring materials) may provide useful guidance in the definition of 'significantly' increased exposures for substances naturally present in botanicals and already a part of the traditional diet. For example, the consumption ratio approach (Stofberg and Kirschman, 1985) permits the comparison of the intakes of substances added as flavouring materials with those consumed as part of the traditional diet. This approach can serve as a useful tool with which to prioritise substances for toxicological evaluation.

High consumption of food chemicals is generally considered in the literature to be between the 90th and 95th percentile per capita intake. The use of maximum intakes derived from dietary studies is generally not considered due to the probable temporary nature of

Table 3
Guidance on how product comparison may be used to define safety data requirements

No difference in composition beyond natural variability from an accepted traditional food, food ingredient or food additive: proceed to intake assessment (see Section 5)
One or a few well-defined specific characteristics that are different from an accepted traditional food, food ingredient or food additive: safety assessment should focus on these characteristics (see Section 6)
The counterpart of the product under investigation is a traditional health product (medicinal product or food supplement). In this situation, the relevance to the new food application of the safety data available on the existing product has to be assessed on a case-by-case basis. Further data to ensure the safety of the food application of the product may be required
The product under investigation does not have any traditional food or health product counterparts. Such a product requires a comprehensive safety assessment (see Decision Tree in Scheme 1)

such high intakes (Kroes et al. 2002). Chambolle (1999) proposed the 95th percentile as an appropriate representative high intake that did not give unsustainable high intake values. When appropriate intake data is not available, Chambolle (1999) estimates the 95th percentile as three times the mean. The method of Bernier et al. (1994) identified high consumption as three times the mean, and very high as five times the mean (for an habitual consumer of a particular food).

Using the above approaches, it might be possible for example to use as a basis for comparison, high consumption from traditional sources in a comparator (sub)population, [not necessarily the target (sub)-population], provided the (sub)populations are comparable (e.g. size of dataset, data compatibility, social classes, level of healthcare, nutritional status, etc.). However, it is impossible to use exposure considerations only in determining the need for safety data as the shape of the dose–response curve and the nature of any adverse effects will also determine what constitutes a toxicologically significant increase in intake. Therefore, it is necessary to conduct such analyses on a case-by-case basis. In view of the scarcity of intake data for botanicals in the diet, exposure calculations could be particularly assisted by the use of probabilistic models (see Kroes et al., 2002) that integrate both the variability in food intake with the variation in amounts of botanicals and their constituents in traditional foods.

5.1. Recommendations

Required information on the intended use of the product under consideration is listed in Table 4.

Table 4
Information required on the intended use of the product and consequent exposure

Intended use
Description of the product i.e. food or food supplement as consumed
Product composition (list of ingredients, concentration of active ingredients)
Purpose of the product, anticipated health effects
Proposed use: frequency, duration, level of consumption, population/defined target groups
Dietary exposure
Estimated dietary exposure of the active ingredient(s) via product and habitual diet taking into account geographical/cultural variation
Estimated dietary exposure in non-target groups taking into account geographical/cultural variation
Assessment of consequences of aggregate exposure (combined exposure from other products in which the active ingredient(s) is/are added and/or present naturally)
Dietary consequences
Assessment of the nutritional consequences of the introduction of the new product

6. Hazard identification and characterisation

Hazard identification (identification of the inherent biological activity of the material) and hazard characterisation (assessment of relevance to humans and dose–response analysis) are necessary before the risk associated with a particular exposure can be characterised. Information on possible hazards may be obtained from a wide variety of sources. A particular difficulty in the case of botanical products will be a clear separation of those physiological effects that are beneficial, those that are simple physiological changes without adverse effects, and those that represent a hazard and would be the basis for safety evaluation. All data that are used to support the safety in use of a botanical preparation should be of a high standard, and if possible should comply with GAP (good agricultural practice), GMP (good manufacturing practice), GLP (good laboratory practice) and GCP (good clinical practice).

Consideration of the nature of the material that was studied, or will be studied, is essential prior to using data for hazard identification and characterisation.

6.1. Test material

The safety evaluation is focused on the material as consumed and, therefore, data applicable to the finished products are of critical importance. If the material of commerce is not tested, then there should be sufficient analytical data to give assurance that available toxicological test data on related preparations are relevant to the final product as it will be marketed. In designing toxicity tests and considering the nature of the material to be tested, it should be recognised that there may be significant differences in pharmacokinetic behaviour, such as the rate or extent of absorption, and pharmacological/toxic potency between a whole botanical and the equivalent amount of an isolated active principle. As a consequence, it is difficult to extrapolate in quantitative terms between the different materials. An example of this is preparations of *Stevia rebaudiana,* a botanical material proposed for use as a sweetener, and of the active principle, stevioside. Both the JECFA (1999) and the SCF (1999) were unable to allocate an ADI to such materials because of the lack of specifications and the high variability of the material under consideration. More recently, the SCF have had similar difficulties in determining whether toxicological data on an active principle, hypericin, could be used in the safety evaluation of extracts of *Hypericum perforatum* used for flavouring purposes (SCF, 2002).

6.1.1. Use of toxicity data on unrefined extracts

Data from toxicological testing on a less pure or unrefined material have been used for the safety assessment of more pure/refined preparations (e.g. toxicity

data on turmeric oleoresin were used to establish the ADI for curcumin, JECFA, 1996). Implicit in this approach is the assumption that the toxicity of the active ingredient is not reduced in the less pure preparation due to interactions with other constituents. An interaction could occur if the matrix of the less refined material influenced the absorption of the active constituent, thereby altering its bioavailability and systemic toxicity. Such questions could be resolved by appropriate ADME studies (see Technical Annex B).

6.1.2. Use of toxicity data on purified ingredients

It is possible that information may be available on a known active constituent of a botanical preparation, because of its clinical use as a therapeutic agent. The use of such information for the assessment of a less pure extract, would be a good starting point for safety assessment, but is unlikely to be considered sufficient in the absence of other data. Botanical extracts will normally contain a large number of plant non-nutrients; some may have similar activities to the active constituent, while others may possess properties totally unrelated to that of the isolated compound that had been studied for its clinical use. Safety data on the purified active constituent could be used as the only source of information provided that the production process leaves negligible residues of all other potentially active constituents, but the resulting product would be equivalent to the therapeutic agent, and this would raise issues related to its regulation. Interactions between constituents will be extremely important when data for highly purified preparations are used to assess relatively unpurified material. Interactions could either reduce or enhance toxicity, for example purification might remove an inhibitor of toxicity. In some cases such questions could be resolved by appropriate ADME studies (see Technical Annex B). All available toxicity data on highly purified components, together with compositional data on the preparation of potential commercial use, should be taken into account in determining safety—but such data may not be sufficient without additional toxicity data on the material of commerce.

In general, it will probably be more reliable to extrapolate from toxicity data on less pure preparations to more refined preparations, than to use data on highly purified components and apply them to less pure materials.

6.2. Human data

Data from studies in humans may come from occupational health observations in the growers and producers of the plant, or in the workers exposed during preparation of a plant extract. Some botanicals and botanical preparations used for medicinal applications have well established use, recognised efficacy and an acceptable level of safety, demonstrated by published literature. Therefore no additional toxicity testing maybe required unless there is a markedly increased intake as a result of food or supplement use.

6.2.1. Experimental studies

The development of a new functional botanical product is likely to involve human studies to demonstrate physiological activity (Diplock et al., 1999). For example, the cholesterol-lowering property of phytosterol-esters in fat spread was demonstrated in several human studies (Weststrate and Meijer, 1998; Hendriks et al., 1999; SCF, 2000). When human studies are conducted, they should conform to the principles of GCP/GLP; any deviations from this approach should be justified on an individual study basis. The experimental design of such studies should be decided on a case by case basis. It may involve mechanistic, parallel, crossover or epidemiological intervention protocols. The appropriate study design is governed by the beneficial effects to be tested. Studies may determine either direct health endpoints or biomarkers of health outcomes. These studies provide important data for safety assessment such as active dosages, conditions of use, anticipated consumer exposure and possibly mechanism of action for the beneficial health effect. In addition they can provide data relevant to safety assessment related to adverse effects detected at high doses in animals as well as the identification of unexpected safety or nutritional issues. Unexpected observations may have to be investigated further by additional special studies. For example, investigations on phytosterol-esters revealed a reduction in plasma levels of lipid-soluble vitamins such as beta- carotene (SCF, 2000). In addition, it was considered important to confirm that phytosterol-esters did not possess oestrogenic activity (Waalkens-Berendsen et al., 1999) which had been reported for some preparations of plant sterols. Further special studies that integrated relevant specific endpoints to address both concerns confirmed the safety of the product (Ayesh et al., 1999). This example highlights the usefulness of incorporating safety parameters in human studies. Endpoints should be targeted on specific questions raised by previous evaluations.

On a case-by-case basis, depending on previous knowledge, special studies may have to be conducted to address issues that cannot be solved adequately by non-human studies or by existing data. Examples include the investigation of the effects of the test material on appetite or on the development of headaches. In the course of the development of a fat spread containing phytosterol-esters, studies were performed in human volunteers in order to address the potential effects of the product on faecal bile acids (which may promote colon cancer) (Weststrate et al., 1999) and gut microflora (some bacterial enzymes have been implicated in colon cancer) (Ayesh et al., 1999).

Comparative toxicokinetic studies may assist in the evaluation of the significance to human health of toxico-

logical and/or beneficial effects observed in animal models. In addition, kinetic investigations may play an essential role in addressing potential matrix effects. However, toxicokinetic studies can provide meaningful data only when the constituents responsible for the beneficial and toxic effects have been identified and are measured.

To perform studies in human volunteers with newly developed products raises ethical issues that must be addressed before initiating a study (Stargel et al., 1996; Dixon, 1998). Subjects must not be exposed to unreasonable risks and, therefore, adequate preclinical investigations, including toxicity tests in animals where necessary, must have been completed and evaluated before initiating any study in humans. If studies in humans are necessary, these should be conducted with the objective to confirm and support the safety previously established by other means. They are clearly not intended to characterise the toxicological properties of a novel product.

The quality of evidence available from published human studies should be analysed according to Cochrane-type reviews (Cochrane Collaboration, 2002).

6.2.2. Recommendations

General recommendations regarding the justification and the generation of experimental human data are provided on Table 5.

Data from studies in humans are only part of the necessary database and additional data may be needed based on structure, activity, metabolism and tests in vivo on laboratory animals as indicated below and in Table 6 and Technical Annex B (also see Decision Tree in Scheme 1).

6.2.3. Epidemiological data

Epidemiological data can provide indirect evidence for either beneficial or adverse effects in humans under the conditions of the investigation. There are a number of different study designs that can be applied to establish a relationship between exposure and effect. Specific considerations and criteria necessary in the interpretation of epidemiological data include assessment of exposure, and consideration of possible bias and confounding factors. A possible example of confounding was the assumption that the protective effects of fruit and vegetables in reducing the risk of cancer in heavy smokers were due to the beta-carotene constituent, an assumption not supported by supplementation studies, which indicates that other plant constituents may be of greater importance. Therefore data related to history of use need to be evaluated carefully in relation to exposure, and also in relation to the sensitivity of the study to detect adverse effects. Providing that such data are adequate, epidemiology can be an important source of safety data (see section on history of use).

6.3. Animal data

In cases where there is insufficient background information from existing human data to give an assurance of safety in use, additional data may be needed. In vivo tests with laboratory animals might be necessary for hazard identification and characterisation.

A range of studies is used for hazard identification and hazard characterisation of chemicals present in, or added deliberately to, human food (see Technical Annex B). There are internationally accepted guidelines for the conduct of such studies (FDA, 1993; OECD, 1993). For food additives, a battery of tests is required including sub-acute/sub-chronic dietary studies, chronic toxicity/carcinogenicity studies together with ADME studies, genotoxicity, reproductive/developmental toxicity and teratogenicity (WHO, 1987). The range of tests is described in more detail in Technical Annex B. The list is a guide to what may be required but is not a checklist, and the studies that are necessary for risk characterisation have to be judged on a case-by-case basis. In some cases, for example, compounds that are metabolised to nutrients or are consumed at very low levels, not all of the tests listed will be necessary, while in other cases, additional tests may be considered essential to provide a complete toxicological profile. Where indicated by the nature of the substance or the outcome of the routine tests, special studies addressing the endpoints of immunotoxicity or neurotoxicity may also be required.

Table 5
General recommendations on the justification of experimental human studies and generation of data (further details are provided in Technical Annex C)

Scientific rationale and clear objectives have to be formulated
Information, including appropriate toxicity tests, dose-response data and appropriate markers of exposure should be available showing that no adverse effect is expected in the healthy volunteers enrolled in the study
Official guidelines focusing on the protection of humans involved in scientific research and new product testing have to be strictly followed: scientific and ethical review of the experimental protocol application of the principle of informed consent
High scientific and quality standards (including adequate statistical power), should be included
Specific endpoints providing safety confirmation should be considered. Endpoints should address specific questions raised by previous information

Toxicity studies are normally conducted on rodents or other small laboratory animal species. They serve two purposes (WHO, 1987); hazard identification, which requires the use of very large doses in order to optimise the detection of adverse biological properties, and hazard characterisation, the aim of which is the determination of a dosage level on a body weight basis (the no observed adverse effect level or NOAEL) that does not produce the effects detected at higher exposures. A problem with the use of high dose levels for hazard identification of compounds with low inherent toxicity is that major effects may be produced on the overall nutritional quality of the diet due to incorporation of the test material. If this situation arises, it may be difficult to distinguish between toxic and nutritional effects. To minimise nutritional imbalances, the maximum dose of a non-toxic substance that would be tested is normally 5% by weight in the diet of the test animals. To reduce the likelihood of non-specific effects, it is usual to set the highest dose level tested in chronic toxicity studies as that which produces a 10% or less decrement in body weight or growth rate; this is called the maximum tolerated dose or MTD. In addition to the toxicity tests, food additives would normally be subjected to comparative ADME studies in the test species and humans.

The NOAEL that is used as the basis for the safety assessment is usually derived from the most sensitive test and species, and the acceptable daily intake (ADI) for a food additive (WHO, 1987) or pesticide (WHO, 1990) is usually set at an intake 100 times lower than the NOAEL. Clearly, a different approach is required for the evaluation of novel foods and macro-ingredients, because it is not feasible to incorporate such materials into the diet of experimental animals at 100 times the potential human intake without causing nutritional imbalance and physiological disturbances not related to toxicity (Allison et al., 1999). For such foods, the SCF has adopted a decision tree approach (EC, 1997a). The logic behind the application of a 100-fold uncertainty factor and its application to botanicals are discussed below.

Not all of the studies in Technical Annex B are considered necessary for chemicals that are present in the diet at very low concentrations, for example packaging migrants (Matthews and Machuga, 1995) and flavouring substances (Munro et al., 1999). A simplified safety assessment is adopted for flavours, in which the metabolism and potential for toxicity, using data for structural analogues, are weighed against the very low human exposures. In the case of botanicals and botanical preparations, although the average per capita exposure on a weight basis could in some cases be very low (e.g. in the microgram range for a particularly potent preparation), it would be reasonable to assume that such an intake would produce beneficial biological effects. The absence of other unwanted effects cannot be assumed at biologically active doses, and specific risk characterisation will always be necessary for botanicals and botanical preparations.

Data on adverse effects in animals may arise from observations in animals consuming the plant as a part of their diet, or as a result of planned laboratory experiments. Guidelines have been proposed for the pre-clinical testing of botanicals proposed for use as medicinal products by, for example, WHO (1993), and European Medicines Evaluation Agency (EMEA, 1999); the FDA in the USA and others are currently working on guidelines. These guidelines relate to the use of botanical products as medicinal products, and give standard methods for non-clinical toxicological studies related to assessing the safety of herbal products. Not all tests are necessarily required for each herbal product (WHO, 1993; EMEA, 1999), and the need for each toxicity test should be evaluated depending on the amount of literature data related to quality, safety, efficacy, established and traditional uses, and intended uses. Toxicity studies on potential medicinal products involve giving exaggerated dosages of the product to laboratory animals. For dose-response assessment of chronic effects of therapeutic drugs, the top dose in animal studies is usually set as the MTD, or it can be based on the plasma concentrations of the active constituent in test animals and humans receiving therapeutic doses. In either case a significant margin of safety would be expected before large-scale clinical trials could proceed on a medicine for treatment of a minor ailment. It would be reasonable to expect that similar considerations, including an adequate margin of safety, would apply to the incorporation of a botanical preparation into foods or food supplements. Indeed, a high standard of safety should be available given the potential for uncontrolled or unrecognised intake if the same plant or plant preparation were to be present in more than one type of food. The available human data and results from animal studies should be considered as complementary, because data from studies in humans may make some animal studies redundant, while hazards identified during animal studies may trigger the need for additional human investigations.

Comprehensive safety data should be available for a botanical or a botanical preparation used in foods or food supplements, which are sufficient to allow assurance of safety in relation to the anticipated intake and expected duration of intake. When appropriate, the available safety data should cover potentially vulnerable life stages such as pregnancy. In the absence of adequate data following human exposure, special studies may be necessary in animals to fill any data gaps relating to specific life stages. The nature of the beneficial effect and its mode of action can act as a trigger for additional special studies, for example at higher intakes or with specific sub-groups or life stages. Acute toxicity tests may be required for botanicals that contain active prin-

ciples that are likely to have adverse side effects in the short term.

It is not possible to provide a specific checklist of tests for diverse botanical preparations, since the testing strategy will depend in part on the availability of existing background data on history of use, and compositional and nutritional data. A decision tree (Scheme 1) has been proposed to allow a logical decision on the nature and extent of testing necessary for different types of botanical preparation.

6.4. Other considerations

A number of other issues need special consideration in the hazard characterisation of botanicals and botanical preparations.

6.4.1. Matrix effects

The elucidation of possible matrix effects on the bioavailability and biological activity of key constituents may play a critical role in the safety assessment of a botanical preparation. Botanicals and botanical preparations are likely to consist of a complex mixture of components such as macromolecules, flavour compounds and other secondary metabolites, some of which may be responsible for adverse and/or beneficial health effects. In addition to naturally occurring constituents, other components may be generated as a result of processing. For example, metabolic transformation by plant enzymes may activate or detoxify toxic compounds. An important matrix effect would be where the complex matrix, or one constituent, alters the absorption of an active principle, particularly when there is a lower than normal safety margin. Slow and possibly incomplete release of the active principle from the matrix may result in a low bioavailability of a constituent from a complex matrix compared with the absorption of the pure compound. Such an interaction would raise serious questions about the use of toxicity data of the complex material compared with a more purified preparation (questions that could be addressed by comparative ADME studies). ADME and toxicokinetic studies performed on individual active principles in botanicals can provide valuable information for safety assessment, but such measurements are not readily applied to complex biological materials, for example the complex mixture of polyphenols (theaflavins, theacitrins and thearubigins) in black tea infusions. However, some indication of the bioavailability of such substances would be valuable in the assessment of both efficacy and safety. This may be particularly important where the toxicity data are on a different preparation to the material of commerce. Where direct measurement of the substances in vivo is not feasible, surrogate biomarkers of absorption and internal exposure might be appropriate (e.g. total antioxidant activity in blood).

6.4.2. Interactions between different constituents of a botanical preparation

In principle, interactions can arise when one constituent affects the toxicokinetics (absorption, distribution and elimination), or toxicodynamics (mode of action) of another constituent (Dybing et al., 2002). Interactions could result in increased or decreased biological activities (adverse or beneficial) when a combination is given. Probably the most important toxicokinetic interaction is through an effect on xenobiotic metabolising systems (either inhibition or induction). Toxicodynamic interactions are most important for compounds that share the same target organ and/or mode of action (Dybing et al., 2002). The issue of interactions may be important for functional botanical products, because biologically active components in a preparation may consist of a family of similar substances that may share a common mode or site of action and, therefore, may interact. Since some constituents are present in botanical preparations at levels associated with physiological effects in the consumer, the chances of biologically important interactions is likely to be higher than other classes of chemicals, such as food additives and pesticide residues which are usually present in food at levels devoid of any biological activity.

6.4.3. Modes or mechanisms of toxicity

Identification of the mode(s) of toxic action may be very useful for inter-species comparison and extrapolation from animals to humans. A mechanism observed in the animal model used for the toxicological studies might not be relevant for the human situation. Such a case was observed with limonene, which induces kidney tumors in male Sprague Dawley rats through a mechanism involving the α-2u-globulin (Hard and Whysner, 1994). Studies have indicated that this mechanism was highly specific for this animal species and sex, and that it was not relevant for other species including humans. Mechanistic information can be used to refine the uncertainty factor, or margin of safety, used to define an acceptable level of intake for humans (see below).

6.5. Recommendations

General recommendations regarding the rationale and need for studies in animals are provided in Table 6.

7. Risk characterisation

A review of risk characterisation as the final step in risk assessment of exposures to food chemicals is provided by Renwick et al. (in press). In principle, risk characterisation can either provide a quantitative estimate of the risk of a particular adverse effect associated with a particular exposure, or it can identify a level of

exposure that would be without significant adverse health effects. The latter is the approach adopted to derive an ADI (for an additive or a pesticide) or a TDI (tolerable daily intake; for a contaminant). In the case of botanicals and botanical preparations, the overall database should provide an adequate assurance of safety for the intakes and duration of anticipated uses. As indicated in the Codex paradigm, the overall assessment of safety/risk (risk characterisation) requires a knowledge or estimate of potential human exposure as a result of use in food or as food supplements, and adequate dose–response data for the adverse effect(s).

A major issue in the case of botanicals added intentionally to food will be the determination of a suitable margin of safety between doses producing adverse effects (in animals or humans) and the intake from foods or food supplements. The evaluation of the safety margin should account for the possibility of high intakes by some consumers.

In principle, all compounds added intentionally to foods undergo a risk characterisation process that ensures an adequate margin of safety between human intakes and doses producing adverse toxic effects. For food additives and pesticide residues a large uncertainty factor is applied to the NOAEL from studies in animals (usually 100-fold) or studies in humans (usually 10-fold) (WHO, 1987, 1990). The use of such factors has been the subject of numerous reviews, which have generally supported these values as being appropriate default values to allow for species differences and human variability in toxicokinetics and toxicodynamics (Renwick, 1991, 1993; Naumann and Weidemann, 1995; Hattis and Minkowitz, 1996; Renwick and Lazarus, 1998; Silvermann et al., 1999). Clearly, there would be no health concerns if there were an adequate toxicity database on a botanical and the margin of safety was 100 or more.

Because an effect on consumer physiology is the rationale for the addition of a botanical to human food, the margin between the exposure corresponding to an intake expected to be physiologically active and the "safe" level of intake is likely to be smaller than the classical safety factor of 100. Therefore, the approach of applying a large default safety factor to the NOAEL for a botanical may result in a "safe" level of exposure too low to give any useful intended biological activity and the material would not be a functional botanical product.

A lower uncertainty factor could be justified if there was adequate toxicokinetic or toxicodynamic data. The use of such data to move away from default factors has been recognised by the International Programme on Chemical Safety (IPCS), and a scheme developed by which chemical-specific data can be used to replace part of the 10-fold interspecies, or human variability factors. Critical to the development of this approach was the subdivision of each 10-fold factor into appropriately weighted factors for toxicokinetics and toxicodynamics (WHO, 1994). This subdivision is illustrated in Fig. 1 (WHO, 2001). The aim of this approach is that chemical-specific data on one aspect of uncertainty, for example differences in toxicokinetics between test animals and humans, could be used to calculate a chemical-specific adjustment factor, which would then replace the appropriate default uncertainty factor (4.0 in this case) in Fig. 1. The total factor applied to the NOAEL from the animal study would be the product of the chemical-specific adjustment factor and the remaining default values. The scheme was developed primarily as a refinement to the use of uncertainty factors in the risk assessment of single chemicals, and it will be less readily applicable to botanicals that contain more than one active constituent. The main advantage of this approach is that the product of all four defaults is the usual value of 100-

Table 6
Recommendations regarding the rationale and need for experimental animal studies for hazard identification and characterisation

The extent of experimental investigation necessary depends on the adequacy of the history of use and the product comparability to traditional foods or food ingredients
The modes of action of beneficial and adverse effects should be assessed to identify possible safety issues including the possibility of interactions between the botanical product and other biologically active ingested compounds
Special studies may be necessary depending on the history of use and the mode of action
Safety studies should normally be performed on the characterised product of commerce as consumed, at a range of doses including those that produce adverse effects
Studies (in vitro and in vivo) on specific biologically active sub-fractions can provide valuable information for the design of safety studies and/or the interpretation of relevant endpoints
The influence of the matrix on the rate and extent of absorption of active constituents should be studied, especially when extrapolating from one level of purification to another
Studies based on purer preparations may underestimate or overestimate the potential for adverse effects of less pure preparations

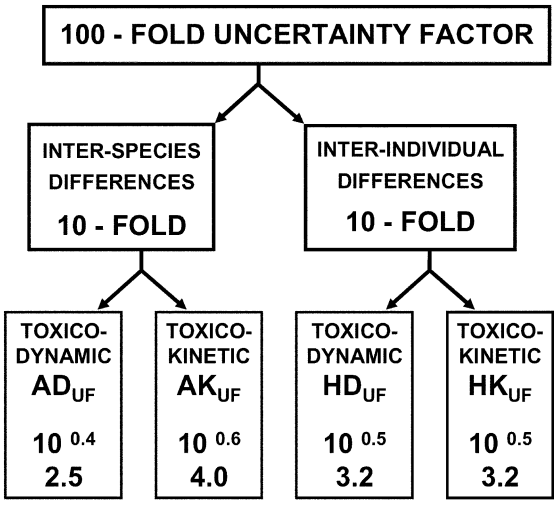


Fig. 1. Chemical specific data can be used to replace a default uncertainty factor (UF) by an adjustment factor (AF). $\mathbf{AD}_{UF}$ – interspecies toxicodynamic uncertainty factor; $\mathbf{AK}_{UF}$ – interspecies toxicokinetic uncertainty factor; $\mathbf{HD}_{UF}$ – human variability toxicodynamic uncertainty factor; $\mathbf{HK}_{UF}$ – human variability toxicokinetic uncertainty factor.

fold, so that the approach reverts back to the current default in the absence of toxicokinetic or toxicodynamic data. While the introduction of chemical-specific data could give a value lower than the 100-fold overall default, it is also possible that the composite value would be greater than 100-fold. This may well be the case with some botanicals for which the active constituent is an alkaloid, because an alkaloid could well be a substrate for CYP2D6 and other enzymes showing genetic polymorphism. Substrates for such enzymes show very large interindividual variability and the default factor for human variability in toxicokinetics (3.2) could be considered too low (Dorne et al., 2002). The adequacy of the default factor will depend on the extent of involvement of the polymorphic pathway in the overall elimination of the alkaloid (Dorne et al., 2002). This possibility could be studied by in vitro expressed enzyme systems and by molecular modelling, providing that the identities and structures of all active constituents were known.

In cases where application of the default 100-fold factor, or chemical-specific factor results in an intake devoid of physiological effects, or comparison of the human adverse effect data with potential intakes results in an inadequate margin of safety, then consideration would have to be given to other strategies such as:

1. modify the material to reduce its toxic potential
2. obtain human data to address specific endpoints
3. obtain mechanistic data to allow a better interpretation of the relevance of certain endpoints (to develop a chemical-specific toxicodynamic adjustment factor)
4. obtain toxicokinetic data to refine the risk assessment for species-differences and inter-individual variability (to develop a chemical-specific toxicokinetic adjustment factor).

An alternative approach to the use of pre-determined default uncertainty or safety factors is to compare the available dose–response data for the adverse effect(s) with the predicted or known human exposure. The ratio between the two estimates is termed the margin of safety. In reality, this is essentially what is done by the application of a 100-fold uncertainty factor (margin of safety) to the NOAEL derived from an animal study in order to define an intake considered to be without significant adverse health effects if consumed daily over a lifetime (the ADI). This "safety margin" of 100-fold would therefore provide a suitable starting point for consideration of the adequacy of any margin of safety for a botanical product added to food or used in food supplements. A smaller safety margin might be justified if the beneficial effect was substantial and the adverse effect was both minor, and reversible on cessation of intake. However, it should be appreciated that weighing the risk against the benefit moves the risk assessment approach away from that usually adopted for substances added to food and towards the approach adopted for an herbal medicinal product.

The establishment of a suitable margin of safety may be particularly difficult for functional botanical-derived products added to food, because an adverse effect for one individual (e.g. a lowering of blood pressure in an elderly subject) could be a benefit for another (e.g. a subject with marginal hypertension). However in such cases the hazards could be addressed by indicating contraindications in product literature or labels. An important issue for botanicals will be clear hazard characterisation, and the separation of adverse effects from physiological adaptation.

Other risk characterisation considerations may be necessary in the case of botanicals and botanical preparations because the intakes can be expected to be associated with biological activities, and because intake may not be restricted to individuals who consume it intentionally for the beneficial biological activity.

There are a number of other considerations that need to be taken into account as part of risk characterisation.

7.1. Nature of the food to which the botanical is added

In addition to an investigation of stability, it could be important to establish that the food matrix did not affect the rate or extent of absorption of either beneficial or potentially toxic constituents, compared with the formulation/material that was used in animal or human studies, or for which there was an adequate history of safe use.

7.2. Interactions with drugs

Many components of the diet can affect the pharmacokinetics or pharmacodynamics of prescribed medicines, and any effects are usually investigated in pre-marketing drug studies. For example grapefruit juice is known to contain an inhibitor of cytochrome P450 isoenzymes (Fuhr, 1998), which are responsible for the oxidation of most drugs in the body. Because of the biological activities of botanicals, pharmacodynamic interactions between botanicals and therapeutic drugs would be considerably more likely than between, for example, a food additive and a drug. Cases of detrimental interactions between botanical products and medicines have been recently recognised, for example St. John's Wort (Di Carlo et al., 2001). According to the biological properties of the botanical product under consideration, the potential for interactions with drugs should be addressed. Effects on the activity of key xenobiotic metabolising enzymes may be addressed by in vitro and in vivo studies in both animals and humans. Effects on expression of drug metabolizing enzymes may also be worth investigation in experimental systems.

7.3. Special target populations

A physiological effect mediated by a botanical product may be considered as beneficial for one group of individuals, but adverse for another sector of the population. The identification of potential sensitive subgroups is, therefore, important and can be based on the known effects of the product: for example, based on an understanding of its mechanism of action (both beneficial and adverse), or on the metabolism and toxicokinetics of the active constituents. For example, phytoestrogens may have a beneficial effect in post-menopausal women, but represent a possible risk to others (Setchell, 2001).

7.4. Mechanism of benefit

For botanical-derived products established to produce beneficial physiological effects on consumers, the elucidation of the mechanism involved and its significance for safety may be critical for risk characterisation. It may allow the design of special studies to address specific issues. The example of functional products aimed at reducing blood cholesterol illustrates the importance of identifying the mechanisms involved. Cholesterol-lowering products may act through different basic mechanisms. An inhibition of dietary cholesterol absorption has been identified for some food products such as phytosterol esters, for which toxicological studies did not raise any significant safety issues except a possible effect on absorption of fat-soluble vitamins (SCF, 2000). Another reported mechanism to reduce blood cholesterol involves an inhibition of its biosynthesis. Based on data for therapeutic drugs, the liver is the key organ in the regulation of blood cholesterol concentration. Efficient regulatory mechanisms acting through the modulation of synthesis, excretion and uptake from the blood allow this organ to maintain adequate levels of cholesterol in the blood. It is known that certain organs, such as the brain, may not utilise blood cholesterol, and depend for their cholesterol supply on in situ biosynthesis (Morell and Jurevics, 1996; Turley et al., 1996). In addition, it has been documented that an inhibition of cholesterol synthesis in muscle cells is associated with myotoxicity (Tobert et al., 1990). Consequently, a cholesterol-lowering botanical product acting through an inhibition of cholesterol biosynthesis, especially if the effects were not restricted to the liver, would raise significantly higher safety concerns than a product affecting absorption from the intestine (Saheki et al., 1994; Reijneveld et al., 1996; Tobert et al., 1990).

7.5. Special intake considerations

The nature of the exposed population and subgroups may be particularly important, because the food will be selected by consumers who are seeking a benefit from the presence of the product, and by definition these may probably not be healthy adults. However, it should be recognised as part of risk characterisation that once a food containing a botanical enters the home, it may be consumed by members of non-target population groups.

7.6. Recommendations

Some of the aspects that need to be considered as part of risk characterisation and the determination of an adequate margin of safety are given in Table 7.

8. Post launch monitoring and subsequent investigations

8.1. Post launch monitoring (PLM)

PLM has received much attention in the context of genetically modified crops and functional foods. In the European Union (EU), PLM can be requested as suggested in the Novel Foods Regulation (EC, 1997a,b). The objective and justification of PLM should not be to replace or reduce pre-market safety studies. The conventional 'reasonable certainty of no harm' criterion should apply before launch and indeed, it would be ethically unacceptable to launch a product where there was a large degree of uncertainty with respect to human adverse health effects. Similarly, PLM is not a risk management tool.

Significant differences may exist between subjects studied in an institutional setting or in a nutritional

Table 7
Elements of risk characterisation and establishment of the margin of safety

All available relevant data should be considered in assessment of the adequacy of the difference between NOAELs in the database and projected intakes (the margin of safety)
The nature of any toxicity should be taken into account in assessing the adequacy of the margin of safety
It should be recognised that the available margin of safety may be limited if the nature of the effect may change with increase in dose, i.e. the effect may be beneficial at low doses but be adverse at higher doses
The margin of safety should take into account both intentional and non-intentional consumers
For botanicals incorporated into food, consideration of the margin of safety should take into account known or predicted human variability, and potentially sensitive subgroups, such as infants and children
Special studies on toxicokinetics or toxicodynamics (mode of action) may provide a rationale for the establishment of the appropriate margin of safety
The possibility of adverse effects should be considered in population subgroups who may be exposed but in whom the physiological effect may not be beneficial

research facility and free-living individuals where dietary behaviour patterns are less controlled. For these and other reasons, it may be advisable to confirm through PLM the absence of adverse effects resulting from the consumption of certain new products. However, it has to be kept in mind that linking health effects with the introduction of a new product is extremely difficult due to many confounding factors. To monitor for acute and severe health effects or for endpoints triggered by concerns identified in premarketing investigations is possible, but to address potential long-term or delayed effects is much more difficult.

Two types of PLM can be defined: passive collection of data and active surveillance targeting consumers (Borzelleca, 1995; Slough et al., 2001). Passive-type post-launch monitoring of food products has been compared with pharmacovigilance. For medicines, several governmental and company reporting schemes exist, stimulated by the expectation of side effects, and facilitated by the fact that products are frequently used under medical supervision. Adverse reactions to foods are, by definition, excluded but unlicensed medicines and in certain countries, such as the USA, special nutritional products (defined as dietary supplements, infant formulae and medical foods) are included.

Procedures within food companies to handle consumer complaints and/or contacts may allow the identification of post-launch safety issues. These procedures include recording, analysis and categorisation of the complaints and/or contacts. They also cover recall procedures, corrective actions, follow-up and archiving aspects. The efficiency of these procedures to identify adverse health effects resulting from the launch of a new product is difficult to evaluate. In the past they have been designed to address quality issues and extrinsic safety problems (e.g. product contaminations). They are not intended to deal with the intrinsic safety of ingredients or novel foods. In addition, complaint data may not provide reliable information since they are highly dependent upon complaint habits, which vary significantly according to country and culture. The complaint handling would probably pick-up relatively severe adverse effects occurring within a short period after the consumption of implicated products. The value of conventional passive surveillance programs and spontaneous complaint reporting may be enhanced by the incorporation of carefully designed procedures for data collection.

Until recently, the application of formal passive and/or active PLM programmes for food products had not been judged necessary, except in rare cases such as for the artificial sweetener aspartame (Butchko et al., 1994) and the fat replacer Olestra (Allgood et al. 2001; Slough et al., 2001). In contrast to drugs, intrinsic risks are not tolerated for foods, whatever the benefit from its consumption may be. New food products are introduced on the market only if a reasonable certainty of no harm for the consumers is demonstrated through pre-market investigations. In consequence, most of the approved new food products pose few safety concerns and do not justify any post-marketing surveillance. The situation may be different for functional botanical products that may contain potent active constituents.

This issue was recently raised within the EU and active PLM programmes were recommended such as for the use of phytosterol esters in cholesterol-lowering fat spreads (SCF, 2000).

Notably, the information demanded of such post-launch studies was very specific, informed by the review of pre-clinical and clinical trial data furnished by the manufacturers. Two objectives were proposed: collection of consumption data and investigation of 'possible health effects' especially the effect on plasma β-carotene levels.

8.2. Recommendations

There are no general rules on how to conduct PLM in the food context. Pre-launch animal and human studies can be used to identify targets for PLM, the need for and nature of which necessitate a case-by-case evaluation. Table 8 provides some guidance to decide on the need for PLM.

Under exceptional circumstances further investigations may be necessary (Table 9). This may arise from anecdotal evidence or when PLM studies generate new

Table 8
Criteria to decide the need for post-launch monitoring (PLM)

There may be a need to reconsider predicted use and exposure scenarios applied in the pre-launch assessment:
If there is evidence of inappropriate patterns of consumption
If aggregate exposure from other dietary sources is likely
If there is evidence of consumption by non-target populations considered at increased risk of adverse effects
There may be a need to confirm the absence of adverse effects of a new product under actual conditions of use:
Under some circumstances where the establishment of a large safety margin is impractical due to technical limitations of animal study design or inherent activity of test material[a]
Where there are prior indications of possible adverse nutritional consequences due to the introduction of the product
If previous considerations (e.g. allergenicity as suggested by composition, novelty or by analogy with known products) indicate the possibility of any hypothetical adverse effects

[a] There are practical limitations as to how much material can be fed to animals without distorting the nutritional properties of the diet (Dybing et al., 2002; Raiten, 1999).

Table 9
Rationale for further studies based on post launch monitoring results

To investigate validity of anecdotal evidence
To investigate in laboratory, clinical and/or epidemiological studies, as appropriate, possible mechanisms and occurrence of unanticipated adverse effects possibly associated with the product
To investigate in laboratory, clinical and/or epidemiological studies, as appropriate, possible mechanisms to explain a marked unanticipated variability in response to the product
To investigate new scientific evidence that may be relevant to the safety assessment of the product

questions that cannot be answered by analysis of existing clinical and non-clinical data or by additional PLM studies. Occasionally media reports may influence consumer behaviour and perception of a product that may be expressed in complaint or enquiry data.

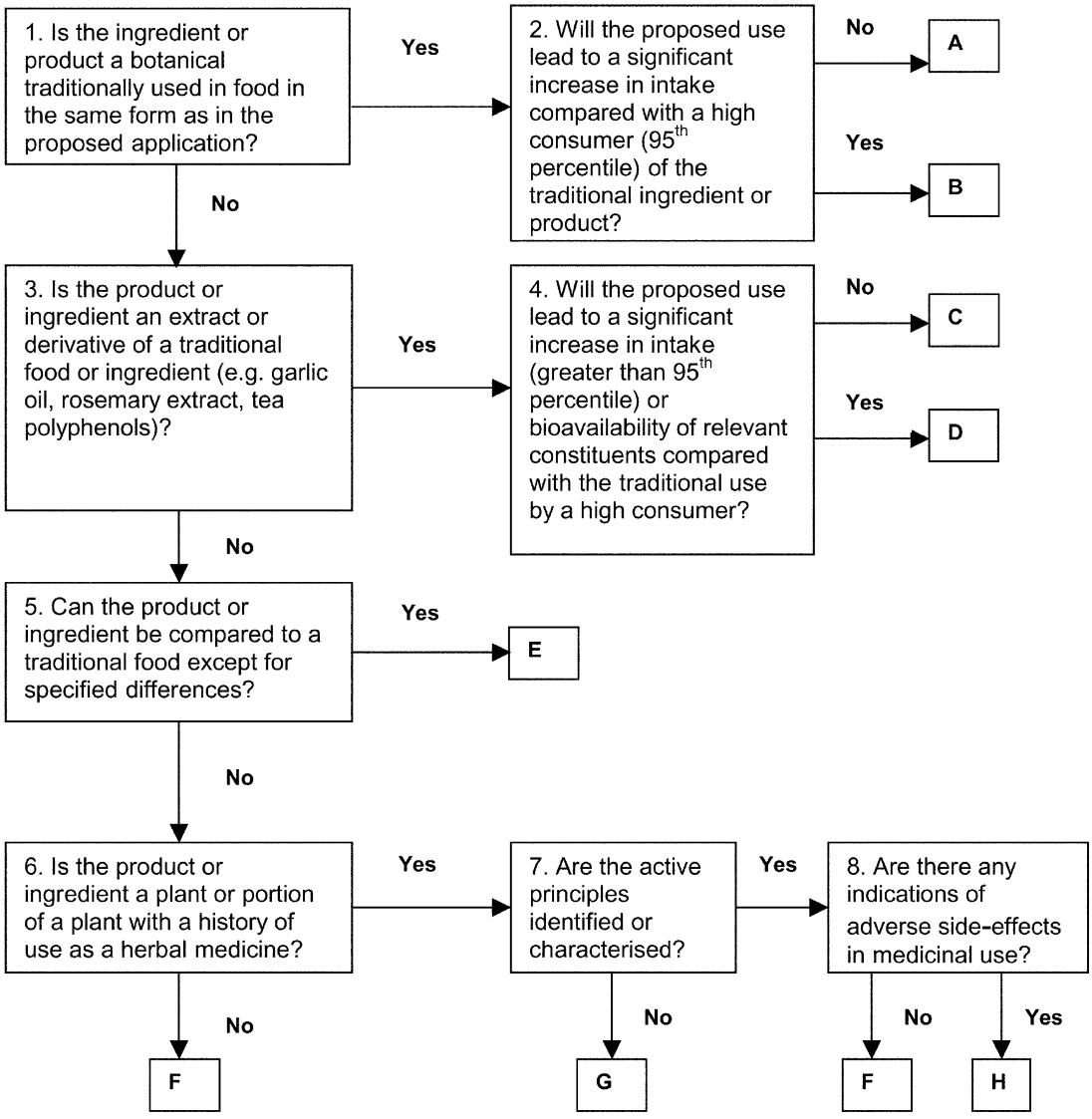
9. The decision tree approach

A decision tree has been designed as a guidance tool to assist in determining the information that needs to be considered for the safety evaluation of functional botanical ingredients or products (Scheme 1). It is not intended as a mandatory checklist. The decision tree allows the classification of new products or ingredients according to their previous history of human uses: botanicals with a traditional food use, extracts from botanicals with traditional food use, botanicals or botanical preparations with a history of medicinal applications, botanicals with no history of human exposure. For each case, key general information required to demonstrate safety is defined.

The first question (box 1) identifies botanical ingredients or products used traditionally in food and which are used as such without significant processing. For such ingredients or products, the key question is whether the intake resulting from the new application is comparable with the intake from traditional use (box 2). If the intake is within the 95th percentile of the intake from traditional use, the information necessary to provide assurance of safety consists in an appropriate characterisation of the ingredient or product and documentation of the traditional uses and resulting intake. If the intake is significantly higher than the 95th percentile of the intake from traditional food use, additional information allowing nutritional and toxicological assessment is required.

The second set of questions deals with extracts derived from botanicals traditionally used in food (box 3). In such a case, the key question is to compare the intakes of relevant constituents of the extract with the intakes resulting from traditional food products (box 4). Since the extraction of a component from the food matrix may alter significantly its absorption, bioavailability may also be an important aspect to consider. If the intakes of the key components are within the 95th percentile of the intakes from traditional food products and if no significant increased bioavailability is expected, the information necessary to provide assurance of safety consists in an appropriate characterisation of the ingredient or product including a thorough description of the extraction process, and documentation of the traditional uses and resulting intakes of key components. If the intake of one or several key components is significantly higher than the 95th percentile of the intake from traditional products, additional information allowing nutritional and toxicological assessment is required. In addition, on a case-by-case basis, ancillary data to assess the safety significance of the desired beneficial effects may be needed.

The next steps of the decision tree refer to products or ingredients, which do not have any history of food application. If the new product or ingredient has no history of human use but is comparable with a traditional food product except for specified differences (box 5), information should be available to permit an assessment of the safety significance of such differences. If the new product or ingredient is a plant, or a portion or extract of a plant with no history of food use but with a history of application as a herbal medicine (box 6), information on the identity of the active principles is considered necessary to conduct a proper safety assessment (box 7). If not available, such information should be generated. For products or ingredients sufficiently characterised, information on the potential side effects associated with medicinal applications should be



Scheme 1. Decision tree to assist in determining the information that needs to be considered for the safety evaluation of a botanical ingredient or product. Information required on herbal product as indicated by exit point of decision tree: A – I, II, III, IV; B – I, II, III, IV, VI, VII, VIII, (IX); C – I, II, III, IV, V; D – I, II, III, IV, V, VI, VII, VIII (IX); E – I, II, III, IV, V, VI, VII, VIII, IX (particular emphasis on the reasons for, and significance of, the differences from traditional comparator e.g. changes due to process); F – I, II, III, IV, V, VI, VII, VIII, IX; G – The product needs to be characterised before a specification can be produced and a safety assessment made for its use in a food or as a food supplement; H – Do not add to food or use in supplements at intakes comparable to medicinal use without a comprehensive risk: benefit analysis. Summary of information required to demonstrate safety of botanical food supplements: I – Specification of the ingredient or product; II – Details of the source organism (Genus, species, portion of the plant consumed); III – Evidence from previous human exposure through food and/or other sources; IV – Extent of use and estimated intake; V – Technical details of any processing and stability in formulation; VI – Nutritional assessment (including assessment of any effects of the active principles on the bioavailability of other dietary components); VII – Toxicological assessment (including assessment of any effects of other dietary components on the bioavailability of the active components); there is not a check-list of essential toxicity tests, and the studies performed should be selected on a case-by-case basis; VIII – Human clinical data including variability of response, adverse effect reports and contra-indications; IX – Ancillary data (mode of action of beneficial effect to allow consideration of special studies, aggregate exposure, susceptible groups etc).

critically analysed (box 8). If no side effect has been reported, the full package of information relevant for safety (specification, evidence of previous human exposure, extent of use and estimated intake, processing, nutritional and toxicological information, human clinical data and ancillary data) should be thoroughly reviewed to ensure that it fully supports the safety of the specific new food application. In case of documented side effects, the botanical preparation should not be added to food or food supplements at intakes comparable to medicinal use without a comprehensive risk: benefit analysis.

For botanicals with no history of human exposure (box 6), a complete package of information is necessary to demonstrate safety.

10. Conclusions

There is an increasing number of products with perceived health benefits available on the food market and efforts are made to develop new concepts. Botanical products are an important part of the health food market. The present document provides guidance on the data necessary to establish their safety for human consumption. Although it is not intended to address regulatory issues, it can provide scientific advice on the development of a risk assessment strategy consistent with due diligence under existing food legislation. In addition, the procedure developed may be used as a basis to undertake risk assessment for existing products identified as having potential safety issues.

It is not possible to give a simple checklist of tests and studies that will be appropriate for establishing the safety of any compound or material added to foods, whether it is a food additive, processing aid or a botanical product with health benefits. A decision tree approach is however suggested as an aid in guiding the safety evaluation process.

The guidelines and studies that are recognised internationally as appropriate for the testing of a food additive are designed to study all life stages, and to ensure that an intake can be established which can be consumed daily throughout life without appreciable health risk. Studies are undertaken to investigate effects on reproduction and development, as well as long-term effects that would be seen in older animals at the end of a chronic study. In addition, special studies may be required depending on the effects detected in acute, subchronic, chronic and reproductive and developmental studies, as well as the biological properties of structural analogues. Hazard characterisation is undertaken against a background of an absence of previous data or prior human exposure, and when there is no direct health benefit to the exposed individual. Large uncertainty factors are applied to toxicity data in order to provide an adequate margin of safety, and to calculate an acceptable daily intake.

In principle, consumers should be able to expect the same degree of safety for both foods containing botanicals and botanical preparations and food supplements, but the situation is different in that there is probably a history of prior human exposure and experience, and the material will be consumed for a health benefit to the individual consumer. It addition, it is anticipated that the material will produce physiological or metabolic changes as part of the beneficial effect, and therefore the wide safety margins used to establish an ADI for an additive will not be available. Hazard identification and characterisation should consider all available animal and human data to ensure that there is adequate information to cover all relevant life stages and all potential biological properties of the botanical material. This may entail the undertaking of special studies in experimental animals to investigate life stages for which there are inadequate human data, or of special epidemiology investigations to study the relevance of effects revealed in animal studies. In general data from human studies would be used in preference to animal data, providing that the methods and endpoints measured were appropriate. In addition the mode of action giving rise to the health benefit may give rise to specific safety questions that would need to be addressed.

Risk characterisation of botanicals and botanical preparations for use in food and food supplements should assess all of the available hazard characterisation data in relation to the potential or predicted human intake, both the daily intake and the duration of intake. At this stage, special attention may need to be given to aspects such as non-target groups, and possible interactions with medicines, which are aspects that would not normally be considered in relation to a food additive. The assessment of botanicals and botanical preparations under food regulations requires the establishment of adequate safety, and any health benefit to the individual would not be taken into account, as this would make the risk characterisation equivalent to a medicinal botanical product. Because of their inherent biological activity, i.e. the benefit, it is unlikely that a wide safety margin will be available for botanicals and botanical preparations, and therefore the advice to risk managers may not be as the equivalent of an ADI, but may take the form of specific advice on the safety margin available for specific groups of the population in relation to intake, duration and contraindications.

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Technical Annex A. Characterisation and quality control of the material

Several analytical methods are available for a correct identification of the raw material. They include macroscopic and microscopic examinations, chemical identity tests, chromatographic techniques, such as thin layer chromatography (TLC), high-pressure liquid chromatography (HPLC), gas chromatography (GC), etc. If the intact plant material is available DNA-based techniques, such as RAPD (randomly amplified polymorphic DNA) fingerprinting can be used to identify or characterise (genetically) the plant.

Methods available to determine the quality and the purity of extracts include:

- Determination of ash and moisture values.
- Analysis for foreign matter of mineral, animal or other vegetable origins.
- Analysis for potential adulterants which may involve analysis for extraneous toxic substances, in particular, alkaloids.
- Analysis for pesticide residues, heavy metals, radionuclides and mycotoxins.
- Analysis of residual solvents.
- Analysis for microbiological contaminants.
- Chemical and microbial analyses following a decontamination operation such as sterilisation, irradiation (before the treatment, immediately after and later).
- Evaluation of the loss on drying.

The chemical analysis may be performed on the botanicals that are used to produce standardised extract and on the extract itself. The analytical techniques chosen to establish chemical profiles should be the most suitable, taking into account the composition of the material. Chromatographic techniques are most frequently used: thin layer chromatography (qualitative and semi quantitative), combined or not with densitometry, gas chromatography, high-pressure liquid chromatography, etc. Quantitative data can be obtained by various approaches such as spectrophotometric techniques or by chromatographic methods (HPLC or GC), and sometimes also by more traditional chemical techniques. The choice depends on the nature of the constituents that are present.

The chemical profile (“fingerprint”) of the raw material, of the intermediate product (specific extract) and of the finished product may serve as a reference to define rigorously the preparations. Such an approach can be applied in routine testing to ensure the quality of the product.

When powdered vegetable is involved as a constituent of the finished product, a chemical profile may be obtained, for example, by extraction with successive solvents of increasing polarity, allowing the detection of most of the constituents present.

Technical Annex B. Toxicological information

Many official guidelines have been established for toxicity testing (e.g. FDA, 1993; OECD, 1993). The following section is not an attempt to redefine guidelines but to provide summarised information on the types of experimental protocols available to characterise the toxic potential of chemicals.

Mutagenicity/genotoxicity

Short-term tests designed to determine activity at a variety of end-points; utilise both bacterial and mammalian systems. Assessment of mutagenicity in vitro, e.g. reverse mutation test, chromosomal aberration test, micronucleus test. These tests may not be feasible on the botanical directly and may need to be performed on appropriate extracts or individual principles. Where there are positive indications in vitro, these tests may need to be supplemented with in vivo tests such as Unscheduled DNA Synthesis (UDS), mouse micronucleus assay, bone marrow clastogenicity.

Acute oral toxicity

Single dose studies are used to define the extent of toxicity in the absence of other data. Assessment of acute oral toxicity. Acute toxicity may be estimated by a tiered approach or as a simple limit assay. Data on acute toxicity would only usually be necessary for supplements if they contained potent biologically active components and the margin of safety is relatively small. The LD_{50} is not considered appropriate.

Sub-acute toxicity

Repeated daily doses for 14–28 days; provide useful indicators of toxic potential when allied to sensitive clinical and pathology tests. Assessment of sub-acute oral toxicity for 1–4 weeks in rodents, with the following parameters:

- Food intake.
- Body weight.
- Weight of essential organs (e.g. brain, heart, liver, kidneys, thyroid gland, spleen, gonads).
- Macroscopic pathology of essential organs (e.g. brain, gastrointestinal tract, liver, kidneys, thyroid gland, spleen, gonads).

Sub-chronic toxicity

Assessment of sub-chronic systemic toxicity for 90 days in rodents or 1 year in non-rodents provide information on major site(s) of toxicity and effects; useful for designing chronic studies

- Food intake
- Body weight
- Weight of essential organs (e.g. brain, heart, liver, kidneys, thyroid gland, spleen, gonads)
- Macroscopic pathology of essential organs (e.g. brain, heart, liver, kidneys, thyroid gland, spleen, gastrointestinal tract, gonads)
- Histopathological examination of essential organs (e.g. brain, peripheral nerve, heart, liver, kidneys, thyroid gland, spleen, gonads, sections of gastrointestinal tract, bone marrow (femur) etc.)
- Haematological examination (RBC, Hb, leucocytes, differential count, prothrombin time etc.)

Chronic toxicity

Two years in rodents; data from these studies are frequently the basis of risk assessment. Assessment of chronic toxicity would normally be by 2-year studies in rodents. (Carcinogenicity is usually assessed during the same studies, but this would not be needed in cases where there is no suspicion of carcinogenic potential or chronic accumulation and where there are no proliferative changes in the 90-day study.). Chronic organ toxicity leading to proliferative changes can result in cancer during long-term studies, and apparently reversible effects, such as veno-occlusive liver damage with pyrrolizidine alkaloids found in short-term tests, may be linked to cancer in the same organ during a life-time study.

Reproductive and developmental toxicity

Repeated daily doses before, during, and after gestation to determine effects on the developing foetus and neonate and possible inheritable effects. Assessment of reproductive toxicity and teratogenicity (developmental toxicity) is important for all chemicals added to human food. Segments II and III testing is normally only required if the product is indicated for use during pregnancy and lactation; however, in the case of food supplements it must be assumed that exposure might occur at all life stages, including during pregnancy).

Segment I. Study on administration of the test substance prior to and in the early stages of pregnancy.
Segment II. Study on administration of the test substance during the period of organogenesis.
Segment III. Study on administration of the test substance during the perinatal and lactation periods.

ADME studies

Studies on absorption, distribution, metabolism and excretion (ADME) should be performed on structurally identified active principles. Such studies should define the extent of absorption of the compound from the intestine, tissue distribution (especially to any organs adversely affected), and the rate and principal routes of elimination. The use of a radio-labelled compound can provide valuable information on the formation and nature of metabolites; such information can be particularly important when metabolites are responsible for, or contribute to, the biological effects. Studies on the plasma concentration-time relationship for the active principle can provide valuable toxicokinetic parameters, such as bioavailability (the fraction of the dose absorbed into the general circulation as the parent compound), the plasma clearance and the half-life (which can be used to predict accumulation). The plasma concentrations and effects of a compound can be influenced by the matrix in which it is given, and this could influence the validity of extrapolating toxicity data from one dosage form to another. Appropriate toxicokinetic data can be used to extrapolate across dosage forms, and if human data are available between species.

Special studies

Special studies are those that are not a normal part of toxicity screening, but are undertaken to investigate in detail some finding, or concern, that has arisen during literature reviews and general toxicity studies. Such studies are usually hypothesis driven and are designed to provide data that can aid in the interpretation of toxicological phenomena. Special studies on immunotoxicity or neurotoxicity may be a focus of attention if the botanical or a related species is known to show such biological activities. Investigations on the structure and/or functioning of the tissues and cells responsible for the activity and integrity of the immune response can be integrated into short-term and sub-chronic studies. Investigations of structure and functioning of the nervous system, e.g. tests of behaviour are also integrated into short-term and subchronic studies; developmental neurotoxicity studies are of increasing importance. In cases where the toxicity of the active principle has been characterised in animal studies, there may be questions about the relevance of effects to humans because of biochemical or physiological differences; studies on the activity of the compound on the relevant system could aid inter-species extrapolation. Biomarkers of toxicity can be particularly useful in special studies. In cases where a botanical is known to produce toxicity at high doses, the role of a postulated active component could be assessed by studying the dose–biomarker response relationship at different levels of purification of the botanical. Similarly dose–biomarker response data for an active principle could change as a botanical product was refined during processing because of the removal of another compound that potentiated or inhibited the actions of the main active principle. For some botanicals dose–response data on the main physiological effect,

such as a cardiovascular response, may be an important part of risk characterisation for the whole population. Knowledge of the biological actions of related botanical species may also indicate the need for special studies.

Technical Annex C. Human data

The purpose of these studies may be to demonstrate the physiological effects of the products and/or to further confirm the absence of adverse effects. The design of the study should be decided on a case-by-case basis, depending upon the product and its expected physiological effects. However, some basic guidelines can be highlighted. The test materials should reflect as closely as possible the product that will be marketed. It should be administered in a way typical of the expected consumer use (food matrices, consumption pattern, daily frequency). The dose levels should cover a range from recommended to upper levels of intakes such as the 95th percentile. Duration depends upon the questions to be addressed, the mechanism involved and the time course of the expected effects. It will also depend upon the anticipated product application. A sequence of clinical tests, starting with short-term studies, may be advised to maximise the safety of the investigation. The selection of an appropriate study population is essential for the successful outcome of the study. Initially, studies should be conducted on healthy adult individuals. Subsequently, studies may focus on selected sub-populations for whom the product is intended as well as non-target sub-populations who may be at special risk (e.g. elderly, obese, diabetic individuals). Inclusion (e.g. age, sex, etc.) as well as exclusion criteria (e.g. pregnancy, medication use, etc.) have to be specified. The study should be designed to have adequate statistical power. Many factors can affect statistical power such as the number of subjects, the variability of the endpoints and the precision of the measurement. Systematic randomisation should be done to avoid bias in assigning subjects to a particular test group. Study design should include a double-blind approach using placebo control. Study design (parallel or crossover) has to be chosen depending upon the objectives and the population involved. Cross-over designs where subjects can serve as their own control is often advisable since they can be used for evaluating factors known to have large inter-individual variability. In addition, they require fewer subjects compared with the parallel design. Safety-related endpoints to be analysed should be selected on a case-by-case basis. Beside clinical examinations and routine laboratory analyses (e.g. blood chemistry, haematology, urinanalysis), special tests may have to be performed according to preclinical information, desired biological effects and mechanisms of action involved. The application of biomarkers of effects may be very helpful, although the predictive value of the biomarker should be validated.

Use of appropriate biomarkers of exposure and effect in stored biological examples may be useful to elucidate previous patterns of exposure. The potential value of archiving samples from larger clinical studies should always be considered.

References

Allgood, G.S., Kuter, D.J., Roll, K.T., Taylor, S.L., Zorich, N.L., 2001. Postmarketing surveillance of new food ingredients: results from the program with the fat replacer Olestra. Regulatory Toxicology and Pharmacology 33, 224–233.

Allison, D., Clancy, K.L., Fennema, O., Miller, S., Munro, I.C., 1999. The Life Sciences Research Office (LSRO). In: Raiten, D.J. (Ed.), Report on Alternative and Traditional Models for Safety Evaluation of Food Ingredients. LSRO, Bethesda, MD, USA.

Ayesh, R., Weststrate, J.A., Drewitt, P.N., Hepburn, P.A., 1999. Safety evaluation of phytosterol esters. Part 5 Faecal short chain fatty acid and microflora content, faecal bacterial enzyme activity and serum female sex hormones in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester enriched margarine. Food Chemical Toxicology 37, 1127–1138.

Bast, A., Chandler, R.F., Choy, P.C., Delmulle, L.M., Gruenwald, J., Halkes, S.B.A., Keller, K., Koeman, J.H., Peters, P., Przyrembe, H., de Ree, E.M., Renwick, A.G., Vermeer, I.T.M. Botanical health products. Positioning and requirements for effective and safe use. Environ. Toxicol. Pharmacol. (in press).

Bernier, J.J., Causeret, J., Hoellinger, H., Suschetet, M., 1994. Méthode pour assurer, au sein d'une population, le non-dépassement de la dose journaliére admissible des additifs alimentaires. Med. Nutri. 1, 9–17.

Betz, J.M., Eppley, R.M., Taylor, W.C., Andrzejewski, D., 1994. Determination of pyrrolizidine alkaloids in commercial comfrey products (*Symphytum* sp.). Journal of Pharmaceutical Sciences 83 (5), 649–653.

Borzelleca, J.F., 1995. Post-marketing surveillance of macronutrient substitutes. Food Technology (September) 107–113.

Burkhard, P.R., Burkhard, K., Haenggeli, C.-A., Landis, T., 1999. Plant-induced seizures: reappearance of an old problem. Journal of Neurology 246, 667–670.

Butchko, H.H., Tschanz, C., Kotsonis, F.N., 1994. Postmarketing surveillance of food additives. Regulatory Toxicology and Pharmacology 20, 105–118.

Chambolle, M., 1999. Assessment of extreme levels of chronic food intakes. Regulatory Toxicology and Pharmacology 30, S13–S18.

Clydesdale, F.M., 1998. Science, education, and technology: new frontiers for health. Critical Reviews in Food Science 38 (5), 397–419.

Cochrane Collaboration, 2002. Available from http://www.cochrane.org.

Codex Alimentarius Commission, 2000. Procedural Manual, eleventh ed. FAO/WHO, Rome.

Couet, C.E., Hopley, J., Hanley, A.B., 1996. Metabolic activation of pyrrolizidine alkaloids by human, rat and avocado microsomes. Toxicon. 34 (9), 1058–1061.

De Smet, P.A.G.M. Towards safer herbal medicines. European Phytojournal (in press).

Di Carlo, G., Borrelli, F., Ernst, E., Izzo, A.A., 2001. St. John's Wort: Prozac from the plant kingdom. Trends in Pharmacological Sciences 22 (6), 292–297.

Diplock, A.T., Aggett, P.J., Ashwell, M., Bornet, F., Fern, E.B., Roberfroid, M.B., 1999. Scientific concepts of functional foods in Europe: consensus document. British Journal of Nutrition 81 (Suppl. 1), S1–S27.

Dixon, R.J., 1998. The international conference on harmonization good clinical practice guideline. Quality Assurance 6, 65–74.

Dorne, J.L.C.M., Walton, K., Slob, W., Renwick, A.G., 2002. Human variability in polymorphic CYP2D6 metabolism: is the kinetic default uncertainty factor adequate? Food and Chemical Toxicology 40, 1633–1656.

Dybing, E., Doe, J., Groten, J., Kleiner, J., O'Brien, J., Renwick, A.G., Schlatter, J., Steinberg, P., Tritscher, A., Walker, R., Younes, M., 2002. Hazard characterisation of chemicals in food and diet: dose response, mechanisms and extrapolation issues. Food Chemical Toxicology 40, 237–282.

European Commission (EC), 1997a. Recommendations concerning the scientific aspects of information necessary to support applications for the placing on the market of novel foods and novel ingredients. Official Journal of the European Communities, L253/1–L253/36.

European Commission (EC), 1997b. Regulation (EC) N0 258/97 of the European Parliament and the Council of 27 January 1997 concerning novel foods and novel ingredients. Official Journal of the European Communities L43, 1–7.

European Agency for the Evaluation of Medicinal Products (EMEA), 1999. Report from the Ad Hoc Working Group on Herbal Medicinal Products (EMEA/HMPWG/25/99). Available from http://www.emea.eu.int/htms/human/hmpwp/hmpwpprj.htm.

Ernst, E., 2002. The risk-benefit profile of commonly used herbal therapies: Ginkgo, St John's Wort, Ginseng, Echinacea, Saw Palmetto, and Kava. Annals of Internal Medicines 136, 42–53.

Food and Drug Administration (FDA), 1993. Toxicological principles for the safety assessment of direct food additives and color additives used in food. "Redbook II", draft. US FDA, Center for Food Safety and Applied Nutrition.

Food Safety in Europe, 2002. Food Safety in Europe (FOSIE): Risk Assessment of Chemicals in Food and Diet. In: Barlow, S., Dybing, E., Edler, l., Eisenbrand, G., Kroes, R., van den Brandt, P. (Eds.). Food and Chemical Toxicology 40(2/3), 137–427.

Fuhr, U., 1998. Drug interactions with grapefruit: extent, probable mechanism and clinical relevance. Drug Safety 18 (4), 251–272.

GAO (United States General Accounting Office), 2000. Improvements needed in overseeing the safety of dietary supplements and "functional foods" (GAO/RCED-00-156).

Graves, S., Burback, B., Smith, C., 2003. Characterisation of ginseng for use in toxicity studies (abstract). The Toxicologist 72 (Suppl. 1), 344.

Greger, J.L., 2001. Dietary supplement use: consumer characteristics and interests. The Journal of Nutrition Supplement 1339S–1343S.

Haller, C.A., Benowitz, N.L., 2000. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. The New England Journal of Medicine 343 (25), 1833–1838.

Hard, G.C., Whysner, J., 1994. Risk assessment of *d*-limonene: an example of male rat-specific renal tumorigens. Critical Reviews in Toxicology 24 (3), 231–254.

Harkey, M.R., Henderson, G.L., Gershwin, M.E., Stern, J.S., Hackman, R.M., 2001. Variability in commercial ginseng products: an analysis of 25 preparations. The American Journal of Clinical Nutrition 76 (6), 1101–1106.

Hattis, D., Minkowitz, W.S., 1996. Risk evaluation: Criteria arising from legal traditions and experience with quantitative risk assessment in the United States. Environmental Toxicology and Pharmacology 2, 103–109.

Hendriks, H.F., Weststrate, J.A., van Vliet, T., Meijer, G.W., 1999. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. European Journal of Clinical Nutrition 53, 319–327.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1996. Toxicological evaluation of certain food additives. WHO Food Additives Series 35, pp. 173–189. Geneva.

Joint FAO/WHO Expert Committee on Food Additives (JECFA), 1999. Toxicological evaluation of certain food additives. WHO Food Additives Series 42, pp. 119–143. Geneva.

Kroes, R., Muller, D., Lambe, J., Löwik, M.R.H., van Klaveren, J., Kleiner, J., Massey, R., Mayer, S., Urieta, I., Verger, P., Visconti, A., 2002. Assessment of intake from the diet. Food and Chemical Toxicology 40, 327–385.

Kwak, N.S., Jukes, D.J., 2001a. Functional foods. Part 1: the development of a regulatory concept. Food Control 12, 99–107.

Kwak, N.S., Jukes, D.J., 2001b. Functional foods. Part 2: the impact on current regulatory technology. Food Control 12, 109–117.

Matthews, E.J., Machuga, E.J., 1995. Threshold of estimated toxicity for regulation of indirect food additives. Toxicology Letters 79, 123–129.

Munro, I.C., Kennepohl, E., Kroes R., 1999. A procedure for the safety evaluation of flavouring substances. Food Chem. Toxicol. 37, 207–232.

Morell, P., Jurevics, H., 1996. Origin of cholesterol in myelin. Neurochemical Research 21 (4), 463–470.

Naumann, B.D., Weidemann, P.A., 1995. Scientific basis for uncertainty factors used to establish occupational exposure limits for pharmaceutical active ingredients. Human and Ecological Risk Assessment 1, 590–613.

New Nutrition Business, 2002. Strategies in Functional Foods and Beverages 2002, Special Report from New Nutrition Business, vol. 7, December/January 2002.

Nortier, J.L., Muniz Martinez, M.-C., Schmeiser, H.H., Arlt, V.M., Bieler, C.A., Petein, M., Depierreux, M.F., De Paw, L., Abramowicz, D., Vereerstraeten, P., Vanherwegheim, J.-L., 2000. Urothelial carcinoma associated with the use of a Chinese herb (*Aristolochia fanghi*). The New England Journal of Medicine 342 (23), 1686–1692.

Organisation for Economic Co-operation and Development (OECD), 1993. OECD Guidelines for the Testing of Chemicals, vols. 1–2. OECD, Paris, France.

Raiten, D.J., 1999. Alternative and traditional models for the safety evaluation of food ingredients: summary report. Regul. Toxicol. Pharmacol. 29 (2), 182–183.

Reijneveld, J.C., Koot, R.W., Bredman, J.J., Joles, J.A., Bär, P.R., 1996. Differential effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on the development of myopathy in young rats. Pediatric Research 39 (6), 1028–1035.

Renwick, A.G., 1991. Safety factors and establishment of acceptable daily intakes. Food Additives and Contaminants 8, 135–159.

Renwick, A.G., 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. Food Additives and Contaminants 10, 275–305.

Renwick, A.G., Lazarus, N.R., 1998. Human variability and noncancer risk assessment—an analysis of the default uncertainty factor. Regulatory Toxicology and Pharmacology 27, 3–20.

Renwick, A.G., Barlow, S.M., Hertz-Picciotto, I., Boobis, A.R., Dybing, E., Edler, L., Eisenbrand, G., Greig, J.B., Kleiner, J., Lambe, J., Müller, D.J.G., Smith, M.R., Tritscher, A., Tuijtelaars, S., van den Brandt, P.A., Walker, R., Kroes, R., 2003. Risk characterisation of Chemicals in Food and Diet. Food Chem. Toxicol. 41 (9), 1211–1271.

Roberfroid, M.B., 1999. What is beneficial for health? The concept of functional food. Food Chemical Toxicology 37, 1039–1041.

Saheki, A., Terasaki, T., Tamai, I., Tsuji, A., 1994. *In vivo* and *in vitro* blood–brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Pharmaceutical Research 11 (2), 305–311.

SCF (European Scientific Committee on Food), 1999. Opinion of the Scientific Committee on Food on stevioside as a sweetener. Available from http://europa.eu.int/comm/food/fs/sc/scf/index_en.html.

SCF (European Scientific Committee on Food), 2000. Opinion of the Scientific Committee on Food on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads. Available from http://europa.eu.int/comm/food/fs/sc/scf/index_en.html.

SCF (European Scientific Committee on Food), 2002. Opinion of the Scientific Committee on Food on the presence of hypericin and extracts of *Hypericum* sp. in flavourings and other food ingredients with flavouring properties. Available from http://europa.eu.int/comm/food/fs/sc/scf/index_en.html.

Schilter, B., Cavin, C., Tritscher, A., Constable, A., 2001. Coffee: health and safety considerations. In: Clarke, R.J., Vitzthum, O.G. (Eds.), Coffee: Recent Developments. Blackwell Science, London, pp. 165–183.

Setchell, K.D.R., 2001. Soy isoflavones—benefits and risks from nature's selective estrogen receptor modulators (SERMs). Journal of the American College of Nutrition 20 (5), 354. S-362S.

Silverman, K.C., Naumann, B.D., Holder, D.J., Dixit, R., Faria, E.C., Sargent, E.V., Gallo, M.A., 1999. Establishing data-derived uncertainty factors from published pharmaceutical clinical trial data. Human and Ecological Risk Assessment 5, 1059–1090.

Slough, C.L., Miday, R.K., Zorich, N.L., Jones, J.K., 2001. Post-marketing surveillance of new food ingredients: design and implementation of the program for the fat replacer Olestra. Regulatory Toxicology and Pharmacology 33, 218–223.

Stargel, W.W., Sanders, P., Tschanz, C., Kotsonis, F.N., 1996. Clinical studies with food additives. In: Tschanz, C., Butchko, H.H., Stargel, W.W., Kotsonis, F.N. (Eds.), The Clinical Evaluation of a Food Additive, Assessment of Aspartame. CRC Press, Boca Raton, FL, USA, pp. 11–22.

Stofberg, J., Kirschman, J.C., 1985. The consumption ratio of flavouring materials: a mechanism for setting priorities for safety evaluation. Food and Chemical Toxicology 23, 857–860.

Tobert, J.A., Shear, C.L., Chremos, A.N., Mantell, G.E., 1990. Clinical experience with lovastatin. The American Journal of Cardiology 65 (12), 23. F-26F.

Turley, S.D., Burns, D.K., Rosenfeld, C.R., Dietschy, J.M., 1996. Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in sheep. Journal of Lipid Research 37 (9), 1953–1961.

Turnbull, D., Whittaker, M.H., Frankos, V.H., Jonker, D., 1999. 13-Week oral toxicity study with stanol esters in rats. Regulatory Toxicology and Pharmacology 29, 216–226.

Waalkens-Berendsen, D.H., Wolterbeek, A.P.M., Wijnands, M.V.W., Richold, M., Hepburn, P.A., 1999. Safety evaluation of phytosterol esters. Part 3 Two generation reproduction study in rats with phytosterol esters—a novel functional food. Food Chemical Toxicology 37, 683–696.

Weststrate, J.A., Meijer, G.W., 1998. Plant sterol enriched margarines and reduction of plasma total and LDL cholesterol concentrations in normocholesterolemic and mildly hypercholesterolemic subjects. European Journal of Clinical Nutrition 52, 334–343.

Weststrate, J.A., Ayesh, R., Bauer-Plank, C., Drewitt, P.N., 1999. Safety evaluation of phytosterol esters. Part 4 Faecal concentrations of bile acids and neutral sterols in healthy normolipidemic volunteers consuming a controlled diet either with or without a phytosterol ester enriched margarine. Food Chemical Toxicology 37, 1063–1071.

World Health Organisation (WHO), 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food. IPCS Environmental Health Criteria 70. World Health Organisation, Geneva.

World Health Organisation (WHO), 1990. Principles for the Toxicological Assessment of Pesticide Residues in Food. IPCS Environmental Health Criteria 104. World Health Organisation, Geneva.

World Health Organisation (WHO), 1993. Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. World Health Organisation, Regional Office for the Western Pacific, Manila.

World Health Organisation (WHO), 1994. Assessing Human Health Risks of Chemicals: Derivation of Guidance Values for Health-based Exposure Limits. IPCS Environmental Health Criteria 170. World Health Organisation, Geneva.

World Health Organisation (WHO), 2001. Guidance document for the use of data in development of chemical-specific adjustment factors(CSAFs) for interspecies differences and human variability in dose/concentration-response assessment. Available from http://www.ipcsharmonize.org/documents/CSAFGuidance5.pdf (accessed on 7 October 2002).

Glossary

ADI: Acceptable Daily Intake; An estimate of the amount of a substance in food or drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk

ADME studies: studies conducted to examine the absorption, distribution, metabolism and excretion of a substance in vivo either in humans or animals

Biomarker: measurement conducted on a biological system that can be used to give an estimate of the exposure, biological effects or susceptibility to a substance

Botanical: of or deriving from plants

Cochrane reviews: system for conducting systematic reviews named after the British epidemiologist Archie Cochrane

Comparator product: traditional product with well documented established use that can be used as a reference to guide the safety evaluation of a novel food product

Culinary ingredient/product: material added during food manufacture or preparation to modify the technological or sensory properties of the food product

EMEA: European Medicines Evaluation Agency

Exposure assessment: The quantitative or semi-quantitative evaluation of the likely exposure of man and/or the environment to risk sources from one or more media

Well-Established Use: Products that have an established efficacy and an acceptable level of safety as demonstrated by published scientific data

Food: "Any substance, whether processed, semi-processed or raw, which is intended for human consumption, and includes drink, chewing gum and any substance which has been used in the manufacture, preparation or treatment of 'food' but does not include cosmetics or tobacco or substances used only as drugs" (Codex Alimentarius Commission, 2000)

Food/dietary supplement: Food product usually containing concentrated sources of nutrients and other ingredients alone or in combination and marketed in dose form and consumed for health maintenance purposes

Functional ingredient/product: ingredient or product with the capacity to produce positive health effects beyond those associated with good nutrition

Genotoxicity: capacity to produce damage by direct action against DNA molecules

Good Agricultural Practice (GAP): auditable system using work instructions, specific documented procedures (standard operating procedures), corrective actions etc for the safe/hygienic production of foods at farm level

Good Clinical Practice (GCP): system of good practices certification for which a clinical institution is generally subject to a successful inspection/audit by a national regulatory authority

Good Laboratory Practice (GLP): system of good practices certification for which a laboratory is generally subject to a successful inspection/audit by a national regulatory authority

Good Manufacturing Practice (GMP): auditable system using work instructions and specific documented procedures (standard operating procedures), corrective actions etc for the safe/hygienic manufacture of foods

Hazard characterisation: The quantitative or semi-quantitative evaluation of the nature of the adverse health effects to humans and/or the environment following exposure to a risk source(s). This must where possible include a dose–response relationship

Hazard identification: The identification of a risk source(s) capable ofcausing adverse effect(s)/event(s) to humans or the environment, together with a qualitative description of the nature of these effect(s)/event(s)

Herb: Non-woody plant; plant of which leaves etc are used for food, medicine, scent, flavour, etc.

JECFA: Joint FAO/WHO Expert Committee for Food Additives

Margin of safety: factor derived by dividing exposure demonstrated to be safe from a combination of clinical and pre-clinical studies by exposure in use

Material of commerce: product as purchased and used by the consumer

Medicinal products/medicines/drugs: substances used to diagnose, cure or mitigate disease

MTD: Maximum Tolerated Dose; normally defined as the dose that produces a reduction in body weight of 10% in the absence of other toxic effects

Mutagenicity: capacity to produce a heritable change in the genetic code (DNA) of somatic or germ cells

NOAEL: No Observed Adverse Effect Level; describing the highest dose in an animal or human study that does not produce an adverse effect

Plant-Extract ratio (PER): A relative measure of the concentration of active principle(s) in an extract normally expressed as the ratio of the weight of plant material to the weight of extract. Unfortunately, the use of the term is not standardised and caution is necessary in its interpretation and use

Post-launch monitoring: (normally) observational studies of food products following market launch

Risk assessment: A process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s). A risk assessment comprises hazard identification, hazard characterisation, exposure assessment and risk characterisation

Risk characterisation: The quantitative or semi-quantitative estimate, including attendant uncertainties, of the probability of occurrence and severity of adverse effect(s)/event(s)in a given population under defined exposure conditions based on hazard identification, hazard characterisation and exposure assessment

SCF: EC Scientific Committee on Food

Toxicodynamics: studies of the elicitation of toxic effects by a substance

Toxicokinetic studies: studies to determine the rates of absorption, metabolic, distribution and excretion processes of a substance

Traditional Use: used to describe products with a long history of use (decades), sometimes in a limited geographical region, and for which limited published scientific data may be available

Uncertainty factor: safety factor applied to the NOAEL to allow for inter-species (animal to human) and intra-species (human to human) variability in toxic responses. In absence of specific data a default uncertainty factor of 100 is normally applied