Ann Ist Super Sanità 2010 | Vol. 46, No. 4: 370-388
DOI: 10.4415/ANN_10_04_05

Plants and parts of plants used in food supplements: an approach to their safety assessment

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Summary. In Italy most herbal products are sold as food supplements and are subject only to food law. A list of about 1200 plants authorised for use in food supplements has been compiled by the Italian Ministry of Health. In order to review and possibly improve the Ministry's list an *ad hoc* working group of Istituto Superiore di Sanità was requested to provide a technical and scientific opinion on plant safety. The listed plants were evaluated on the basis of their use in food, therapeutic activity, human toxicity and in no-alimentary fields. Toxicity was also assessed and plant limitations to use in food supplements were defined.

Key words: food supplements, botanicals, herbal products, safety assessment.

Riassunto *(Piante o parti di piante usate negli integratori alimentari: un approccio per la valutazione della loro sicurezza d'uso)*. In Italia i prodotti a base di piante utilizzati a scopo salutistico sono integratori alimentari e pertanto devono essere commercializzati secondo le normative degli alimenti. Le piante che possono essere impiegate sono raccolte in una "lista di piante ammesse" stabilita dal Ministero della Salute. Allo scopo di rivedere ed eventualmente incrementare tale lista, un gruppo di lavoro dell'Istituto Superiore di Sanità è stato chiamato ad esprimere un parere tecnico-scientifico sulla sicurezza d'uso delle piante elencate. Le piante sono state valutate sulla base del loro uso a scopo alimentare o terapeutico o in campi diversi. In funzione delle eventuali sostanze potenzialmente tossiche contenute in tali piante sono state individuate delle limitazioni per il loro uso negli integratori alimentari.

Parole chiave: integratori alimentari, ingredienti vegetali, prodotti salutistici, sicurezza d'uso.

INTRODUCTION

Botanicals and botanical preparations are widely available to consumers through several distribution channels in the EU and elsewhere. They are sold over the counter in pharmacies and can also be bought in supermarkets, herbalists and other shops, or via the Internet. Their ready availability and widespread use are such that they have almost become part of the common diet, thus generating a significant level of human exposure from a public health point of view. This heterogeneous group of preparations includes both unprocessed and processed plant parts (bark, leaves, flowers, fruits, root and stem) in the form of extracts and/or essential oils. These products are available in a variety of forms, including infusions, powders, tablets, capsules and elixirs, and may be marketed as single substances or in combination with other materials such as vitamins, minerals, amino acids or non-nutrient ingredients. According to their intended use and claims, these products fall under different Community regulatory frameworks, and for some types of products no legal provisions for preliminary risk assessment are yet in place. The main regulations that are relevant in this field are Directive 2002/46/EC on food supplements and Directive 2004/24/EC on traditional herbal medicinal products for human use [1, 2].

Food supplements are foodstuffs whose purpose is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form. Legally, food supplements have to be considered unambiguously as food, although they possess some unique characteristics and specific legislation is devoted to this category of products.

A medicinal product is defined as any substance or combination of substances presented for treating or preventing disease in human beings. Any substance or combination of substances which may be administered to human beings with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in human beings is likewise considered a medicinal product.

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At present botanicals and botanical preparations are partially harmonised at EU level; nevertheless there is still considerable discretion at the national level, and Member States may classify a product as a food supplement or as a drug. It is not surprising, therefore, that difficulties arise and tensions exist because of the differences among EU Member States in their approaches, particularly to the classification of some herbal products as food supplements or as traditional or well established or as other types of medicinal products.

In Italy the majority of herbal products are sold as food supplements and are subject only to generic food law, in particular to Italian Legislative Decree n. 169/2004 [3] incorporating EU Directive 2002/46/EC [1]. In Italy 41 000 products had been notified to the Ministry of Health up to March 2009, only 24 000 of which were included in the Register of food supplements; 10 000 of these contain plants and herbal extracts, 2000 include nutrients and plants. The Ministry of Health has compiled a list of plants which may be added to food supplements, and recently the competent department decided to review and possibly improve it. For this reason, the Istituto Superiore di Sanità (ISS) was requested to provide a technical and scientific opinion on the list of approximately 1200 plants and/or plant parts for use in food supplements. The aim of this report was to describe the methodology adopted by an ISS working group to evaluate the safety of these listed plants.

METHODOLOGY

For several years the Italian Ministry of Health has maintained a list of plants in herbal food supplements which have to be used for salutistic aims. This list is continually updated with data generated by scientific research, so that some plants may be excluded and others added.

In July 2008 the Italian Ministry of Health asked ISS to review an updated list of plants whose presence in herbal food supplements is authorised. To this end a working group of eight researchers (ISS working group) was formed. The members of the group were drawn from two ISS departments: Department of Veterinary Public Health and Food Safety and Department of Therapeutic Research and Medicines Evaluation. The working group discussed various aspects that should be considered in order to determine the safe use of these types of ingredients.

The safety evaluation of food constituents such as additives and/or pesticides is based on data derived from internationally accepted standard approaches (*e.g.* admissible dietary intake, ADI) which incorporate the use of large uncertainty factors and provide a wide margin of safety. For plant materials this approach may not always be appropriate, as the margin between the desired beneficial effect and adverse effects may be narrower.

No harmonised approach is currently in place to assess the risks associated with plants or plant parts authorised for use in food supplements, so the working group decided to start from the reports developed by the Scientific Committee and Advisory Forum Unit of the European Food Safety Authority (EFSA) regarding the safety assessment of botanical ingredients based on contributions from industry, food supplement manufacturing organisations and similar bodies.

In this work the two compendia drawn up in 2008 by a working group of the EFSA's Scientific Committee on Botanicals, were taken into account: the first is a Draft compendium of botanicals that have been considered for a food and/or food supplement use and that have been reported to contain toxic, addictive or psychotropic substances [4]; the second is a Draft compendium of botanicals and botanical preparations that have been considered for food and/or food supplement use and have been reported to have also a medicinal use [5].

The two EFSA documents [4, 5] were compiled by incorporating lists of plants that have been studied and evaluated by various international and European agencies and by other National Food Safety authorities of European Union member states.

The ISS working group selected 121 plants from the list of 1200 plants compiled by the Italian Ministry of Health in accordance with the first EFSA Compendium, plants that contain toxic, or addictive or psychotropic substances.

The criteria for the evaluation of the selected 121 plants were:

- the use of plant or of its specific part;
- the high therapeutic activity of the secondary metabolites of the examined plant;
- the reported human toxicity;
- the use of plant other than for food or medicinal reasons (*i.e.* its use in cosmetics, processing materials, etc.);
- the toxicity data adopted to assess safe levels or risks associated with intake.

The main criteria for inclusion in the list of food supplements was the food use of plants.

In this evaluation the consulted literature sources and web sites of relevant scientific agencies were: *Drogen Kunde*, Heinz A Hoppe, 1975, Band 2 [6]; *Chemotaxonomie der Pflanzen*, R Hegnauer, 1992 and 2001 [7]; *The ayurvedic pharmacopoeia of India*, 2001 [8]; *Traditional Chinese medicines*, GWA Milne, 2003 [9]; *Handbook of medicinal herbs*, James A Duke, 2000 [10]; *Handbook of ayurvedic medicinal plants*, LD Kapoor, 2000 [11]; *Physicians' desk reference for herbal medicines* [12]; Monographs of European Scientific Cooperative on Phytotheraphy (ESCOP); Joint FAO/WHO Expert Committee on Food Additives (JECFA); Committee of Experts on Flavouring Substances of the Council of Europe (CEFS); Commissione Unica per la Dietetica e la Nutrizione, Italian Ministry of Health [13]; European Medicines Agency (EMA) [14]; European Food Safety Authority (EFSA's Scientific Documents on Botanicals) [15].

Plants for which no dietary and/or therapeutic uses are found in the available literature but which are used in other fields such as the manufacture of paints, lacquers, perfumes etc., were excluded from the list.

The "flavouring" compounds present in significant quantities in many of the plants examined were taken into account. It was necessary to consider the qualitative-quantitative composition of plants, as some flavouring components do not require special precautions for their use in food, while for others, due to their potential toxicity, a maximum daily intake (MDI) expressed in mg/kg bw/day has to be defined. If a full MDI cannot be set, a temporary maximum daily intake (TMDI) should be set, based on the available toxicological data [16, 17].

Some of the substances considered are described by the Council of Europe in *Active principles (constituents of toxicological concern) contained in natural sources of flavourings*; these are chemically defined substances that, in certain natural flavourings of vegetable origin and on the basis of existing toxicological data, should not be used as flavouring substances in their own right [16]. Another report of the Council of Europe, *Natural sources of flavourings*, published in 2008 was also consulted [17]. Three classes of "active principles" are reported (I, II, III). The first comprises substances suspected of being genotoxic carcinogens, for which no MDI is established because they should not generally be detectable by modern analytical test methods in foodstuffs and beverages. For the second class of substances no MDI could be set because existing toxicological data are insufficient, however a temporary MDI (TMDI) may be set in certain circumstances. Active principles of the third class are substances which are considered toxic and for which an MDI has been set.

Examples of substances belonging to class I are estragole and methyleugenol; examples of class II substances are eucalyptol, hydrocyanic acid and thujone; class III: menthofuran and pulegone.

Maximum limits in foodstuffs as consumed have been set on the basis of existing toxicological data and/or with reference to a MDI or a TMDI [16, 17].

JEFCA, the panel on food contact materials, enzymes, flavourings and processing aids (CEF) of EFSA, the Council of Europe and other competent organisations have defined an acceptable daily intake (ADI) for other substances that are not included in the list of "active principles" of flavourings.

In this study the ISS working group defined for plants containing flavourings the limits within which it is possible to consider them suitable for use in food supplements, on the basis of available scientific information.

Other secondary metabolites taken into account were coumarins for which the EFSA limits were considered [18].

Plants containing furocoumarins were excluded from the list on account of the possible occurrence of acute phototoxic effects following their oral intake in combination with sunlight or UVA light [19].

Plants containing alkaloids were mostly judged unfit for use in food, and were generally omitted from the list [20, 21].

RESULTS AND DISCUSSION

From the evaluation process some of the 121 selected plants were approved for use, others were considered suitable only within certain limits, and the remaining plants were considered not suitable for use in botanical supplements. The ISS working group defined the limitations for the use of some plants linked to a series of chemical components which may represent a danger for the health of the consumer. The limitations to use were set for the plants containing potentially dangerous substances listed in the second 2008 EFSA compendium of botanicals for food and/or food supplements and that have been reported to have also a medicinal use [5].

During the performance of the present study, after one year of activity the EFSA's Working Group on Botanicals and Botanical Preparations merged the two compendia into a single volume: the resulting compendium now focuses on toxicity aspects, listing botanicals reported to contain toxic, addictive, psychotropic or other substances of toxicological concern [22]. It was therefore necessary to update the list of plants prepared by the ISS working group in light of the information contained in the new 2009 EFSA compendium.

For plants containing anthraquinones (*e.g. Aloe* spp., *Cassia* spp., *Harungana madagascariensis*, *Picramnia antidesma*, *Rhamnus frangula*, *Rhamnus purshiana* (Cascara), *Rheum hybridum* (Rhubarb), *Rheum officinale, Rheum palmatum*) no evaluation was performed because the Italian Ministry of Health is already preparing its own assessment.

Plants with limitations to use

This section discusses chemical components for which it has been necessary to set some quantitative limitation to their presence in the herbal raw material of dietary supplements. Most such substances are "potentially toxic chemically defined substances" which occur in natural flavouring source materials that are periodically evaluated by CEFS, EFSA and other competent agencies on the basis of existing data.

Table 1 shows the limitations to use for the substances that the ISS working group decided to include in this category.

With regard to the presence of sinefrine and hypericin in plants and/or botanical extracts, the limitations to use were established by the Commission on Nutrition and Dietetic Products of the Italian Ministry of Health.

<u>*Coumarin*</u> - Coumarin is a naturally occurring flavouring substance present in numerous plants included in the list: *Anthoxanthum odoratum, Eryngium campestre, Ferula assa foetida, Fortunella species, Gallium odoratum, Gaultheria procumbens, Herniaria glabra, Melittis melissophyllum.*

Table 1 | *List of the natural substances evaluated by the ISS working group and their limit to use*

Substance/plant	Limit
Coumarin	0.1 mg/kg bw/die [18]; (*e.g.* 6 mg/die at body weight of 60 kg)
Estragole	for category of food [17]
Eucalyptol	10 mg/kg of food with some exceptions [17]
Eugenol	2.5 mg/kg bw/die [25]; (*e.g.* 150 mg/die at body weight of 60 kg)
Glycyrrhizinic acid	100 mg/die [27]
Hydrocyanic acid	0.023 mg/kg bw/die [17]; (*e.g.* 1.4 mg/die at the body weight of 60 kg)
Menthofuran and pulegone	1.1 mg/kg bw/die [17]; (*e.g.* 6 mg/die at body weight of 60 kg)
Menthol	4 mg/kg bw/die [37]; (*e.g.* 240 mg/die at body weight of 60 kg)
Methyleugenol	Advise:contraindicated for pregnant women, infants, children [17, 40]
Quillaja saponaria	1 mg/kg bw/die [43]; (*e.g.* 60 mg/die at body weight of 60 kg)
Quinine	Advise: contraindicated for pregnant women, infants, children and individuals that are taking anticoagulant preparations [44]
Safrole	Advise: contraindicated for pregnant women, infants, children [47]
Saponaria officinalis	1 mg/kg bw/die [43]; (*e.g.* 60 mg/die at body weight of 60 kg)
Smilax spp	1 mg/kg bw/die [43]; (*e.g.* 60 mg/die at body weight of 60 kg)
Thujone	1.01 mg/kg bw/die [17]; (*e.g.* 0.6 mg/die at body weight of 60 kg)
Trans-anethole	2 mg/kg bw/die

Coumarin is listed as an "active principle" by the Council of Europe [17], and the EFSA's Scientific Panel on food additives, flavouring, processing aids and materials in contact with food has recently review its toxicity, focusing on its potential to induce DNA-adduct formation in kidney and liver of rats [18].

On the basis of additional *in vivo* studies of coumarin metabolism in different species, including humans, the EFSA panel demonstrated that *in vivo* coumarin does not bind covalently to DNA in target organs, supporting a non-genotoxic mechanism of action for tumour induction [23]. The panel therefore concluded that the available data allowed the derivation of a tolerable daily intake (TDI) that would take into account the hepatotoxic responses. After applying safety factors to the no-observed-adverse-effect level (NOAEL) of 10 mg/kg bw/day for liver toxicity in a 2-year study on dogs, a TDI of 0-0.1 mg coumarin/kg bw/day was established.

Taking the estimated theoretical added maximum daily intake (TAMDI) of coumarin via food, the total daily intake would be 1.3-1.5 mg/day (0.02 mg/kg bw/day), this value is below the TDI [18].

Estragole - Estragole occours naturally in many common plants and culinary herbs, including the following listed plants: *Anethum graveolens, Cinnamonum verum, Commiphora mukul, Cuminum cyminum, Foeniculum vulgare, Glycyrrhiza glabra, Hyssopus officinalis, Illicium verum, Melilotus officinalis, Myrrhis odorata, Ocimum basilicum, Ocimum gratissimum, Paullinia cupana, Pimenta dioica, Pimenta racemosa, Pimpinella anisum, Salvia sclarea, Syzygium aromaticum.*

The highest concentrations of estragole, approximately 5-85%, are found in volatile oil derived from *Ocimum basilicum* [24].

Evidence of estragole's carcinogenic potential includes observations of genotoxicity in several short-term tests, DNA adduct formation *in vivo* and *in vitro*, chemical-structural analogies with recognized carcinogens. The formation of DNA adducts *in vivo* and *in vitro* by metabolites of estragole has been demonstrated and the major hepatic DNA adducts have been characterised. Administration of estragole to adult female mice in the diet for 12 months induced increased incidence of hepatocellular carcinomas compared with control mice [24].

Estragole was classified as a weak genotoxic carcinogen (type I active principle) for which no (T)MDI can be set, and in general it should be non-detectable using modern analytical test methods; however, long-term carcinogenicity studies in rats and mice of both sexes are needed.

An approximate estimate of the total intake of estragole from all sources appears to be in the order of one milligram per person per day; this is below the LD50 derived from available carcinogenity studies in mice by a factor of about 3000. Nevertheless, further studies are needed to define both the nature and implications of the dose response curve in rats at low levels of exposure to estragole. In any case, efforts should be made to reduce the amount of estragole in food as far as possible [16, 17].

Eucalyptol - Eucalyptol is widely distributed in plants. In the list the main sources are: *Achillea millefolium, Alpinia galanga, Artemisia abrotanum, Artemisia absinthium, Artemisia vallesiaca, Artemisia vulgaris, Crocus sativus, Cuminum cyminum, Cupressus sempervirens, Curcuma longa, Cymbopogon citratus, Cymbopogon flexuosus, Cymbopogon laniger, Cymbopogon martini, Cymbopogon nardus, Cymbopogon schoenanthus, Cymbopogon winterianus, Elettaria cardamomum, Eucalyptus citriodora, Eucalyptus globulus, Eucalyptus odorata, Eucalyptus smithii, Hyptis suaveolens, Laurus nobilis, Melissa officinalis, Mentha piperita, Myristica*

fragrans, Rosmarinus officinalis, Satureja montana, Thymus serpillum, Thymus vulgaris. In some plants eucalyptol is the primary constituent of essential oil *(Eucalyptus, Rosmarinus, Elettaria)* and is also present in considerable quantities in others too. Eucalyptol can be hazardous via ingestion, skin contact or inhalation, and it can have acute health effects on behaviour, respiratory tract and nervous system.

The available toxicological studies relating to eucalyptol are limited and inadequate to derive a TMDI: eucalyptol has been classified as a class II active principle. The estimated daily intake of eucalyptol from foods and beverages of approximately 4.5 mg per person is a factor of 1000 below the lowest exposure of eucalyptol by ingestion of eucalyptus oil reported to result in severe intoxications or death in humans (2.45-6.25 g eucalyptol per person) [16].

For a more precise risk characterisation to set an MDI, further data on exposure and toxicity would be needed.

The general limit in food and beverages is 10 mg/kg with some exceptions [17].

Eugenol - Eugenol is a chemically defined flavouring substance used in foodstuffs and is a component of the essence of *Croton eluteria, Dianthus caryophyllus, Ocimum gratissimum*, included in the list.

On the basis of the latest evaluations performed by JECFA in 2005 (65th meeting) the initially established ADI of 0-2.5 mg/kg bw/day (1982) for eugenol was maintained [25]. The Committee considered the results of a 19-week study in rats and a later bioassay in rodents in which the NOEL was 300 mg/kg bw per day, which is more than 16 000 and 5000 times the estimated daily exposure to eugenol from its use as a flavouring agent in Europe (18 μg/kg bw) and in the US (56 μg/kg bw/day), respectively [26]. The Committee therefore concluded that eugenol, as a flavouring substance, should not present a safety concern at the estimated daily exposure and the Panel of EFSA agreed with the JECFA's conclusions [25, 26].

The results concerning acute toxicity indicate that eugenol and derivates given orally have little acute toxicity [25, 26].

Glycyrrhizinic acid - Glycyrrhizinic acid (glycyrrhizin), a triterpenoid saponin glycoside, is one of the compounds obtained from the root extract of the liquorice plant: *Glycyrrhiza glabra*, included in the Italian Ministry's list. The crude dried aqueous extracts may contain 4-25% glycyrrhizinic acid in the form of salts. Humans may be exposed to glycyrrhizinic acid via dried crude root extract or via food into which the extract of *Glycyrrhiza glabra* has been incorporated.

Glycyrrhizinic acid and its ammonium salt are listed in the Community register of chemically defined flavouring substances, on account of their sweet taste, and liquorice has been well known for centuries, in traditional medicine, for its anti-inflammatory efficacy.

Moderate chronic or high acute exposure to glycyrrhizinic acid, ammonium glycyrrhizinate and their metabolites has been demonstrated to cause transient systemic alterations, including increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of the rennin-angiotensis-aldosterone system, hypertension and muscular paralysis. Glycyrrhetinic acid and its derivates block gap junction intracellular communication in a dose-dependent manner in animal and human cells, at high concentrations it is cytotoxic. Glycyrrhizinate and its hydrolysis products were considered to be non-genotoxic in *in vivo* and *in vitro* cytogenetic assays. Glycyrrhetinic acid and glycyrrhizinic acid have anti-infiammatory effects in rats and mice [27, 28].

In 1991 the Scientific Committee on Food of the European Commission did not derive an ADI because of inadequate data, but considered it prudent that the regular ingestion of glycyrrhizinic acid should not exceed 100 mg/day. In the light of subsequent toxicological information the Committee recommended an upper limit of 100 mg/day for ingestion of glycyrrhizin, while noting that human toxicity studies were still insufficient to derive a definite ADI for glycyrrhizinic acid [27].

Hydrocyanic acid - Hydrogen cyanide occurs naturally as cyanogenic glycosides in at least 2000 plants, of which the following are listed: *Amygdalus communis, Indigofera tinctoria, Linum usitatissimum, Prunus armeniaca, Prunus laurocerasus, Prunus persica, Sambucus ebulus, Sambucus nigra.*

The most important cyanogenic glycosides are linamarin, found in *Linum usitatissimum* (over 500 mg HCN/kg of seed), prunasin and amygdalin in *Prunus* spp. (up to 470 mg/kg of kernel) [17].

Hydrogen cyanide can be produced by a hydrolytic reaction catalysed by one or more enzymes from the plants containing cyanogenic glycosides. When the enzymes are activated by crushing and moistening or chewing of kernels the hydrolysis reaction to cyanide is rapidly completed, particularly in an alkaline environment [29].

Fruit cyanogenic glycosides, such as amygdalin and prunasin in almonds, in seeds of apricots, plums and peaches, are hydrolysed by the gut microflora to release cyanide slowly and incompletely, with subsequent adsorption, so that the acute toxicity level for cyanogenic glycosides will be lower.

However, oral LD50 values have been reported in the range of 3-4 mg cyanide/kg bw for rats, 6 mg cyanide/kg bw for mice and about 2.6 mg cyanide/kg bw for rabbits. Symptoms of acute intoxication following oral doses of cyanide are cardiovascular, respiratory and neurological alterations. Several studies have shown that the brain is the most sensitive organ.

The panel on contaminants of EFSA in its latest opinion (September 2007) concluded that the data for hydrogen cyanide were not adequate to identify a NOAEL for chronic exposure in humans, because

they were highly confounded by other nutritional and environmental factors. Adequate long-term toxicity studies to derive a NOAEL were also lacking and therefore a TDI could not be derived [29].

In 2008 the group of experts on flavouring substances of the Council of Europe placed hydrogen cyanide in the category of "active principles II" (constituents of toxicological concern) for which a TMDI may be set. Based on the NOAEL of 4.5 mg cyanide/kg bw/day found in the 13-week study in rats and on a safety factor of 200, a TMDI of 0.023 mg cyanide/kg bw/day was established [17].

Maximum use level for hydrocyanic acid in foods was set at 1 mg/kg in Italian Decree n. 107/92 [30]; in the following Regulation (EC) No. 1334/2008 [31] maximum level was not reported, with exceptions for specific classes of foodstuffs; in the report of 2008 the expert committee on flavouring substances of the Council of Europe defined a limit of 0.5 mg/kg for hydrocyanic acid in foods [17] .

Menthofuran and pulegone - Pulegone and menthofuran show a qualitatively similar hepatotoxicity so that the evaluation of these two substances should be considered together. Metabolic studies have established the role of pulegone in the cytochrome P450-catalyzed bioactivation that occurs via at least two independent pathways: 1) the formation and subsequent activation of menthofuran from pulegone; and 2) the formation of reactive intermediate(s) from pulegone, but not menthofuran, which can be detoxified through a mechanism requiring reduced glutathione.

These substances are contained in the following list plants: *Barosma betulina, Barosma crenulata, Barosma serratifolia, Nepeta cataria, Hedeoma pulegioides, Mentha arvensis, Mentha piperita.*

Mint oil (Ph Eur) contains maximum 2.0% pulegone and peppermint oil contains maximum 4.0% pulegone and between 1.0 and 9.0% menthofuran [32].

Pulegone and menthofuran may not be added as such to foodstuffs; maximum levels in beverages and special kinds of foodstuffs, to which flavourings or other food ingredients with flavouring properties have been added, are set in European Regulation (EC) No 1334/2008 but no limit is given for generic food [31] .

The Committee of Experts on Flavouring Substances of the Council of Europe [17] classifies pulegone and menthofuran under "active principles III" for which an MDI has been set: in the report of 2008 a limit of 20 mg/kg is indicated in foods and beverages, with some exceptions, and an MDI of 0.1 mg/kg bw/day, based on a no-observed-effect-level (NOEL) of 20 mg/kg bw/day in the 28-days oral toxicity study in rats [33] with a safety factor of 200 was reported. However, no ADI can be derived because of limited and inadequate toxicological studies, in the opinion of the EFSA's panel on food additives, flavouring, processing aids and materials in contact with food [34].

In 2005 the EFSA panel evaluated pulegone and menthofuran and found that the available database for the two substances was still inadequate to establish an ADI, even though the new 95-day toxicological data on pulegone enabled the establishment of NOAELs in rats and mice [34].

Menthol - This substance is a naturally occurring compound of plant origin, which gives plants of the mentha species *(Mentha piperita, Mentha arvensis)* their typical flavour. The chemical substance exists in four pairs of optical isomers, but in nature the (-)-menthol (or L-menthol) is the most commonly found isomer. Exposure to menthol occurs through the use of peppermint oil, since menthol is its primary component (35-60%) [35]. Peppermint oil is used in cosmetic formulations, in the manufacture of chewing gum, confectionery, toothpaste and pharmaceutical products. Potential exposure to menthol isomers from drinking water and ambient air is presumed to be negligible [36].

L-, D/L- and the unspecified menthol isomers are well absorbed by the oral route of exposure, humans metabolize menthol primarily by conjugation with glucuronic acid and elimination in the urine, cytochrome P450-mediated oxidation occurs yielding various alcohol and hydroxy acid derivatives, which would also be eliminated in the urine unchanged or conjugated with glucuronic acid. All menthol isomers are of very low acute oral toxicity with LD50 values normally greater than 2000 mg/kg bw. Clinical signs of intoxication are unspecific and include apathy and reduced activity.

Menthol was first evaluated at the eleventh meeting of the expert committee on food additives, when it was allocated an ADI of 0.2-2 mg/kg bw/day; at the eighteenth meeting, an ADI of 0-0.2 mg/kg bw/day was also established [37]. In 1998 the safety evaluation on menthol was reviewed by the JECFA at its 51st meeting, where it was noted that the highest dose of (±)-menthol tested in long-term studies in mice and rats had no specific toxic effect. As the survival of mice was reduced at the high dose of 600 mg/kg bw/day, the committee allocated an ADI for L-menthol and D/L-menthol in the range of 0-4 mg/kg bw/day on the basis of the NOEL of 380 mg/kg bw per day in the long-term study in rats, applying a safety factor of 100 and rounding to one significant figure [37].

Methyleugenol - Methyleugenol is a natural constituent of many aromatic plants and their essential oils; among the listed plants it occurs in particular in: *Acacia senegal, Alpinia galanga, Artemisia abrotanum, Cinnamonum verum, Cymbopogon citrates, Cymbopogon flexuosus, Cymbopogon laniger, Cymbopogon martini, Cymbopogon nardus, Elettaria cardamomum, Hamamelis virginiana, Hyssopus officinalis, Laurus nobilis, Melaleuca alternifolia, Melaleuca leucadendron, Myristica fragrans, Ocimum basilicum, Ocimum gratissimum, Peumus boldus, Pimenta dioica, Pimenta racemosa, Pistacia lentiscus, Rosmarinus officinalis, Satureja montana, Syzygium aromaticum, Zingiber officinalis.*

The consumer is exposed to methyleugenol through the consumption of foodstuffs flavoured with the mentioned plants, for instance soft candy, non-alco-

holic beverages, meat products etc.

Methyleugenol belongs to the same chemical class as safrole, estragole, eugenol and myristicin.

When administered orally, it was found to be carcinogenic in rats and mice: it significantly increased the incidences of liver neoplasms and neuroendocrine tumours of the glandular stomach in male and female rats. Mechanisms of tumour induction by methyleugenol in other organs (kidney, mammary gland, and skin) or induction of mesotheliomas are not known. Mechanistic data indicate that liver tumours induced by methyleugenol and structurally related allylbenzenes such as safrole, result from the metabolism of these compounds to DNA-reactive intermediates [38].

Methyleugenol is rapidly absorbed following oral administration to rats and mice; its metabolism occurs via the cytochrome P450 system and involves side-chain hydroxylation, side-chain epoxide diol formation, and *O*-demethylation. Studies carried out to investigate the metabolism of methyleugenol have suggest that the risk due to dietary ingestion varies in the human population: it seems that methyleugenol is eliminated more rapidly in males than in females, suggesting that metabolic induction is greater in males [39].

No studies on the potential carcinogenicity of methyleugenol in humans have been reported, no data are available that would suggest the mechanisms thought to account for tumour induction by methyleugenol in experimental animals would not also operate in humans [38]. In the *Tenth Report on Carcinogens* (2002), published by the Department of Health and Human Services, methyleugenol is defined as *"reasonably anticipated to be a human carcinogen"* based on sufficient evidence of carcinogenicity from studies in experimental animals, which indicate an induction of liver tumours (malignant and/or combination of malignant and benign) in mice and rats via DNA-adduct formation.

The Scientific Committee on Food of the European Commission in its opinion of 26 September 2001 confirmed the genotoxicity and carcinogenicity of methyleugenol and recommended that reductions in exposure and restrictions in use levels should be ensured [40].

In 2008 the latest evaluation of the experts on flavouring substances of the Council of Europe considered methyleugenol as an active principle I, for which no (T)MDI has to be set, on account of its genotoxic potential, but whose limit has to be considered as the limit of determination [17].

Quillaja saponaria extracts - Quillaia extracts (QE) are obtained by aqueous extraction of the milled inner bark or whole wood of *Quillaja saponaria* Molina (family Rosaceae), included in the list. The extracts contain over 100 triterpenoid saponins, the glycosides of the chief hydrophobic aglycone quillaic acid (about 8.5-17% in the freshly prepared unpurified extract); other compounds are tannins, polyphenols and calcium oxalate [12].

There are two types of purified extracts (types 1 and 2) with differing contents of saponins: type 1 contains 10-30% and type 2 may contain 65-90% quillaia saponins on a dried basis [41].

Based on the ability of saponin micelles to form insoluble aggregates with cholesterol Qujllaia saponins can be used for the production of low-cholesterol dairy food products; they are also used internally for coughs and other conditions of the respiratory tract because of their expectorant and purgative effects. The biological activities and the potency of individual saponins *in vivo* depend primarily on the route of administration.

JECFA established a number of specifications for QE, including limits for colour absorbance, for a pH of fresh aqueous extracts between 5 and 5.5 and set a limit no greater than 14% for ash on the dried basis for type 1 and 5% for type 2. The latter limit reflects the high levels of calcium oxalate in type 1 (about 11% by weight) [41].

Some of the more toxicological effects include haemolysis (strong *in vitro*, much weaker *in vivo*), local irritation, inflammation; more severe toxic effects are liver damage, respiratory failure, convulsions, coma [42].

QE have a wide range of industrial applications: their major use is as foaming agents in beverages such as soft drinks and as emulsifiers in foods.

Quillaia is recognised as a natural flavouring substance for use in food and beverages by the United States Food and Drug Administration (US FDA); in the European Union the Codex Committee on Food Additives and Contaminants lists unpurified QE as suitable for use as a foaming agent in "water-based flavoured drinks", including sport or electrolyte drinks, and sets a maximum use level of 500 mg/kg [42].

In 2006 the JECFA Committee, in its sixty-fifth report, established an ADI of 0-1 mg/kg bw/day (corresponding to 60 mg/die for a person weighing 60 kg) for type 1 and type 2 extracts expressed as Quillaia saponins and the previously established temporary ADI of 0-5 mg/kg bw was withdrawn [43].

Quinine - Quinine and its isomer quinidine are the most important alkaloids of the barks of *Cinchona* spp. present in the list: *Cinchona calisaya, Cinchona cordifolia, Cinchona ledgeriana, Cinchona officinalis, Cinchona succirubra*. In medicine quinine is used to treat malaria and nocturnal calf muscle cramp; because of its characteristic bitter flavour, it is also used in foods, particularly soft drinks. Quinine is also used as a flavouring, mainly in tonics and bitter lemonades.

When large amounts of quinine are consumed, it can constitute a health problem, especially for specific groups of persons. The German Federal Institute for Risk Assesment (BfR) has recently published an updated health assessment *Quinine-containing beverages may cause health problems* [44]. The BfR identified potential risks for quinine intake in particular for pregnant women, who should therefore

be advised to avoid quinine-containing beverages on precautionary grounds because of the possible link between the consumption of these beverages during pregnancy and the occurrence of health disorders in the mother and in the child. Quinine is also counter-indicated for people with tinnitus, pre-existing damage to the optic nerve, certain types of haemolytic anaemia or who take medicinal products such as anticoagulant medicines. The main adverse reactions to quinine intake are tinnitus, visual disturbances, disorientation, haematomas.

The 2008 *Report on natural sources of flavouring* of the Council of Europe [17] reported a NOEL of 1.2 mg quinine base/kg bw/day in humans and, based on a safety factor of 10 for inter-individual variability, an MDI of 0.1 mg/kg bw/day was proposed.

Directive 2002/67/EC of 18 July 2002 on the labelling of foodstuffs containing quinine, in accordance with the opinion of the EU Scientific Committee on Food (SCF), decided "to continue the use of quinine at a certain maximum level in bitter drinks. However, consumption of quinine may be counter-indicated for certain people for medical reasons". Moreover, quinine and its salts must be mentioned by name in labelling, in the list of ingredients after the term "flavouring". The SCF concluded that exposure to quinine (at up to 100 mg/l) has no adverse effects on reproduction and is not teratogenic [45].

However, the panel on food additives of EFSA, in their opinion of 22 May 2008, recommended that the toxicological database of quinine should be reconsidered, adding that more reliable exposure data are required in order to finalise the evaluation [45].

Safrole - This substance occurs naturally in a variety of spices in the Italian Ministry list such as *Hamamelis virginiana, Illicium verum, Myristica fragrans*, cinnamon, nutmeg, pepper and herbs such as basil. The most important dietary sources are nutmeg, mace and their essential oils. Safrole is also present in cola drinks [17]. Its intake estimates in flavouring substances are generally very poor because of the lack of data on the concentrations of these chemicals occurring naturally or voluntarily added in foodstuffs.

The latest evaluation of safrole by the International Agency for Research on Cancer (IARC) resulted in a classification in Group 2B: possibly carcinogenic to humans [46]. The Scientific Committee on Food of the European Commission, in its opinion of 12 December 2001, classified safrole as a genotoxic and carcinogenic substance for which no safe exposure limit could be established; consequently, reductions in the exposure and restrictions in use levels are indicated [47].

Regulation (EC) No 1334/2008 of the European Parliament sets a maximum level of safrole for specific kinds of foods but does not indicate specific limits for foodstuffs in general to which flavourings or other food ingredients with flavouring properties have been added [31].

Safrole has not been approved by the US Food and Drug Administration for use in foods (21 CFR 121-106) [47].

Safrole and isosafrole are carcinogenic in mice and rats; they produce liver tumours following oral administration [48].

The oral LD50 was reported to be 1950 mg/kg bw for rats and 2350 mg/kg bw for mice [49]. Safrole is metabolically activated through the formation of intermediates able to react directly with DNA; DNA adducts (two major and two minor) were detected in rat liver DNA after single doses of safrole at 1 or 100 mg/kg bw. These results suggest that the cytogenetic effects may result from covalent DNA modification in the rat liver [50].

No adequate human studies of the relationship between exposure to safrole and human cancer have been reported.

Saponaria officinalis - The root active compounds of this plant consist of triterpene saponins (2 to 8%) [12]. Closely similar to the Quillaia active principles, Saponaria is used to treat inflammation of the mucous membranes of the upper respiratory tract and also as an antibiotic, antiphlogistic and cholesterol-lowering agent.

On the basis of toxicological evaluations of Quillaia the ISS group decided to adopt the same recommendations for *Saponaria* sp., *i.e.* a maximum use level is established at 1 mg/kg bw/day of plant (corresponding to 60 mg/die for a person weighing 60 kg) [42, 43].

Smilax spp. - The roots of *Smilax* spp., known as Salsaparilla and included in the Italian Ministry's list, are reported to contain steroid saponins in 0.5-3% content, with sarsaponin as principal aglycone [12]. The steroid saponins are responsible for the drug's irritant effect on the skin and its strong diuretic and diaphoretic effects in high doses [12]. The German Commission E reported side effects following oral administration of preparations containing Salsaparilla, including gastric irritation and temporary renal damage. Saponins also increase the adsorption of digitalic glycosides and bismuth and the elimination of some hypnotic drugs. Particular attention should therefore be paid when saponins are ingested simultaneously with these medicinal products. For this reason the Commission E considered that the therapeutic index of the roots of *Smilax* is too low and the substance should only be used within certain limits.

On the basis also of the toxicological evaluation of saponin-containing Quillaia extracts, the ISS working group decided to adopt the same limitation to use, *i.e.* a maximum level of 1 mg/kg bw/day of plant (corresponding to 60 mg/die for a person weighing 60 kg) [42, 43].

Thujone (alpha- and beta-) - Thujone is a terpenoid ketone that exists in nature as a mixture of alpha- and beta- isomers which occur widely in essential oils in varying proportions, most notably in *Achillea millefolium*, *Artemisia* spp., *Cymbopogon* spp., *Croton eluteria, Salvia officinalis, Satureja montana* of the list.

Essential oils are used in traditional herbal medi-

cine (as abortifacient, carminative, anthelmintic, and for digestive problems, female hormone activity, fever, cough) and in food as flavourings in the alcoholic drink industry. Thujone has a neurotoxic potential and produces convulsions in animals and humans, acting on the central nervous system. No data are available on long term toxicity, carcinogenity or reproductive toxicity.

Several studies on the mechanism of the neurotoxicity of alpha-thujone indicate that it is a modulator of the GABA type A receptor; the effects appear to be due to the parent compound and metabolism leads to detoxification [51, 52].

Little is known about the pharmacokinetics of these terpenes in humans and no epidemiological studies investigating the association of exposure to thujone and cancer risks in humans were available, but several case study reports of the acute effects of essential oils containing thujone causing seizures in humans indicated that the animal data are of relevance to humans [53, 54].

Thujone is banned as a food additive in the US and its presence in foods and beverages is regulated in several countries.

Annex III of Regulation (EC) No 1334/2008 on flavourings sets levels for thujone in some beverages as a naturally occurring substance present in flavourings or other food ingredients to which flavouring properties have been added, but it may not be added as such to food [31].

In 2002 thujone was evaluated by the Scientific Committee on Food of EFSA, which considered that the amounts of thujone isomers in foods and beverages resulting from the addition of thujone-containing flavouring agents (*e.g.* sage) should be reduced to the lowest practicable level [55].

The Council of Europe confirmed in its 2008 report [17] the limit in food and beverages of 0.1 mg/kg set in 2005 [16], and allocated a TMDI of 0.01 mg/kg bw/day based on a NOAEL of 5 mg/kg, derived from a 14-week study in female rats, to which a safety factor of 500 (due to the poor quality of the database) was applied [56]. The toxicological data on thujone are limited and the quality of the available studies was considered insufficient to set a TDI/ADI. However, the total intake of thujone from all sources appears to be well within the established TMDI value.

<u>*Trans-anethole*</u> - Trans-anethole is an alkenylbenzene or para-propenylanisole (t-anethole). It provides the characteristic sweet aroma of anise seeds and leaves. T-anethole is an aromatic oxidant, present in a variety of medicinal plant extracts used by the food and beverage industry, especially anise-flavoured alcoholic beverages. The most important listed plants containing t-anethole are: *Pimpinella anisum* (Anise), *Foeniculum vulgare* (Fennel Fruit, Fennel Plant), *Illicium verum, Artemisia dracunculus, Anethum graveolens, Ocimum basilicum, Origanum majorana, Agastache foeniculum, Coriandrum sativum, Apium graveolens*.

The most recent ADI approved by JECFA is 0-2 mg per kg of body weight.

Trans-anethole is liposoluble and rapidly absorbed by passive diffusion from the digestive tract. Despite the widespread use of this product, there is little scientific information about side effects of high doses in experimental animals: it seems to have very weak mutagenic activity and produces slight hydropic changes in the liver of male animals [57]. Trans-anethole is also considered a biocide because of its nematicidal activity [58] and was found to be the major insecticidal agent present in anise oil. Rare side effects are reported, *e.g.* a case of stomatitis after use of denture cream containing oil of anise and a case of erythema and vesiculation after cutaneous application of a cream with the essential oil of anise [57]. Recent studies demonstrate that anethole suppresses T-cell proliferation and IL-2 production in mouse splenocyte cultures. This inhibition is mediated, at least in part, through the down-regulation of NF-AT and AP-1 [59].

Plants not admitted for use in food supplements

Plants or their parts not admitted for use in food supplements are divided into four groups: plants containing substances that are definitely toxic to humans *(Table 2)*; plants of proven therapeutic efficacy, even at low doses, which can not be used in food supplements *(Table 3)*; plants used in the preparation of cosmetics, repellents and other no-food products *(Table 4)*; plants whose active principles are unknown and on which the toxicological studies are insufficient to express an opinion regarding their use in food.

A brief summary of these four classes of plants is given below.

<u>*Plants that contain substances which are definitely toxic to humans*</u> <u>(Table 2)</u> - Among the plants that are not suitable for food use because of their content of toxic principles [60-128], there are those that contain furan diterpenoids. These plants, when ingested, have toxic effects on the liver. They are found in the genera: *Perilla* (*P. frutescens* Britton) and *Teucrium (T. marum, T. montanum, T. polium, T. scordium, T. scorodonia)*. Their toxicity can be effective directly in the case of oxidation of furanoditerpenes by the P450 enzyme system (the metabolites produced lead to a depletion of reduced glutathione, causing bubbles in the plasma membrane and death of hepatocytes) or indirectly, when it is mediated by activation of CYP3A4, leading to the formation of reactive epoxides, which inactivate or alter the human microsomal epoxide hydrolase, triggering an immune response against this enzyme (autoimmune hepatitis).

Plants containing toxic alkaloids should be excluded from the preparation of food supplements. Among these, the genus *Alkanna (A. tinctoria)* contains pyrrolizidinic alkaloids and is responsable for serious liver poisoning (liver cirrhosis and ascites), and suspected of causing liver tumours, right ventricular hypertrophy and pulmonary hypertension.

Table 2 | *List of not admitted plants in food supplements and their constituents of toxicological concern*

Botanical name	Parts of plants of possible concern	Constituents of toxicological concern	References
Alkanna tinctoria Tausch.	Radix	Pyrrolizidine alkaloids	61
Discorea villosa L.	Rhizoma	Alkaloid:dioscorine; saponins: dioscin, diosgenine; phytoestrogens with haemolytic activity	6, 12, 19, 62-64
Eschscholtzia californica Cham.	Herba	Pyrrolizidine alkaloids	19, 65-69
Gossypium herbaceum (L.)	Radix	Ergot alkaloids, terpenoids.	6, 10, 19, 70-83
Gossypium spp.	Semen	Gossypol, 1,3-dicloropropene.	6, 10, 12, 70, 72, 74-84
Juglans regia L.	Folium	Nicotine, p-cumaric acid, eugenol, cyanidine, juglone.	85
Perilla fruttescens Britton	Fructus	Furano diterpenoids	86
Pimpinella major	Radix	Furano coumarins	6, 19, 87, 88
Pimpinella saxifraga	Herba, radix, rhizoma	Furano coumarins	6, 19, 87, 88
Physalis peruviana L.	Folium	Physaline, tropane alkaloids	86, 89
Polygonum multiflorum Thunb.	Rhizoma, Radix	Liver toxins	60, 90-92
Ruta graveolens	Oleum, herba, floribus	Alkaloids, coumarins	12, 93.
Sassafras albidum Nees	Cortex ex radicibus, radix, folium	Safrole	12, 94, 95
Spigelia marilandica	Floribus, radix, rhizoma	Neurotoxin	12
Stachys officinalis Trev.	Herba, folium	Aucubin, aucubigenine.	96-103
Teucrium marum L.	Folium, summitas	Furano diterpenoids	104-107
Teucrium montanum L.	Herba, floribus	Furano diterpenoids	108-112
Teucrium polium L.	Herba, floribus	Furano diterpenoids	113-116
Teucrium polium L. ssp. *Aureum arcangeli*	Herba	Furano diterpenoids	
Teucrium polium L. ssp. *Capitati arcangeli*	Herba, floribus	Furano diterpenoids	
T*eucrium scordium* L.	Herba, floribus	Furano diterpenoids	117
Teucrium scorodonia L	Herba, floribus	Furano diterpenoids	117
Viscum album L.	Folium, herba	Viscotoxin	118-128

Plants with clear therapeutic efficacy, even at low doses, which can not be used in food supplements (Table 3) - Some plants can not be used in food supplements because of their therapeutic effects [129-213]: among these the ISS working group identified the genera *Annona (A. muricata, A. reticulata* and *A. squamosa)* and *Asimina (A. triloba)*, which contain acetogenins, which are active against the parasites *Leishmania braziliensis* and *L. panamensis*. These plants cause depletion of ATP levels in many cells, including tumour cells, thereby inactivating the mechanisms of cellular resistance. The seeds of *Asimina triloba* also contain the alkaloids asimicina and asimina, which have emetic and narcotic effects, respectively.

Plants containing the alkaloid berberine, such as the genus *Coptis* (*C. japonica* and also *C. teeta* and *C. trifolia* are in the list) are used effectively as hypolipidaemic agents on account of their plasma cholesterol-reducing activity.

Plants used in the preparation of cosmetics, repellents and other no-food products (Table 4) - Some plants are used for non-food applications for the presence of active odour and/or emollients can be used as natural insect repellents, or for cosmetic preparations [214-253].

Plants of the genus *Calophyllum* contain polyunsaturated fatty acids, lipoproteins, coumarins, flavonoids, tocopherols, tocotrienols, and xanthones, and are used in cosmetics for the treatment of skin and hair conditions and so can not be permitted for use in dietary supplements.

Furanocoumarins, active ingredients consisting of a furan ring conjugated to a coumarin, are compounds with high phototoxicity. These substances, synthesised by plants for defence against insect pests, are present in the genera *Heracleum (H. sphondylium)* and *Opopanax (O. chironium)* reported in the list.

Some plants used in the field of cosmetics contain toxic principles, such as the gender *Nigella (N. da-*

Table 3 | *List of not admitted plants in food supplements due to their therapeutic effect*

Botanical name	Parts of plants of possible concern	References
Annona muricata L.	Folium, radix, semen	129-136
Annona reticulata	Folium, radix, semen	
Annona squamosa L.	Folium, radix, semen	
Asimina triloba Dunal	Semen	19, 137-141
Brunfelsia hopeana Benth.	Radix	12
Callitris articulata Link.	Gummi	12
Canarium commune L.	Resina, semen	142
Cedrela toona Roxb.	Folium, lignum, semen	143-145
Combretum micranthum Don.	Folium	12, 19, 146-153
Copaifera officinalis Linn.	Balsamum	154-160
Coptis japonica Makino	Radix	161-164
Eryngium campestre L.	Radix	12, 18, 19, 165-170
Evernia prunastri Ach. (L.)	Thallus	171, 172
Fagus sylvatica L.	Fructus,lignum, semen	6, 173
Ferula galbaniflua Boiss.-B.	Gummi, resin	12,19, 174-181
Levisticum officinale Koch.	Radix	6, 12, 182, 183
Mahonia aquifolium Pursh.	Radix	6, 12, 184
Momordica charantia	Fructus, balsamum	6, 19, 185, 186
Paeonia officinalis L.	Flos, radix	10
Papaver sonniferum **L.**	Semen	187-194
Piper betle L.	Folium	6, 195-198
Ptychopetalum olocoides Benth.	Cortex, cortex ex radicibus, lignum	199-203
Punica granatum L.	Cortex	204
Quassia amara L.	Lignum	205-207
Saussurea lappa Clarke	Radix	8, 208, 209
Scutellaria laterifolia	Herba	12, 210, 211
Scutellaria baicalensis Geor.	Folium, radix	
Scutellaria galericulata	Herba	
Selenicereus grandiflorus (L.) Britt et Rose	Flos, herba	12
Smilax officinalis H.B.K.	Radix	212, 213

Table 4 | *List of not admitted plants in food supplements due to their use in no alimentary fields*

Botanical name	Parts of plants of possible concern	References
Arnica montana L.	Capitula, herba c., floribus, radix folium, summitas	214-218
Calophyllum inophyllum L.	Resina	219-223
Convolvulus scoparius L.	Lignum, radix	6
Heracleum sphondylium (L.)	Fructus	6, 12, 19, 178, 224-229
Ledum palustre	Herba	230-235
Liquidambar styraciflua L.	Balsamum	6
Malaleuca alternifolia	Oleum, summitas	236, 237
Nigella damascena L.	Semen	238, 239, 240
Opopanax chironium Koch.	Gummi, resina	241, 242
Pogostemon cablin Benth.	Folium	243-246
Tanacetum cinerariaefolium Sch. Bip.	Capitula	247-250
Tanacetum creticum		251
Tanacetum vulgare L.	Capitula, herba, floribus, semen	252, 253

mascena), which synthesises damascenine, while the genus *Arnica (A. montana)* is used for external use, in the treatment of bruises and sprains. These plants contain sesquiterpene lactones and are therefore toxic for humans when ingested orally.

Plants used as natural repellents belonging to the genus *Tanacetum (T. cinerariaefolium, T. creticum* and *T. vulgare)* contain pyrethroids, compounds known for their activity against insects, but toxic in foods.

Plants whose active principles are unknown, and whose toxicological studies are insufficient to enable their safe use in food supplements - In examining the herbal drugs used in food supplements, we found that there is insufficient evidence to enable a proper assessment of the leaves of *Croton eluteria*. It was therefore deemed prudent to preclude the use in food of the leaves of this plant.

CONCLUSIONS

The criteria for the preparation of this paper were intended to safeguard the health of consumers of botanicals and botanical preparations as food supplements. To this end, the plants were studied from a phytochemical point of view in order to highlight the components with any kind of activity in humans. Where the activity of a plant is expressed and there is sufficient documentation regarding its toxicity, the plant has been excluded from the list of admitted plants. Where the activity of one substance present in plant has one treshold level, this quantity was taken as the maximum admitted dose.

However it is clearly stated that the safety in the use of botanical preparations should be assessed considering the toxicity and the increased exposure of the consumers as main criteria. If in the future, new plants will be studied by ISS working group, the same criteria and limits will be used also for them if they contain the same constituents of the plants examined in this study.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

Received on 1 March 2010.
Accepted on 17 June 2010.

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