European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on safety evaluation of Ephedra species for use in food[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The ANS Panel provides a scientific opinion evaluating the safety in use of Ephedra herb and its preparations originating from *Ephedra* species when used in food, e.g. in food supplements. *Ephedra* species contain alkaloids of biological relevance: ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine (cathine), methylephedrine and methylpseudoephedrine. They have sympathomimetic activity and some of them are also used as active ingredients in medicinal products for which adverse effects are described. The use of Ephedra herb in food supplements is prohibited in several European countries, however food supplements containing Ephedra herb, also in combination with caffeine, are offered for sale via the internet. The Panel reviewed the available scientific data on a possible association between the intake of Ephedra herb, its preparations or its alkaloids and potential harmful effects on health. There is wide variation in the concentration of the individual ephedra alkaloids in different *Ephedra* species and in their preparations for use in food. No adequately specified individual preparations of Ephedra herb are known for which sufficient toxicological data for hazard characterisation exist. Due to the absence of adequate data on genotoxicity, short-term, long-term and reproductive and developmental toxicity the Panel could not provide advice on a daily intake of Ephedra herb and its preparations that do not give rise to concerns about harmful effects to health. Ephedra herb and its preparations in food supplements may result in exposure to total ephedra alkaloids or ephedrine which falls within or may exceed the therapeutic dose ranges for the individual ephedra alkaloids or ephedrine, respectively, in medicinal products. Such an exposure could lead to severe adverse effects, which may be enhanced by the combination with caffeine. The Panel concluded that Ephedra herb and its preparations containing ephedra alkaloids used as food supplements were of significant safety concern at the estimated use levels.

KEY WORDS

ephedra herb, ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine (cathine), ephedra alkaloids

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SUMMARY

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the evaluation of *Ephedra* species in accordance with Article 8 (2) of Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods. This request was triggered by the concerns raised by one of the EU Member States on the possible harmful effects associated with the consumption of foods containing *Ephedra* species and preparations thereof, for example in food supplements.

In particular, EFSA was requested to review the existing scientific data on the possible link between the intake of *Ephedra* species and harmful effects on health, and to provide advice on a tolerable upper intake level (UL) for *Ephedra* species for the general population, and as appropriate, for vulnerable subgroups of the population. In the absence of a tolerable upper intake level, EFSA was asked to provide advice on a daily intake of *Ephedra* species that does not give rise to concerns about harmful effects to health.

The risk assessment was performed by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) in accordance with the 2009 EFSA Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements.

In addressing the Terms of Reference of this request, the ANS Panel noted that the term "tolerable upper intake level", was used by EFSA so far only for nutrients, such as vitamins or minerals, to describe the maximum level of total chronic daily intake judged to be unlikely to pose a risk of adverse health effects to humans. The Panel considered that the term "tolerable upper intake level" for botanicals and botanical preparations could lead to the misinterpretation that they could play a similar role in human nutrition as minerals and vitamins, which differ from them *inter alia* in being in general constituents of the normal diet and in being in many cases essential. This is particularly so in this specific case, where the botanicals and botanical preparations, or their main components, have known medical uses based on scientifically established pharmacological properties and/or where they cannot be regarded as components of the normal diet. Therefore, the Panel considered that the use of the term "tolerable upper intake level" would not be appropriate for botanicals and botanical preparations being constituents of food or food supplements.

The term Ephedra herb comprises herbs which may derive from up to 66 different *Ephedra* species further subdivided into subspecies and varieties. *Ephedra* plants grow in temperate climates of the sub-tropical regions of Asia, Europe, North and Central America, South America, North Africa.

Ephedra species contain a number of alkaloids of biological relevance, (-)-ephedrine and (+)-pseudoephedrine being the main ones. Other ephedra alkaloids of the ephedrine-type present in the herb are (-)-norephedrine, (+)-norpseudoephedrine (also known as cathine), (-)-methylephedrine and (+)-methylpseudoephedrine. Each of these ephedra alkaloids occur in nature only in one of the two possible enantiomeric forms. However, these alkaloids may also be obtained in the forms of the racemates by chemical synthesis, which may qualitatively and/or quantitatively differ in their pharmacological and toxicological potencies from the enantiomers found in nature. In this opinion, the term "ephedra alkaloids" was used to exclusively to refer to these ephedra alkaloids of the ephedrine-type, being closely related in their chemical structure to ephedrine.

Ephedra alkaloids of the ephedrine-type act as sympathomimetics, by evoking the release of noradrenaline at the post synaptic alpha and beta adrenoreceptors, and are also capable of direct activation of these receptors, leading to pronounced cardiovascular and central nervous system stimulating effects. The sympathomimetic effects of the ephedra alkaloids are based on the same mode of action, however there are quantitative differences in their peripheral and central effects. Dose-effect relationships for different target organs are difficult to predict for individual Ephedra herb preparations due to the lack of data on the standardisation of the botanical preparations used with respect to the

ratio of extracted material to starting material, alkaloid content or content of other biologically active ingredients.

In 2004 the US Food and Drug Administration (FDA) banned food supplements containing ephedra alkaloids of the ephedrine-type on the grounds of unreasonable risks. Despite the specific prohibition of Ephedra herb and its preparations in foods or food supplements in several European countries as well as in Canada, Australia and New Zealand, these products are nonetheless offered for sale via the internet, often in combination with other substances (e.g. caffeine). These products are mostly promoted for weight loss and for improving athletic performance.

Ephedra alkaloids are listed as prohibited doping stimulants, whereas ephedrine and pseudoephedrine are monitored worldwide as precursors for the chemical synthesis of the illicit drug methamphetamine.

Quantitative data on the chemical composition of the alkaloids in Ephedra herb are extremely variable, depending on factors such as the plant species and growing/harvesting conditions of the plant. The reported value of total alkaloid content of the herb ranges from about 0.5 to 49.9 mg/g, with the two main alkaloids, (-)-ephedrine and (+)-pseudoephedrine together accounting for 70-99 % of the total alkaloid content. The relative proportion of the individual alkaloids is also extremely variable, with (-)-ephedrine ranging from 0 and 90 % and (+)-pseudoephedrine from 0.1 and 99 %. Concentrations of the other alkaloids (+)-norpseudoephedrine, (-)-norephedrine and (-)-methylephedrine are reported to be $\leq$ 7.3 mg/g, 4.7 mg/g and 3.2 mg/g respectively, whereas (+)-methylpseudoephedrine has only been detected in trace amounts.

The lack of occurrence of ephedra alkaloids in certain *Ephedra* species is reported in some studies, however detailed investigations using state of the art methods of analysis and including a variation of samples within each of the species in question were not available to support these claims.

Besides ephedrine-type alkaloids, other biologically active components, such as ephedroxane and ephedradines and certain glycans (ephedran A, B, C, D and E) have been reported to occur in the aerial parts of *Ephedra* species. Also flavanols have been reported to be components of twigs and barks of *Ephedra* species. Qualitative and quantitative data for these other biologically active components of Ephedra herb preparations in food supplements were not available but would be required for the evaluation.

According to analytical data published in the literature, ephedrine is the major ephedra alkaloid to be found in food supplements containing Ephedra herb, representing more than 50 % of total alkaloids. Pseudoephedrine was reported to range between 10 and 50 % of total alkaloids, whereas more than 60 % of the analysed samples did not contain detectable levels of methylephedrine, methylpseudoephedrine, norephedrine and norpseudoephedrine.

Preparations containing *Ephedra* species, also known as Ma huang, have been used in traditional medicine as stimulants, for the treatment of asthma, cough, cold and mild forms of bronchospasm. However, according to the European Medicines Agency (EMA), no traditional medicinal products containing Ephedra herb and its preparations appear to be authorised or registered in the EU and no assessment report on the safety and efficacy of this botanical was available or currently expected from the Committee on Herbal Medicinal Products (HMPC) of the EMA.

In the European Union, there are authorised medicinal products for oral use, containing (-)-ephedrine, racemic ephedrine, (+)-pseudoephedrine, racemic norephedrine and (+)-norpseudoephedrine as active ingredients. Duration of treatment with these medicinal products is generally restricted. The use of methylephedrine hydrochloride as a bronchodilator and for the relief of cough and nasal congestion was found in the literature, however medicinal products containing methylephedrine seem to be no longer available in the European Union.

It was not possible to assess exposure to Ephedra herb and its preparations due to lack of data. Instead literature data was used in the preparation of an estimate of exposure to total and to five individual ephedra alkaloids (ephedrine, norpseudoephedrine, pseudoephedrine, methylephedrine and norephedrine) from food supplements containing Ephedra herb or its preparations. In the published studies considered for the exposure assessment, the mean content of total ephedra alkaloids in food supplements (capsules and tablets) ranged from 11.5 to 26.8 mg per serving and the maximum content amounted to 75.5 mg total alkaloids per serving. The mean content of the main alkaloid (-)-ephedrine in food supplements (capsules and tablets) ranged from 8.4 mg to 18.3 mg per serving and the maximum content amounted to 65.8 mg per serving. Hence, estimated exposure to total ephedra alkaloids resulting from food supplement use of Ephedra herb and its preparations may be in the same range or even exceed the therapeutic dose range for the individual ephedra alkaloids in medicinal products. Estimated exposure to (-)-ephedrine alone resulting from food supplement use of Ephedra herb and its preparations would fall within or may exceed the therapeutical dose range of ephedrine when used as a single active ingredient in medicinal products.

When comparing estimated exposure to single ephedra alkaloids from use in food supplements to the therapeutic doses of the same alkaloid used in medicinal products, the Panel did not take into account possible combination effects from the other ephedra alkaloids present in food supplements. The Panel was aware that this procedure underestimated the pharmacological effects which could occur following ingestion of a food supplement containing ephedra alkaloids.

In reviewing the toxicological and clinical data available, the Panel gave priority to the studies investigating Ephedra herb and its preparations. Studies with the single ephedra alkaloids found in the different *Ephedra* species and in particular with (-)-ephedrine and (+)-pseudoephedrine being the main alkaloids measured in food supplements containing Ephedra herb and its preparations were also considered.

The absorption and disposition characteristics of ephedrine, when orally administered to humans as Ephedra extract (Ma huang), were found to be similar to those observed for an equivalent oral dose of (-)-ephedrine given as a single substance. When orally administered as a single substance, ephedrine was completely absorbed, widely distributed in the body and metabolised by N-demethylation, oxidative deamination of the side chain and conjugation. Unchanged ephedrine was the major urinary excretory product (53-74 % of the dose administered).

Results of several *in vivo* and *in vitro* toxicity studies with Ephedra herbs or their preparations were of limited relevance due to the lack of an indication of the *Ephedra* species from which the botanical/botanical preparation being tested originated and the lack of specification of the individual ephedra alkaloids contents. No data were available on subchronic or chronic toxicity or carcinogenicity of Ephedra herb or its preparations or of pseudoephedrine.

Mice and rats exposed to ephedrine sulphate via the feed at doses up to 750 mg/kg bw/day (mice) or 200 mg/kg bw/day (rats) for 13 weeks exhibited hyperactivity and excitability with the highest incidence in mice of the 150 mg/kg bw/day group and higher and in rats of the 100 mg/kg bw/day group and higher. In both species, no compound-related histopathological changes were found. Compound-related reduced weight gain was observed in each sex from 75 mg/kg bw/day (mice) or 50 mg/kg bw/day (rats) onwards.

No adequate data were available on the genotoxicity of Ephedra herb, its preparations or pseudoephedrine. Ephedrine sulphate was negative in a limited Ames test both with and without metabolic activation.

Regarding the evaluation of possible reproductive and developmental effects, the only data available were derived from a chick embryo study with ephedrine, which by itself was insufficient for risk assessment in humans.

Ephedrine sulphate did not demonstrate carcinogenic activity in a 103-week feeding study in mice and rats.

Reviews of human case reports described adverse cardiovascular and cerebrovascular events, including hypertension, tachycardia, arrhythmias, myocardial infarction, cardiac sudden death, stroke and intracerebral haemorrhage as possibly associated with the use of dietary supplement preparations containing ephedra alkaloids (including combination products containing caffeine). These effects are consistent with the adverse effects associated with the therapeutic use of medicinal products containing ephedra alkaloids as active ingredients.

In most of the clinical studies investigating Ephedra herb preparations, cardiovascular effects were reported such as increased heart rate and increased blood pressure. In all the studies considered Ephedra herb preparations were always tested with concomitant caffeine administration. Cardiovascular effects were also observed in most clinical studies of oral ephedrine, with or without concomitant caffeine administration. Data from clinical studies indicated that pseudoephedrine was less potent at increasing blood pressure and inducing bronchodilation than ephedrine.

Individuals with underlying cardiovascular diseases, that might be unrecognised, or other conditions, such as those described as contraindications for the medical use of ephedra alkaloids, were identified as vulnerable subgroups of the population.

Caffeine, which is often used in combination with Ephedra herb or its preparations in food supplements, could enhance the cardiovascular and central nervous system effects of ephedrine and other ephedra alkaloids.

In the light of the above the ANS Panel concluded that there is wide variation in the concentrations of the individual ephedra alkaloids in different *Ephedra* species and in their preparations for use in food. There were no adequately specified individual preparations of Ephedra herb for which sufficient toxicological data were available for hazard characterisation. In particular there was an absence of data on genotoxicity, subchronic toxicity, carcinogenicity, and reproductive and developmental toxicity.

The Panel concluded that according to the Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009) Ephedra herb and its preparations belong to the category of botanicals and botanical preparations for which the available data are not sufficient to conclude that there is no safety concern (Level A safety assessment based on available knowledge).

Overall, the Panel considered that the absence of adequate data on genotoxicity, short-term , long-term and reproductive and developmental toxicity would not permit advice on a daily intake of *Ephedra* species or preparations made thereof that does not give rise to concerns about harmful effects to health.

The Panel considered that intake of Ephedra herb or its preparations via food supplements could lead to exposure to ephedra alkaloids within or above the therapeutic dose range. Such an exposure could lead to severe adverse effects on the cardiovascular and central nervous systems, which may be enhanced in combination with caffeine. The Panel further noted that for medicinal products a potential for tolerance and dependency to ephedra alkaloids was identified which would be applicable to food supplements and could lead to significant potential for misuse. The Panel also noted that a series of cases of severe adverse effects including fatalities was reported to have a definitive, probable or possible relationship with the intake of ephedra alkaloid-containing food supplements.

Moreover, despite the data gaps identified in the toxicity database, the available information on the pharmacological and toxicological profile and the severe adverse events reported in humans were judged by the Panel to be sufficient to conclude that Ephedra herb and its preparations containing ephedra alkaloids used as food supplements were of significant safety concern at the estimated use

levels.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The German Authorities have raised concerns regarding a potential risk to consumers linked with the consumption of foods containing *Ephedra* species (*Ephedra L.*) and preparations made from them on the basis of a risk assessment report by the German Federal Institute for Risk Assessment (BfR) on *Ephedra* species. The BfR risk assessment report refers to the use of *Ephedra* alkaloid-containing *Ephedra* haulm in food supplements for weight loss and to enhance athletic performance and in bodybuilding. The report concludes that serious undesirable, sometimes life-threatening effects are associated with the consumption of food supplements containing *Ephedra* alkaloids.

The US Food and Drug Administration (FDA) considers that food supplements containing *Ephedra* alkaloids cannot be considered to be safe at any dose and has banned the sale of such products.

Consequently, the Commission has initiated the procedure under Article 8 (2) of Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods[4], for *Ephedra* species.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[5], the European Commission asks EFSA to:

- Review the existing scientific data on the possible link between the intake of *Ephedra* species and a harmful effect on health.

- Provide advice on a tolerable upper intake level (UL) for *Ephedra* species, for the general population, and as appropriate, for vulnerable subgroups of the population.

- In the absence of a tolerable upper intake level (UL), to provide advice on a daily intake of *Ephedra* species that does not give rise to concerns about harmful effects to health.

INTERPRETATION OF THE TERMS OF REFERENCE

The Panel noted that the term "tolerable upper intake level (UL)" was used by EFSA so far only for nutrients, such as vitamins or minerals, to describe the maximum level of total chronic daily intake judged to be unlikely to pose a risk of adverse health effects to humans. The Panel considered that the use of this term may not be appropriate for botanicals[6] and botanical preparations[7] being constituents of food supplements or other food products, especially in cases where the botanicals/botanical preparations or their main components have known medical uses, based on scientifically established pharmacological properties and/or where the botanicals/ botanical preparations cannot be regarded as a component of the normal diet. Using the term "tolerable upper intake level (UL)" for botanicals and botanical preparations could lead to the misinterpretation that they could play a similar role in human nutrition as minerals and vitamins, which differ from them *inter alia* in being in general constituents of the normal diet and in being in many cases essential.

[4] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26-38.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures on matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

[6] This includes all botanical materials (e.g. whole, fragmented or cut plants, plant parts, algae, fungi and lichens) (EFSA SC, 2009).

[7] This includes all preparations obtained from botanicals by various processes (e.g. pressing, squeezing, extraction, fractionation, distillation, concentration, drying up and fermentation) (EFSA SC, 2009).

ASSESSMENT

1. Introduction

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of *Ephedra* species (*Ephedra L.*) and preparations made from them when used in food, e.g. in the form of food supplements.

The evaluation was based on the published scientific literature as well as monographs and risk assessment reports by national and international authorities, which were available for *Ephedra* species, their preparations and alkaloids, regarding food or drug use.

Concerns have been raised regarding possible severe health risks especially associated with the consumption of food supplements, containing herbs of *Ephedra* species, their preparations or their alkaloids, for weight loss, to enhance athletic performance and in bodybuilding (Health Canada, 2001 FDA, 2004; BfR, 2012). (-)-Ephedrine and its congeners occurring in the herbs of *Ephedra* species act as sympathomimetics with pronounced cardiovascular effects as well as stimulating effects on the central nervous system (CNS) (Sweetman, 2011). In combination products, containing the herbs of *Ephedra* species/ephedra alkaloids together with the stimulant caffeine, the effects of ephedra alkaloids in the body might even be increased (Health Canada, 2002). The US Food and Drug Administration (FDA) considered dietary supplements containing ephedrine alkaloids (ephedrine-type ephedra alkaloids[8]) illegal for marketing (FDA, 2004, 2006, online).

This risk assessment was carried out in the framework of the procedure under Article 8 (2) of Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods, for *Ephedra* species initiated by the European Commission. Article 8 (2) of Regulation (EC) No 1925/2006 is referring to a possible prohibition, restriction or Community scrutiny of a substance or ingredient by placement in Annex III, Part A, B or C of this regulation.

The risk assessment was performed according to the EFSA Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee (SC), 2009).

[8] In this opinion the term 'ephedra alkaloids' is used exclusively for alkaloids being closely related in their chemical structure to ephedrine (ephedrine-type alkaloids), such as those shown in Table 1. Thus, in this opinion the term 'ephedra alkaloids' does not comprise other alkaloids such as ephedroxane (an analogue of ephedrine in which the hydroxyl group and the amino group are bridged by a carbonyl group to form an oxazolidone ring).

2. Technical data

2.1. Identity and nature of source material

Ephedra is a genus of gymnosperm shrubs, the only genus in the family of the *Ephedraceae* and in the order of the Ephedrales. Different Ephedra species are listed below.

Scientific (Latin) name:	
Family	*Ephedraceae*
Genus:	*Ephedra* L.
Species:	Data on the number of species vary from 20 and up to 66. (Kitani et al., 2009; IPNI, online; The Plant List, online; USDA, online). Further divisions in subspecies and varieties are used, resulting in up to 263 taxa. Species[9] that are known to contain alkaloids are the following: *Ephedra californica* S. Watson – California joint fir *Ephedra distachya* L. (synonym: *Ephedra vulgaris* Rich.) – Ma huang[10] - jointfir *Ephedra equisetina* Bunge (synonym: *Ephedra shennungiana* T.H. Tang) – Ma huang *Ephedra gerardiana* Wall. Ex Stapf - Ma huang *Ephedra intermedia* Schrenk & C.A. Mey - Ma huang *Ephedra likiangensis* Florin *Ephedra lomatolepis* Schrenk *Ephedra major* Host *Ephedra minuta* Florin (synonym: *Ephedra minuta* var. Dioica) - Ma huang *Ephedra monosperma* J.G. Gmel. ex C.A. Mey *Ephedra regeliana* Florin *Ephedra rhytidosperma* Pachom. (synonym *Ephedra lepidosperma* C.Y. Cheng) *Ephedra saxatilis* (Stapf) Royle ex Florin *Ephedra sinica* Stapf – Chinese ephedra (Ma huang) *Ephedra viridis* Coville (Cui et al., 1991; Kitani et al., 2009; IPNI, online; USDA, online)
Common names:	Few common names are found for the species in the English language. Some of these are given above after the species name. The Chinese common name "Ma huang" is normally associated with some of the species, where it is indicated above (Leung, 1999; Abourashed et al., 2003).
Part used	herb (Ephedrae herba, Herba Ephedrae, Ephedra herb)
Geographical origin:	Temperate climate and sub-tropical regions of Asia, Europe, North and Central America, South America (along the Andes from Ecuador to Patagonia), North Africa (WHO, 1999; Abourashed et al., 2003; Blaschek et al., 2008; USDA, online).
Growth and harvesting conditions:	Some collected from wild stock, mostly cultivated; air-dried, where possible in the sun (Blaschek et al., 2008; USDA, online).

[9] Accepted names and synonyms according to WCSP (2013) and The Plant List, online.

[10] The term Ma huang is found in the literature in several spellings (e.g. ma-huang, ma huang, Ma Huang) and is associated with a number of different *Ephedra* species. In this opinion when the term 'Ma huang' has been found in the literature, without any further specification, it has been reported as such.

In the WHO monograph (1999) the Ephedra plant is described as: "*Erect or prostrate, green, almost leafless shrub, 20–90 cm high. Branches erect, short, glaucous green, somewhat flat, 1.0–1.5 mm in diameter, with small sparse longitudinal striae, fasciated at the nodes; nodes reddish brown; internode 2.5–5.5 cm long x 2 mm in diameter. Small triangular leaves opposite, reduced to scales, barely 2 mm. Flowers in summer, unisexual, dioecious; male flowers pedunculate or nearly sessile, grouped in catkins composed of 4 to 8 pairs of flowers with about 8 anthers; female flowers biflorous, pedunculate with 3 or 4 pairs of bracts, the naked ovule surrounded by an urn-shaped perianth sheath, fruiting with often fleshy red succulent bracts, 2-seeded*". The WHO monograph (1999) refers to *Ephedra sinica* Stapf or other ephedrine-containing *Ephedra* species.

In the European Pharmacopoeia 7.0 (EDQM, 2011) Ephedra herb plant material (*Ephedrae herba, Herba Ephedrae*) is described as: "*Thin cylindrical pale green or yellowish-green stems up to 30 cm long and 1-3 mm in diameter Thin cylindrical pale green or yellowish-green stems up to 30 cm long and 1-3 mm in diameter; longitudinally striated and slightly rough; internodes varying in length between 1 cm and 6 cm; opposite and decussate leaves reduced to sheaths surrounding the stem, carrying diminutive laminae 1.54 mm long with 2 lobes (rarely 3), acutely triangular, apex greyish-white, base tubular and reddish-brown or blackish-brown. Fracture slightly fibrous*". The European Pharmacopoeia monograph refers to *Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk & C.A. Mey or *Ephedra equisetina* Bunge.

The powdered Ephedra herb has a greenish-yellow colour and the following diagnostic characters: fragments of the epidermis, in surface view, composed of rectangular cells and numerous stomata with a small depression at each end, the guard cells large and broadly elliptical; epidermal fragments, in transverse section, showing a thick cuticle and some of the cells extended to form projections; fibres in groups or single, with thick, usually lignified walls; fragments of lignified tissue composed of small, bordered-pitted tracheids, vessels with spiral thickening and groups of sclereids; groups of parenchyma, some with thickened and pitted walls; scattered prism crystals of calcium oxalate (EDQM, 2011). The European Pharmacopoeia monograph refers to *Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk & C.A. Mey or *Ephedra equisetina* Bunge.

2.2. Chemical composition

Biologically active constituents of toxicological relevance of the Ephedra herb are ephedra alkaloids[11] (see Table 1). The occurrence of other alkaloids and amino compounds which are not closely structurally related to ephedrine, such as ephedroxane, ephedradine A, B, C and D, cyclopropyl-α-amino acids, 6-methoxykynurenic acid, N-methylbenzylamine and tetramethylpyrazine, and glycans, such as ephedrans A, B, C, D and E, has been reported in the aerial parts of *Ephedra* species. Other reported components of the twigs and barks of *Ephedra* species are e.g. flavanols (Abourashed et al., 2003).

The relative composition of the alkaloids in the herb varies considerably between the *Ephedra* species and within individual species (see Table 2) (Lake et al., 2001; Kitani et al., 2009; Wang et al., 2010; Hong et al., 2011). The total alkaloid content, depending on the species, origin and time of harvest has been reported to vary between 0.5 and 49 mg/g (see Table 2) (Cui et al., 1991; WHO, 1999; Lake et al., 2001; Kitani et al., 2009).

The two main alkaloids, (-)-ephedrine and (+)-pseudoephedrine, account usually for around 70-99 % of the total alkaloid content in Ephedra herb (Cui et al., 1991; White et al., 1997; Trujillo and Sorenson, 2003; Long et al., 2005; Kitani et al., 2009). The ephedrine content may be between 0 and 90 % and the (+)-pseudoephedrine content may be between 0.1 and 99 % of the total alkaloid content

[11] In this opinion the term 'ephedra alkaloids' is used exclusively for alkaloids closely structurally related to ephedrine (ephedrine-type alkaloids), such as those shown in Table 1. Thus, in this opinion the term 'ephedra alkaloids' does not comprise other alkaloids such as ephedroxane (an analog of ephedrine in which the hydroxyl group and the amino group are bridged by a carbonyl group to form an oxazolidone ring).

(Cui et al., 1991; WHO, 1999; Long et al., 2005; Blaschek et al., 2006; Kitani et al., 2009). The concentrations of (+)-norpseudoephedrine in the herb may amount up to 7.3 mg/g. (-)-norephedrine and (-)-methylephedrine occur in concentrations up to 4.7 or 3.2 mg/g, respectively, and (+)-methylpseudoephedrine has only been detected in traces (see Table 2). The results of Wang et al. (2010) suggested that samples of *Ephedra sinica* Stapf grown in more alkaline soil and in more arid conditions contain more total ephedrine alkaloids and high pseudoephedrine content.

For the following species, the reported total concentrations of which, where known, are given as mg/g in brackets (Zhang et al., 1989; Cui et al., 1991; Lake et al., 2001; Long et al., 2005; Kitani et al., 2009; Hong et al., 2011): *E. equisetina* (15-49), *E. gerardiana* (10.6), *E. intermedia* (5-39) *E. lepidosperma* (= *E. rhytidosperma;* 0.42), *E. likiangensis* (14.8), *E. lomatolepis* (13.6), *E. monosperma* (28), *E. regeliana* (20-32), *E. saxatilis* (8.0) and *E. sinica* (4-42).

Analysing 45 out of 250 collected samples representing nine species of *Ephedra* for total alkaloids as well as for their content of ephedrine and pseudoephedrine, an investigation in Iran showed *E. major* to have the maximum amount of total alkaloid, the amount of ephedrine and pseudoephedrine making up 90 %. (Faker Baher et al., 1999). Only the abstract being available in English, no numerical concentrations can be given.

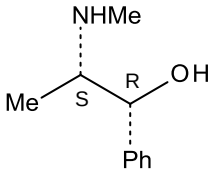
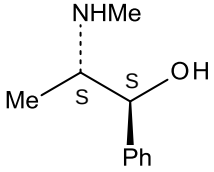
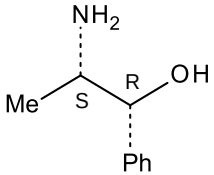
It is reported that not all *Ephedra* species contain ephedra alkaloids (WHO, 1999) but data on *Ephedra* species, which contain no ephedra alkaloids, are contradictory. According to Long et al. (2005), *Ephedra regeliana* contains no ephedra alkaloids, whereas Kitani et al. (2009) demonstrated the existence of 20-32 mg/g of ephedra alkaloids in this species. Cui et al. (1991) found 0.4-0.5 mg/g of total alkaloids in *Ephedra przewalskii* Stapf and *Ephedra lepidosperma* C.Y. Cheng. Other authors assumed that these species do not contain or only contain traces of ephedra alkaloids (Kitani et al., 2009; Long et al., 2005; White et al., 1997; Zhang et al., 1989). Compared with *Ephedra sinica*, which contains up to 42 mg/g, the two latter species have very low concentrations of ephedra alkaloids. According to Trujillo and Sorenson (2003) *Ephedra nevadensis* S. Watson probably contains no ephedra alkaloids. *Ephedra californica* and *Ephedra viridis* contain pseudoephedrine, but no ephedrine (Adams Jr. and Garcia, 2006).

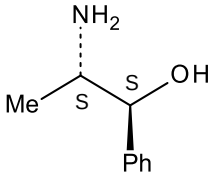
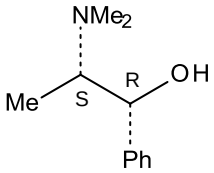
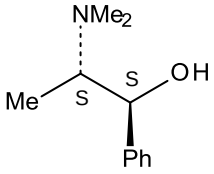
The Panel noted that some studies reported the lack of occurrence of ephedra alkaloids in certain *Ephedra* species. However detailed investigations using state of the art methods of analysis and including a variation of samples within each of the species in question are not available to support this claim.

The presence of ephedra alkaloids has also been reported in the following plants: *Taxus baccata L* (Taxaceae), *Sida cordifolia L*. (Malvaceae), *Roemeria refracta D.C.* (Papaveraceae) and *Aconitum* napellus L (Ranunculaceae). (+)-Norpseudoephedrine (cathine) and (-)-norephedrine are also present in khat (*Catha edulis*, Celastraceae) (Lake et al., 2001, Betz et al., 1997; Marchei et al., 2006). Cathine is formed from cathinone, the main active ingredient contained in khat leaves, when the leaves are dried. Cathinone shows a more pronounced psychostimulant activity than cathine. Khat leaves are widely used in East Africa and the Arab peninsula as an amphetamine-like stimulant (Szendrei, 1980; Brenneisen et al., 1986; Dewick, 2001; Aktories at al., 2009).

In the European Pharmacopoeia 7.0 (EDQM, 2011) ephedrine anhydrous ((-) (1R, 2S); CAS No. 299-42-3) is described as a white, crystalline powder or colourless crystals, soluble in water, very soluble in alcohol. It melts at about 36 °C and has a specific optical rotation of -41 to -43 (2.25 g in 50 mL dilute HCl solution).

Table 1: Ephedra alkaloids and relative synonyms, CAS numbers and structural formulas (WHO, 1999; Hager-ROM, 2006; SciFinder, online).

Name	CAS Registry Number	Molecular formula/ molecular weight	Synonyms[(a)]	Structural formula[(a)]
(-)-ephedrine	299-42-3	$C_{10}H_{15}NO$ 165.23	**Benzenemethanol, α-[(1*S*)-1-(methylamino)ethyl]-, (α*R*)-** Benzenemethanol, α-[1-(methylamino)ethyl]-, [R-(R*,S*)]-;Ephedrine, (-)- (8CI); (-)-(1R,2S)-Ephedrin; (-)-Ephedrin; (-)-Ephedrine; (-)-erythro-Ephedrine; (1R,2S)-(-)-Ephedrine; (1R,2S)-1-Hydroxy-2-(methylamino)-1-phenylpropane; (1R,2S)-2-(Methylamino)-1-phenyl-1-propanol; (1R,2S)-Ephedrine; (αR)-α-[(1S)-1-(Methylamino)ethyl]benzenemethanol; 1(R),2(S)-erythro-(-)-Ephedrine; Efedrina; Ephedrin; Ephedrine; L-Ephedrine; L-erythro-2-(Methylamino)-1-phenylpropan-1-ol;NSC 170951; NSC 8971; Xenadrine; l-Ephedrine	
(+)-pseudoephedrine	90-82-4	$C_{10}H_{15}NO$ 165.23	**Benzenemethanol, α-[(1*S*)-1-(methylamino)ethyl]-, (α*S*)-** Benzenemethanol, α-[1-(methylamino)ethyl]-, [S-(R*,R*)]-; Pseudoephedrine (6CI); Pseudoephedrine, (+)- (8CI); (+)-(1S,2S)-Pseudoephedrine; (+)-Pseudoephedrine; (+)-threo-Ephedrine; (+)-ψ-Ephedrine; (1S,2S)-(+)-Pseudoephedrine;(1S,2S)-Ephedrine; (1S,2S)-Pseudoephedrine; Isoephedrine;L(+)-ψ-Ephedrine; L-(+)-Pseudoephedrine; Lopac E 3250; d-Isoephedrine; d-Pseudoephedrine; d-ψ-Ephedrine; trans-Ephedrine; ψ-Ephedrine; ψ-Ephedrine, (+)-	
(-)-norephedrine	492-41-1	$C_9H_{13}NO$ 151.21	**Benzenemethanol, α-[(1S)-1-aminoethyl]-, (αR)-** Benzenemethanol, α-(1-aminoethyl)-, [R-(R*,S*)]-; (-)-Norephedrin; (-)-Norephedrine; (-)-Phenylpropanolamine; (-)-erythro-2-Amino-2-methyl-1-phenylethanol; (1R,2S)-(-)-Norephedrine; (1R,2S)-2-Amino-1-phenyl-1-propanol; (1R,2S)-2-Amino-1-phenyl-1-propanol; (1R,2S)-Norephedrine; (1S,2R)-2-Hydroxy-2-phenyl-1-methyl-1-aminoethane; (R,S)-(-)-Norephedrine; L-Norephedrine; NSC 17704; Norephedrine, (-)-; erythro-(1R,2S)-Norephedrine; l-Norephedrine; l-Phenylpropanolamine	

Name	CAS Registry Number	Molecular formula/ molecular weight	Synonyms[(a)]	Structural formula[(a)]
(+)-norpseudoephedrine	492-39-7	$C_9H_{13}NO$ 151.21	**Benzenemethanol, α-[(1S)-1-aminoethyl]-, (αS)-** Benzenemethanol, α-(1-aminoethyl)-, [S-(R*,R*)]-; Benzyl alcohol, α-(1-aminoethyl)-, (+)- (8CI); Cathine (6CI); (+)-Cathine; (+)-Norpseudoephedrine; (+)-Pseudonorephedrin;(+)-Pseudonorephedrine; (1S)(2S)-Cathine; (1S,2S)-(+)-Norpseudoephedrine; (1S,2S)-Norephedrine; (1S,2S)-Pseudonorephedrine; (1S,2S)-Pseudonorephedrine; (1S,2S)-threo-Norephedrine; Cathin; D-(+)-Norpseudoephedrine; D-Norpseudoephedrin; Fugoa-Depot; Katine; d-Norpseudoephedrine; d-Pseudonorephedrine	
(-)-methylephedrine	552-79-4	$C_{11}H_{17}NO$ 179.26	**Benzenemethanol, α-[(1S)-1-(dimethylamino)ethyl]-, (αR)-** Benzenemethanol, α-[1-(dimethylamino)ethyl]-, [R-(R*,S*)]-;Benzyl alcohol, α-[1-(dimethylamino)ethyl]-, (-)- (8CI);Ephedrine, N-methyl-, L- (3CI); (-)-Methylephedrine; (-)-N-Methylephedrine; (1R,2S)-(-)-N-Methylephedrine; (1R,2S)-2-Dimethylamino-1-phenyl-1-propanol; (1R,2S)-2-Dimethylamino-1-phenylpropanol; (1R,2S)-3-Dimethylamino-1-phenyl-1-propanol; (1R,2S)-Methylephedrine; (1R,2S)-N-Methylephedrin; (1R,2S)-N-Methylephedrine; L-N-Methylephedrine; Methylephedrine; N-Methyl-L-ephedrine; N-Methylephedrine	
(+)-methylpseudoephedrine	51018-28-1	$C_{11}H_{17}NO$ 179.26	**Benzenemethanol, α-[(1S)-1-(dimethylamino)ethyl]-, (αR)-** Benzenemethanol, α-[1-(dimethylamino)ethyl]-, [R-(R*,S*)]-;Benzyl alcohol, α-[1-(dimethylamino)ethyl]-, (-)- (8CI);Ephedrine, N-methyl-, L- (3CI); (-)-Methylephedrine; (-)-N-Methylephedrine; (1R,2S)-(-)-N-Methylephedrine; (1R,2S)-2-Dimethylamino-1-phenyl-1-propanol; (1R,2S)-2-Dimethylamino-1-phenylpropanol; (1R,2S)-3-Dimethylamino-1-phenyl-1-propanol; (1R,2S)-Methylephedrine; (1R,2S)-N-Methylephedrin; (1R,2S)-N-Methylephedrine; L-N-Methylephedrine; Methylephedrine; N-Methyl-L-ephedrine; N-Methylephedrine	

(a): From SciFinder, online

Table 2: Ephedra alkaloid content in mg/g dry weight and proportion of the two main alkaloids (%) in the total alkaloid content in the Ephedra herb of various species.

Species	No of samples analysed	Ephedra alkaloid total	(-)ephedrine	(+)-pseudoephedrine	(-)-norephedrine	(+)-norpseudo ephedrine	(-)-methyl ephedrine	(+)-methyl pseudoephedrine	Country/ Region	Reference
	-	9.7	6.4 (66 %)	1.4 (15 %)	0.9	0.8	0.3	0.01		Trujillo et al., 2005
	-	21.3	9.1 (43 %)	8.0 (38 %)	0.4	1.6	1.9	0.1		Trujillo and Sorenson,2003
E. sinica	-	16.3	12.4 (76 %)	3.1 (19 %)	n.a.	n.a.	0.8	not analysed		White et al., 1997
	15	18.6-41.6	3.8-21.8 (11-78 %)	2.2-22.4 (8-67 %)	0.7-3.4	0.7-7.3	0.7-3.2	not analysed	Mongolia	Kitani et al. ,2009
	-	12.6-13.8	7.6-8.1 (58-60 %)	2.8 (20-22 %)	1.0	1.1-1.4	0.5-0.7	traces	China	Cui et al., 1991
	34[a]	3.5-26	1.5-15.4 (43-74%)	0-13.2 (0-50 %)	0-4.3	0-2.2	0.2-2.4	not analysed	China	Hong et al., 2011
	2	39.8-49.0	1.4-1.5 (3-4 %)	36.6-44.5(91-92 %)	0.7-1.2	0.7-1.2	0.7-1.0	not analysed	Mongolia	Kitani et al. ,2009
E. equisetina	-	22.1	12.5 (57 %)	5.8 (26 %)	2.0	1.6	0.3	not detected	China	Cui et al., 1991
	7[b]	15.4-33.7	3.0-24.6(20-74 %)	1.6-15.2(6-45 %)	0.1-3.4	0.6-3.7	0-0.9	not analysed	China	Hong et al., 2011
	-	4.6-18.1	0-3.3(0-30 %)	1.5-15.6(3-99 %)	0.1-0.9	not analysed	not detected	not analysed	China	Long et al., 2005
E. intermedia	-	11.2-16.6	1.3-5.5(12-33 %)	8.0-9.1(54-72 %)	0.3-0.8	1.1-1.3	0.1-0.3	not detected	China	Cui et al., 1991
	23[b]	4.6-39.4	0-11.4(0-30 %)	5.3-25.4(35-89 %)	0-4.7	0.2-4.8	0-1.5	not analysed	China	Hong et al., 2011
E. regeliana	3	19.5-31.9	0.7-5.9 (0-21 %)	14.4-27.8(71-89 %)	0.7-1.3	0.7-3.5	0.7-1.0	not analysed	Mongolia	Kitani et al. ,2009
	-	not detected	not detected	not detected	not detected	not detected	not detected	not analysed	China	Long et al., 2005
	-	not detected	not detected	not detected	not detected	not detected	not detected	not analysed	Mongolia	Kitani et al. ,2009
E. przewalskii	-	not detected	not detected	not detected	not detected	not analysed	not detected	not analysed	China	Long et al., 2005
	1	0.5	0.3 (60 %)	0.06 (12 %)	0.03	0.05	0.03	not detected	China	Cui et al., 1991
E. likiangensis	1	14.8	6.3 (43 %)	6.1 (41 %)	0.5	1.7	0.3	traces	China	Cui et al., 1991
E. monosperma	1	28.3	14.1 (50 %)	8.6 (30 %)	1.8	3.3	0.5	0.1	China	Cui et al., 1991
E. gerardiana	1	10.6	7.7 (73 %)	1.0 (9 %)	0.8	0.7	0.4	trace	China	Cui et al., 1991
E. saxatilis	1	8.0	5.9 (74 %)	0.5 (6 %)	0.6	0.2	0.6	not detected.	China	Cui et al., 1991
E. lomatolepis	1	13.6	1.7 (13 %)	8.3 (61 %)	0.4	3.2	0.1	trace	China	Cui et al., 1991
E. lepidosperma	1	0.42	0.12 (29 %)	0.17 (40 %)	0.05	0.07	0.01	trace	China	Cui et al., 1991

(a): 29 wild, 5 cultivated
(b): wild

2.2.1. Biosynthesis of the ephedra alkaloids

^{15}N, ^{3}H and ^{14}C labeled compounds (phenylalanine, methionine and sodium formate among others) were supplied to growing shoots of *Ephedra distachya*. It was demonstrated that ephedra alkaloids are biosynthesized by the condensation of a benzylic C_6-C_1 portion which is derived from phenylalanine via cinnamate and a C_2 fragment derived from the CH_3CO group of pyruvic acid (Yamasaki et al., 1973; Grue-Sørensen and Spenser 1993). Structures of alkaloids and biosynthetic routes are shown in Figures 1-2.

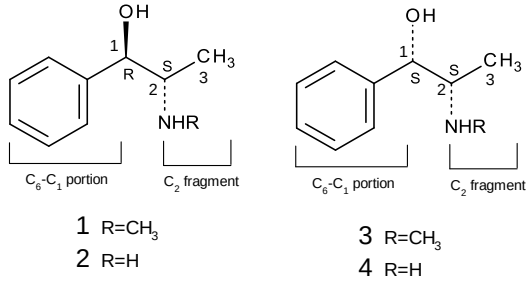


Figure 1: Structures of 1 = (1R,2S)-(-)-ephedrine; 2 = (1R,2S)-(-)-norephedrine; 3 = (1S,2S)-(+)-pseudoephedrine and 4 = (1S,2S)-(+)-norpseudoephedrine, adapted from Grue-Sørensen and Spenser, 1993.

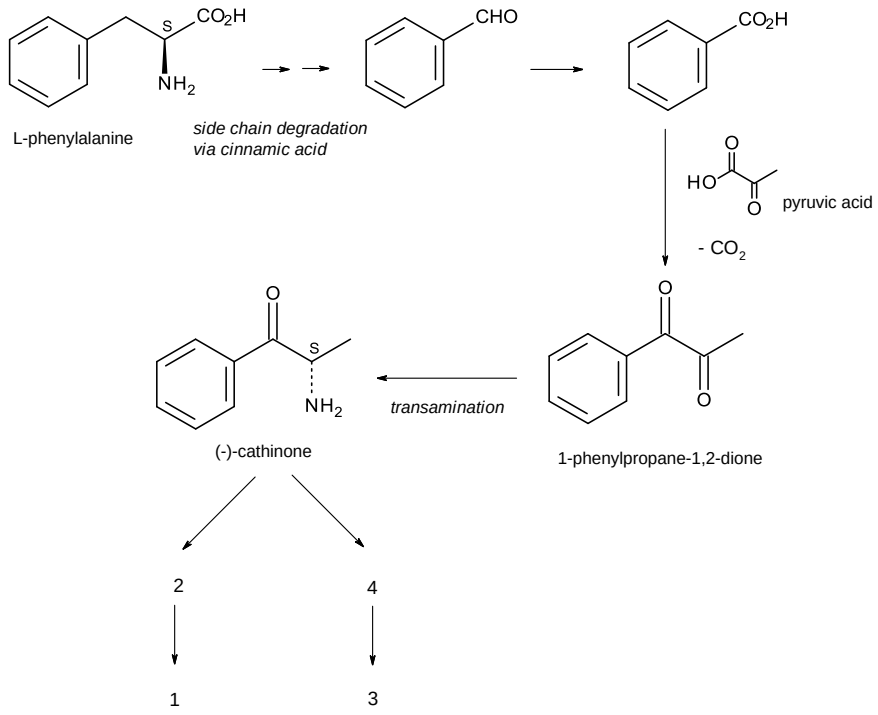


Figure 2: Biosynthesis of 1 = (1*R*,2*S*)-(-)-ephedrine; 2 = (1*R*,2*S*)-(-)-norephedrine; 3 = (1*S*,2*S*)-(+)-pseudoephedrine and 4 = (1*S*,2*S*)-(+)-norpseudoephedrine from phenylalanine adapted from Grue-Sørensen and Spenser, 1993 and Dewick, 2001.

2.2.2. Stereochemical aspects

According to their structural formula, ephedrine and pseudoephedrine contain two chiral C atoms and therefore two sets of optical isomers. The total number of stereoisomers of the two sets is four. All isomers have been successfully synthesised in the period between 1911 and 1920 (Chen, 1928).

The widespread occurrence of differences in biological activities for optical activities has been of particular importance in the development of theories on the nature of drug-receptor interactions. Ephedra alkaloids are asymmetric[12], meaning that they cannot be divided into symmetrical halves. The diastereomers (-)-ephedrine and (+)-pseudoephedrine have different physical properties: (-)-ephedrine with erythro configuration has a melting point of 79 °C and is soluble in water, whereas (+)-pseudoephedrine with threo configuration has melting point of 118 °C and is only sparingly soluble in water. Receptors, that are mostly proteins constructed from L-amino acids, are also asymmetric. Therefore it can be predicted that optical isomers will also have different biological properties (Beale and Block, 2011). (-)-Ephedrine showed 3 times more pressor activity than (+)-ephedrine, 5 times more pressor activity than (+)-pseudoephedrine and 36 times more pressor activity than (-)-pseudoephedrine (Beale and Block, 2011) (see section 3.1).

2.3. Specifications

There are no known specifications for Ephedra herb for use in food supplements.

The European Pharmacopoeia 7.0 (EDQM, 2011) contains a monograph on Ephedra herb (Ephedrae Herba, Herba Ephedrae) and the WHO monographs on selected plants (WHO, 1999) contain a monograph on Herba Ephedrae[13]. The parts referring to the specifications on identity and purity are summarised in Table 3.

Table 3: Specifications for Ephedra herb according to European Pharmacopoeia 7.0 (EDQM, 2011) and WHO Monographs on selected plants (WHO, 199).

	European Pharmacopoeia 7.0 (EDQM, 2011)	**WHO Monographs on selected plants (WHO, 1999)**
Definition	Dried herbaceous stem of *Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C.A.Mey, or *Ephedra equisetina* Bunge	Herba Ephedrae consists of the dried stem or aerial part of *Ephedra sinica* Stapf or other ephedrine-containing Ephedra species
Identification	A. Macroscopic examination B. Microscopic examination of the powder using *chloral hydrate solution R*. C. Thin-layer chromatography	Macroscopic and microscopic examinations and microchemical tests for the presence of alkaloids with Mayer's reagent
Assay	Content: minimum 1.0 % of ephedrine ($C_{10}H_{15}NO$; M_r 165.2) (dried drug) determined by liquid chromatography.	Contains not less than 0.7 % total alkaloids, calculated as ephedrine by high performance liquid chromatography in the Japanese pharmacopoeia; or not less than 0.8 % of total alkaloids, calculated as ephedrine in the Chinese pharmacopoeia. Thin-layer, gas–liquid or high-performance liquid chromatographic analysis for ephedrine and related alkaloids are available.
Major active principles	-	(-)-Ephedrine in concentrations of 40–90 % of the total alkaloid fraction, accompanied by (+)-pseudoephedrine. Other trace alkaloids in the alkaloid complex include (-)-norephedrine, (+)-norpseudoephedrine, (-)-methylephedrine and (+)-methylpseudoephedrine. The total alkaloid content can exceed 2 % depending on the species. Not all Ephedra species contain ephedrine or alkaloids

[12] In this opinion, when the optical activity of the individual ephedra alkaloids is not indicated it means that it was not reported in the literature source used.

[13] If for the term "Ephedra herb" the originating *Ephedra* species are not indicated in this opinion they were not reported in the literature source. When the term refers to the botanical material as specified by the Ph Eur or WHO this would be made clear by quoting these references.

	European Pharmacopoeia 7.0 (EDQM, 2011)	**WHO Monographs on selected plants (WHO, 1999)**
Purity		
Loss on drying	Maximum 10.0 %, determined on 1.000 g of the powdered drug by drying in an oven at 105 °C for 2 hours.	Not more than 9 %
Total ash	Maximum 9.0 %	Not more than 9 %
Acid-insoluble ash	-	Not more than 2 %
Foreign organic matter	-	Woody stems, not more than 5 %. Does not contain stems of Equisetaceae or Gramineae plants, nor any other foreign matter
Heavy metals	As specified in the General monograph	Recommended lead and cadmium levels are no more than 10 and 0.3 mg/kg, respectively, in the final dosage form of the plant material
Pesticide residues	As specified in the General monograph	To be established in accordance with national requirements. Normally, the maximum residue limit of aldrin and dieldrin for Herba Ephedrae is not more than 0.05 mg/kg. For other pesticides, see WHO guidelines on quality control methods for medicinal plants and guidelines for predicting dietary intake of pesticide residues
Microbiology	As specified in the General monograph	The test for *Salmonella* spp. in *Herba Ephedrae* products should be negative. The maximum acceptable limits for other microorganisms are as follows. For preparation of decoction: aerobic bacteria — not more than 10^7/g; fungi — not more than 10^5/g; *Escherichia* coli — not more than 10^2/g. Preparations for internal use: aerobic bacteria — not more than 10^5/g or mL; fungi — not more than 10^4/g or mL; enterobacteria and certain Gram-negative bacteria — not more than 103/g or mL; *Escherichia* coli — 0/g or mL.
Radioactive residues	-	For analysis of strontium-90, iodine-131, caesium-134, caesium-137, and plutonium-239, see WHO guidelines on quality control methods for medicinal plants
Other purity tests	-	Chemical, dilute ethanol-soluble extractive, and water-soluble extractive tests to be established in accordance with national requirements.
Dosage forms	-	Powdered plant material; extracts and other galenicals. Store in well closed, light-resistant containers.

The European Pharmacopoeia 7.0 (EDQM, 2011)contains specifications for ephedrine, anhydrous ((-) (1*R*,2*S*); CAS No. 299-42-3); ephedrine hemihydrate ((-) (1*R*,2*S*); CAS No. 50906-05-3) ; ephedrine hydrochloride ((-) (1*R*,2*S*); CAS No. 50-98-6); ephedrine hydrochloride, racemic ((±)(1*RS*,2*SR*); CAS No. 134-71-4) ; pseudoephedrine hydrochloride ((+) (1*S*,2*S*); CAS No. 345-78-8) and phenylpropanolamine hydrochloride ((±)(1*RS*,2*SR*); CAS No. 154-41-6).

2.4. Manufacturing process

Herbaceous stems of the ephedra alkaloid-containing *Ephedra* species mostly cultivated or from wild stock are collected and air-dried, where possible in the sun (Blaschek et al., 2008; USDA, online).

2.5. Methods of analysis in food

Authentication of *Ephedra* ground plant material can be achieved by the chemical fingerprinting method. Schaneberg et al. (2003) applied reverse phase high performance liquid chromatography with photodiode array detection (HPLC/PDA) for the chemical fingerprinting of *Ephedra* species. The method could distinguish between *Ephedra* species from Eurasia, North America and South America. Sequencing and molecular analysis of purified polymerase chain reaction (PCR) products from different *Ephedra* species demonstrated that the sequences of *trn*K gene and 18S ribosomal RNA gene provide a useful index for identification and taxonomic classification of *Ephedra* plants (Kitani et. al., 2009).

Two inter-laboratory validated methods for the determination of ephedra alkaloids in botanicals and food supplements are identified in the literature. The AOAC official method 2003.13 using high performance liquid chromatography with UV detection (HPLC-UV) gives satisfactory results for the determination of ephedrine (reproducibility standard deviation (RSD_R) 2.1-6.6 %, HORRAT 0.7-2.12) and pseudoephedrine (RSD_R 9.0-11.4 %, HORRAT 1.77-2.78) in the presence of norephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrine in botanicals and food supplements, excluding the results from the high protein drink (Roman, 2004). The second inter-laboratory validated method employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of the above mentioned six ephedrine-type alkaloids in dietary supplements and botanicals (Trujillo and Sorenson, 2003). For food supplements, statistic showed good overall repeatability standard deviation (RSD_r) (3.0-11.8 %), (RSD_R 11.1-16.9 %) values and recovery, but the method did not show acceptable precision according to the HORRAT values that ranged from 1.74 to 14.1 (acceptable HORRAT range is: 0.5-2).

Reported single-laboratory validated methods for the determination of ephedra alkaloids in food supplements include HPLC (Sagara et al., 1983; Gurley et al., 1998a, 2000; Wang et al., 2010; Hong et al., 2011), LC-MS/MS (Jacob et al., 2004), gas chromatography (Betz et al., 1997), gas chromatography-mass spectrometry (GC-MS) (Cui et al., 1991; Marchei et al., 2006), high performance capillary electrophoresis (HPCE) (Avula and Khan., 2004) and PCR (Techen et al., 2006).

Attempts have been made to develop immunoassays methods for the determination of ephedrine in different biological matrices such as urine (Miagkova et al., 1991); however none have so far been successfully developed for food or food supplement matrices.

2.6. Stability of the botanicals or botanical preparation used as ingredient in food supplements and reaction and fate in food

There is no information available on the stability of Ephedra herb and its ingredients in food supplements.

The European Pharmacopoeia 7.0 indicates that ephedrine, anhydrous; ephedrine hydrochloride; ephedrine hydrochloride, racemic and pseudoephedrine hydrochloride should be stored protected from light (EDQM, 2011).

2.7. Uses and use levels (including products marketed via internet)

2.7.1. Common foods

The European Herbal Infusions Association (EHIA) declared that Ephedra is not used, as a plant or part of plant, by the European industry for herbal and fruit infusions (Letter from EHIA, personal communication, April 2013).

There is no indication that parts of the *Ephedra* plant or its preparations are a component of common food in Europe.

2.7.2. Food supplements

Commercial dietary supplements containing Ephedra herbs or their preparations are typically used for weight loss and athletic performance enhancement, often in combination with caffeine or guarana extracts and/or other components.

According to a survey conducted among members of the European Responsible Nutrition Alliance (ERNA), Food Supplements Europe and the European Botanical Forum representative of the relevant EU food sector, *Ephedra* and its preparations are not used in food supplements or other food products. As a result there has been no interest in generating data to demonstrate the safety of this botanical for food use (Letter from ERNA, personal communication, April 2013).

Thus, the Panel noted that these products are not generally marketed in Europe but that at present they can easily be purchased via the internet.

Quantitative determination of ephedra alkaloids and ephedrine in dietary supplements has been carried out in five different studies (see Table 4) and results have been expressed as mg/g and/or mg/serving (tablet, capsule, chewing gum, powder, liquid or herb). A summary of the main results for each of these studies is reported below.

Haller et al. (2004) analysed 35 samples from 15 commercial dietary supplements by means of a LC-MS/MS method. Food supplements considered in this study were presented as tablets or capsules (10 samples), liquid (3 samples), gelcap (1 sample) or powder (1 sample). The total ephedra alkaloid content ranged from 5.97 mg to 29.3 mg per serving. Of the products tested, 31 % contained more than 110 % of the total ephedra alkaloids listed on the label. The ephedrine content ranged from 0.7 to 10.7 mg per serving and the amount determined by analysis was always higher than the one declared on the label (from 102 % to 242 %). Results on the levels of pseudoephedrine, methylpseudoephedrine, norephedrine and norpseudoephedrine were also reported in this paper.

The ephedra alkaloid content of 20 Ephedra herb containing supplements (duplicate samples for 4 products) was determined by high-performance liquid chromatography by Gurley et al. (2000). One sample referred to a liquid extract, all other supplements were in the form of tablets or capsules. In this study total alkaloid content ranged from not detected to 23.5 mg per serving. Half of the products exhibited discrepancies > 20 %, between the label claim for ephedra alkaloid content and actual alkaloid content. One product was devoid of ephedra alkaloids. Ephedrine content ranged from below the limit of detection to 15.3 mg per serving and the amount determined varied from 25 % to 105 % of that declared on the label. Results on the levels of pseudoephedrine, methylephedrine, norephedrine and norpseudoephedrine were also reported in this paper.

Ephedra alkaloids were measured in 35 dietary supplements (duplicate samples for 12 products) to examine variability within and between products as well as for comparison of actual constituents with label claims of manufactured products (Baker et al., 2003). Only supplements served in tablets or capsules were considered in this study. Total alkaloid content ranged from 2.5 to 29.9 mg per serving unit. In most of the cases, total ephedra alkaloid content in dietary supplements was in line with label claims. Out of the 47 products analysed, only eight were found to deviate by > 25 % from the content declared on the label. Ephedrine content ranged from 2.1 to 29.6 mg per serving; no information was given in this paper on the amount of ephedrine reported on the label. Results on the levels of pseudoephedrine and methylephedrine were also reported in this paper.

Lake et al. (2001) performed analytical studies on products containing Ephedra herb on the Dutch market at the request of the Dutch Health Care Inspectorate and the Inspectorate of Health Protection, Commodities and Veterinary Public Health. This is the only study reporting information on food supplements commercialised in Europe since the previous three all referred to the United States. In total, data from 121 samples of dietary supplements were examined in the period 1993 to 1999. Food supplements considered in this study were served in tablets or capsules (99 samples), as liquid (6), chewing gum (2), powder (2) herb (2) and sachet (1). The serving type was not reported for 9 samples.

No information was given in this paper on the amount of total ephedra alkaloid and ephedrine reported on the label. The total ephedra alkaloid and ephedrine content ranged from 0.2 mg to 75.5 mg per serving and from 0.2 mg to 65.2 mg per serving, respectively. Results on the levels of pseudoephedrine, methylephedrine, norephedrine and norpseudoephedrine were also reported in this paper. In addition, Lake et al. (2001) investigated whether synthetic ephedra alkaloids were present, and if so, determined their concentration. The large majority of the 121 samples tested were classified as "probably" (60 %) or "possibly" (17 %) of natural origin. All the other samples were categorised as "unlikely" of natural origin (12 %), or "probably of natural origin but enriched with a synthetic analogue" (2 %). In 9 % of the cases it was not possible to carry out this evaluation.

Trujillo et al. (2003) conducted an inter-laboratory study to evaluate the accuracy and precision of a method for ephedrine-type alkaloids (norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine, methylephedrine, and methylpseudoephedrine) in dietary supplements and botanicals. The amount of ephedrine-type alkaloids present was determined using liquid chromatography with tandem mass selective detection. In this study only 5 dietary supplements (3 tablets or capsules, 1 as liquid and 1 powder) were analysed, total alkaloid content ranged from 0.3 to 98.6 mg/g.

The number and percent of food supplements according to their content of the different ephedrine alkaloids are reported in Table 5.

Table 4: Results from five different studies on quantification of ephedra alkaloids in dietary supplements

Reference		Number of samples reported as		Minimum		Mean		Standard Deviation		Median		Maximum	
		mg/g	mg/ serving	mg/g	mg/ serving	mg/g	mg/ serving	mg/g	mg/ serving	mg/g	mg/ serving	mg/g	mg/ serving
Baker et al. (2003)	Total alkaloids	0	40	.	2.5	.	12.4	.	6.6	.	10.9	.	29.9
	Ephedrine	0	40	.	2.1	.	10.4	.	6.2	.	8.8	.	29.6
	Pseudoephedrine	0	25	.	0.6	.	2.9	.	2.2	.	2.1	.	8.9
	Methylephedrine	0	26	.	0.1	.	0.3	.	0.2	.	0.2	.	0.9
Gurley et al. (2000)	Total alkaloids	0	23	.	1.3	.	11.5	.	4.9	.	11.6	.	23.5
	Ephedrine	0	23	.	1.1	.	8.4	.	4.1	.	9.3	.	15.3
	Pseudoephedrine	0	20	.	0.2	.	3.1	.	2.7	.	2.0	.	9.5
	Methylephedrine	0	11	.	0.2	.	0.7	.	1.0	.	0.3	.	2.7
	Norephedrine	0	6	.	0.2	.	0.2	.	0.0	.	0.2	.	0.3
	Norpseudoephedrine	0	5	.	0.2	.	0.3	.	0.1	.	0.3	.	0.4
Haller et al. (2004)	Total alkaloids	15	15	1.2	6.0	13.0	19.3	14.1	6.4	10.6	21.6	60.7	26.1
	Ephedrine	15	15	0.7	3.9	10.3	13.6	14.2	4.9	7.3	14.7	59.5	19.4
	Pseudoephedrine	15	15	0.3	0.2	1.9	3.8	1.2	2.2	1.6	3.2	4.0	7.7
	Methylpseudoephedrine	15	0	0.1	.	0.4	.	0.5	.	0.2	.	1.7	.
	Norephedrine	15	15	0.0	0.0	0.1	0.3	0.1	0.3	0.1	0.2	0.4	0.8
	Norpseudoephedrine	15	15	0.0	0.0	0.3	0.6	0.2	0.6	0.2	0.3	1.0	2.0
Lake et al. (2001)	Total alkaloids	107	102	0.1	0.2	34.8	26.8	26.2	20.8	33.8	21.6	95.7	75.5
	Ephedrine	107	102	0.1	0.2	24.1	18.3	21.7	16.0	14.4	14.6	83.3	65.8
	Pseudoephedrine	86	84	0.2	0.3	9.5	7.3	7.9	6.9	8.0	4.9	34.8	34.3
	Methylephedrine	25	24	0.0	0.0	5.6	4.9	5.4	4.8	3.0	2.5	16.4	14.4
	Norephedrine	5	5	0.0	0.0	0.2	0.2	0.3	0.2	0.1	0.1	0.6	0.4
	Norpseudoephedrine	12	11	0.1	0.1	1.0	0.8	1.1	1.1	0.6	0.2	3.8	3.6
Trujillo et al. (2003)	Total alkaloids	5	0	0.3	.	34.1	.	38.1	.	20.5	.	98.6	.
	Ephedrine	5	0	0.2	.	27.2	.	33.2	.	15.2	.	83.9	.
	Pseudoephedrine	5	0	0.1	.	4.5	.	4.6	.	3.5	.	12.4	.
	Methylephedrine	5	0	0.0	.	1.3	.	1.2	.	0.9	.	3.2	.
	Methylpseudoephedrine	5	0	0.0	.	0.2	.	0.4	.	0.1	.	1.0	.
	Norephedrine	5	0	0.0	.	0.4	.	0.3	.	0.4	.	0.9	.
	Norpseudoephedrine	5	0	0.0	.	0.5	.	0.6	.	0.3	.	1.6	.

Table 5: Number and % of food supplements according to their content of the different ephedrine alkaloids

	Ephedrine[(a)]		Pseudoephedrine[(a)]		Methylephedrine[(a)]		Methylpseudo ephedrine[(a)]		Norephedrine[(a)]		Norpseudo ephedrine[(a)]	
	Number of samples	**% of samples**	**Number of samples**	**% of samples**	**Number of samples**	**% of samples**	**Number of samples**	**% of samples**	**Number of samples**	**% of samples**	**Number of samples**	**% of samples**
Not detected, traces or not relevant	13	6	52	25	123	60	56	74	134	81	126	77
Less than 1%	0	0	0	0	7	3	5	7	12	7	8	5
From 1 to 5%	0	0	4	2	37	18	10	13	18	11	18	11
From 5 to 10%	2	1	6	3	9	4	3	4	1	1	10	6
From 10 to 20%	3	1	57	28	9	4	2	3	0	0	1	1
From 20 to 50%	19	9	71	35	5	2	0	0	0	0	0	0
From 50 to 90%	126	61	15	7	15	7	0	0	0	0	0	0
Higher than 90%	42	20	0	0	0	0	0	0	0	0	0	0
Total samples tested	205	100	205	100	205	100	76	100	165	100	163	100

(a): stereoisomers not specified. It is assumed that in most of the cases these are the naturally occurring forms.

Ephedrine represented > 90 % of total alkaloids in 42 (20 %) of the above mentioned food supplements; pseudoephedrine mainly ranged between 10 and 50 % of total alkaloids whereas in more than 60 % of the samples methylephedrine, methylpseudoephedrine, norephedrine and norpseudoephedrine were not detected. Among these latter substances only methylephedrine (in 20 samples) contributed > 20 % to total alkaloids.

Information on the maximum number of servings per day, as suggested on the labels of the food supplements included in the above studies, is reported in Table 7 and discussed in section 2.8.1.

Results from the above mentioned papers identified that the declared content of ephedra alkaloid and ephedrine in food supplements on the label, or on the commercial web page, is not always reliable and consequently cannot be used for the assessment of exposure.

No information was reported on the content of ephedra alkaloids in herbal tea infusions. A herbal tea named "Mormon tea" is known to originate from *Ephedra* species, e.g. *E. nevadensis* (Bracher et al., 2010) thus the possibility that consumers may purchase tea from Ephedra herb via the internet cannot be excluded.

The Panel noted that qualitative and quantitative data for compounds other than ephedrine-type ephedra alkaloids for Ephedra herb containing food supplements are missing. Such data would be needed for all biologically active substances present in the herbs. For example, certain glycans (ephedran A, B, C, D and E) have been isolated from *Ephedra distachya* herbs and have been shown to significantly reduce blood glucose levels in normal and alloxan-induced diabetic mice (Konno et al., 1985; Abourashed et al., 2003). Ephedroxane may potentiate the action of noradrenaline in the catecholaminergic nervous system as concluded by Hikino et al. (1985) from studies in which ephedroxane has been injected intraperitoneally to mice (Hikino et al., 1985).

Results of Grippo et al. (2007), who detected isoflavones, e.g. daidzein, in ephedra-containing dietary supplements could not be taken into account in this opinion, because the composition of the dietary supplements analysed for this study was unknown and thus the origin of the isoflavones remains unclear.

2.7.3. Medicinal products

2.7.3.1. Botanical/botanical preparations

Ephedra, or Ma huang has been known in Chinese medicine for more than 5 000 years. The parts of the plant used in traditional medicine are the dried green stems, which are usually boiled in water and administered as a hot tea. The usual daily dose is reported to be 1.5–9 g[14] of the decocted herb (Leung, 1999).

Several textbooks and Pharmacopoeias mention the use of the Ephedra herb, either for the use of tea preparations (decoctions) or the use of ethanolic preparations thereof. Sometimes also the use of the herbal substance[15] (cut or powdered) is described.

In the EB6 (1953) the usage of the Ephedra herb and a tincture prepared from the herb were described; however no indication for use is mentioned.

[14] In this opinion, medicinal use doses are always referred to the quantity (mg, g) administered per person unless otherwise specified.

[15] In this opinion the term herbal substance is used as defined in Directive 2001/83/EC, i.e.: "All mainly whole, fragmented or cut plants, plant parts, algae, fungi, lichen in an unprocessed, usually dried, form, but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal substances. Herbal substances are precisely defined by the plant part used and the botanical name according to the binomial system (genus, species, variety and author)".

The monograph "Ephedra" of the British Herbal Pharmacopoeia (BHP, 1979) reported as indications: "asthma, hay fever, urticaria, enuresis, narcolepsy, myasthenia gravis". The usage of Ephedra herb and of liquid extracts and tinctures thereof were mentioned. For Ephedra herb a single dose of 1-4 g given 3 times daily is recommended (BHP, 1979).

The Monograph of the Commission E[16] described the short-term usage of Ephedra herb in diseases of the respiratory tract with mild bronchospasms in adults and children over the age of six. Regarding Ephedra herb preparations, for adults a single dose corresponding to 15-30 mg total alkaloids, calculated as ephedrine and a maximum daily dose of 300 mg total alkaloids calculated as ephedrine are recommended. In children, Ephedra herb preparations are recommended as a single dose corresponding to 0.5 mg total alkaloids (calculated as ephedrine)/kg bw and a maximum daily dose of 2 mg alkaloids (calculated as ephedrine)/kg bw (Blumenthal et al., 1998).

Teuscher (1997) mentioned the herbal substance as herbal tea and ethanolic extracts to be used in cough and colds with mild forms of bronchospasms.

Within the monograph of the WHO (WHO, 1999) the following medicinal uses were given for preparations from the herbal substance according to the different levels of evidence supporting them: treatment of nasal congestion due to hay fever, allergic rhinitis, acute coryza, common cold and sinusitis and as a bronchodilator in the treatment of bronchial asthma (uses supported by clinical data); treatment of urticaria, enuresis, narcolepsy, myasthenia gravis, chronic postural hypotension (uses described in pharmacopoeias and traditional systems of medicine); analgesic and antiviral agent, an antitussive and expectorant and an antibacterial and immune stimulant (uses described in folk medicine).

From data provided by all Member States during a recent survey conducted by the European Medicines Agency (EMA) on the uptake of the traditional use registration it appears that no Ephedra herbal substances/preparations in (traditional) herbal medicinal products have been authorised or registered in the EU between the implementation of Directive 2004/24/EC[17] and 31 December 2012 (Letter from EMA to EFSA, personal communication, April 2013).

2.7.3.2. Single alkaloids

The individual ephedra alkaloids of the ephedrine-type are used in medicinal products (e.g. (-)-ephedrine hydrochloride, (-)-ephedrine anhydrous, (-)-ephedrine hemihydrate, (+)-pseudoephedrine hydrochloride; (+)-pseudoephedrine hemisulphate, (+)-norpseudoephedrine). Information about their specifications and use was available (EDQM, 2011; Sweetman, 2011).

All the four alkaloids ((-)-ephedrine, (+)-pseudoephedrine, (-)-norephedrine, (+)-norpseudoephedrine) act in the same way. Differences in potency are connected with the slight chemical differences which lead into e.g. a higher lipophilicity of (+)-norpseudoephedrine (cathine) which results in a better transfer across the blood-brain-barrier. Ephedrine acts as a sympathomimetic with direct and indirect effects on α- and β-adrenergic receptors and pronounced stimulating effects on the CNS. It stimulates heart rate and cardiac output and variably increases peripheral resistance. As a result ephedrine usually increases blood pressure. Stimulation of α-receptors of smooth muscle cells in the bladder may increase the resistance of the outflow of urine. Stimulation of the β-receptors in the lung promotes bronchodilation (Brunton et al., 2010; Sweetman, 2011). Pseudoephedrine acts similarly to ephedrine, although it has been reported to exhibit less pressor activity and fewer CNS effects. Norephedrine and norpseudoephedrine are used as appetite suppressants. Norpseudoephedrine is more potent in CNS stimulation than ephedrine (Kalix, 1991).

[16] Commission E is a scientific expert committee for herbal medicinal products to support to the German Federal Institute for Drugs and Medical Devices in drafting monographs for herbal preparations.

[17] Directive 2004/24/EC of the European Parliament and of the Council of 31 march 2004 amending, as regards traditional herbal medicinal products, Directive 2001/83/EC on the Community Code relating to medicinal products for human use. OJ L 136, 30.4.2004, p.85-90.

Information on existing authorised medicinal products containing the individual alkaloids has been gathered by the EMA from 16 Member States and is summarised in Table 6. Combination products (e.g. with ibuprofen) are not included in the table below.

Methylephedrine hydrochloride is another sympathomimetic with effects similar to those of ephedrine which has been used as a bronchodilator and is given orally in combination preparations for the relief of cough and nasal congestion (Gregori, 1970; Wichert and Marwede, 1974; Sweetman, 2011). In the 37th edition of "Martindale: The complete Drug Reference" there was no indication that medicinal products containing methylephedrine hydrochloride are still available in the European Union (Sweetman, 2011).

Table 6: Summary of information reported for the authorised medicinal products for oral use containing ephedra alkaloids as single active ingredient.

Ephedra alkaloid[18] **Posology (oral use)**[(a)]	**Therapeutic indications**[(a)]	**Contraindication/warnings**[(a)]	**Adverse effects**[(a)]
Ephedrine			
adults and adolescents: single dose: 15-60 mg daily dose: 40-200 mg	Airflow obstruction where bronchial mucosal oedema is thought to be contributing factors. Hypotension, collapse, imminent paralysis of the respiratory center, chronic bronchitis. Treatment or prevention of attacks of bronchospasm in asthma. Allergic diseases. Narcolepsia, kinetosis in case of lack of other treatment options.	**contraindications:** hypersensitivity to ephedrine hypertension thyrotoxicosis angina pectoris, cardiac insufficiency, ischaemic heart disease phaeochromocytoma angle-closure glaucoma concomitant treatment with MAO (mono amino oxidase) inhibitors and within 10 days following termination of MAO-inhibitor administration before the use of halogenated anaesthetics prostatic hypertrophy hyperexcitability **warnings:** - diabetes mellitus - lactation Not recommended in children >1 year. Should only be used periodically, 4-5 days at a time.	Cardiac disorders: palpitations, hypertension, tachycardia, arrhythmia, precordial pain, hypotension Gastrointestinal disorders: nausea, vomiting (especially at higher doses) Musculoskeletal and connective tissue disorders: muscle weakness Nervous system disorders: tremor (most frequently in adults) Psychiatric disorders: anxiety, insomnia, confusion, increased irritability, asthenia, psychosis, fear, restlessness, tremor (most frequently in adults) Tolerance with dependence has been reported with prolonged administration. Ephedrine may act as stimulant in children with nocturnal enuresis and cause sleeplessness. It may have sedative effects in some children. Renal and urinary disorders: micturition problems, urinary retention (with prolonged use, especially by prostatic hypertrophy) Respiratory, thoracic and mediastinal disorders: pulmonary oedema, dyspnoea Vascular: cerebral haemorrhage, impaired circulation to the extremities General disorders: dry mouth, thirst, dizziness, headache, increased perspiration, salivation
children: 1-5 years: single dose: 15 mg daily dose: 45 mg 6-12 years: single dose: 30 mg daily dose: 90 mg or: daily dose 0.5-3 mg/kg bw/day in 4-6 divided doses			

[18] Information on the stereochemical isomers ((+)-, (-)- or racemate), salts or forms (anhydrous or hydrated) are mostly not given. Therefore uncertainties regarding dosage of biological active ingredients are resulting.

Ephedra alkaloid Posology (oral use) [(a)]	Therapeutic indication[(a)]	Contraindication/warnings[(a)]	Adverse effects[(a)]
norephedrine (phenylpropanolamine)[19]:			
adults and adolescents: single dose: 50 mg daily dose: 100 mg **children 6-12 years:** single dose: 25 mg daily dose: 50 mg	Allergic swelling of the nasal mucosa. Vasomotoric rhinitis for which nasal congestion is the main symptom.	**contraindications:** hypersensitivity to norephedrine hyperthyroidism phaeochromocytoma concurrent treatment with MAO-inhibitors children <6 years	hypersensitivity reactions sleeplessness, nervousness, nightmares, aggression, confusion, hallucinations dry nose, dry mouth micturition difficulties, urinary retention, increased blood pressure, intracranial bleeds
adults: daily dose: 50 mg (divided into two single doses or as single dose)	Supportive, short-term up to 4 weeks persisting therapy of nutrition-related overweight.	**contraindications:** hypersensitivity to norephedrine hypertension, tachycardia, palpitations, dizziness (cardio-) vascular disorders diabetes renal disorders thyroid disorders enlargement of the prostate with residual urine angle-closure glaucoma phaeochromocytoma pregnancy and lactation children <12 years **warnings:** Not to be taken for longer than 4 weeks.	Cardiovascular system: increase of blood pressure and pulse rate, arrhythmias, heart pain Kidneys and urinary tract: difficulties in urination Nervous system: headache, irritability, drowsiness, restlessness, nervousness, dizziness, sleeping disorders Psychiatric disorders: In single cases personality changes cannot be excluded Long-term use is not recommended and there is a risk of dependency and severe mental changes (disregards, psychoses) as well as depressive mood change in case of discontinuation.

[19] According to relevant literature norephedrine is used therapeutically only in the form of the racemate (±)-norephedrine (Phenylpropanolamine Hydrochloride Ph. Eur., CAS-No. 154-41-6) (EDQM, 2011; Sweetman 2011).

Ephedra alkaloid Posology (oral use)	Therapeutic indication[(a)]	Contraindication/warnings[(a)]	Adverse effects[(a)]
Pseudoephedrine:			
adults and adolescents: single dose: 60 mg daily dose: max. 240 mg At intervals of not less than 4 hours. In delayed release formulas: single dose: 120 mg daily dose: max. 240 mg **children 2-6 years:** single dose: 15 mg daily dose: 45-60 mg **children 6-12 years:** single dose: 30 mg daily dose: 90-120 mg	For the symptomatic relief of conditions such as allergic rhinitis, vasomotor rhinitis, nasal, sinus and upper respiratory congestion, common cold and influenza.	**contraindications:** hypersensitivity to pseudoephedrine; severe cardiovascular disease; severe hypertension severe renal impairment; diabetes mellitus closed angle glaucoma; hyperthyroidism prostatic enlargement; phaeochromocytoma in patients receiving monoamine oxidase inhibitors or have taken them in the preceding two weeks patients receiving other sympathomimetics (such as decongestants, appetite suppressants and amphetamine-like psychostimulants) children under 2 years of age **warnings:** Not to be used for more than five days without the advice of a doctor. Use with caution in patients suffering from mild to moderate hypertension. Use with caution in patients with heart disease, diabetes, hyperthyroidism, elevated intra-ocular pressure or prostatic enlargement. Patients with severe hepatic or moderate to severe renal difficulties, especially with concurrent cardiovascular disease, should be treated with caution, as there is an increased risk of drug accumulation and adverse effects. Use with caution in occlusive vascular disease. If any of the following occur, this product should be stopped: hallucinations, restlessness, sleep disturbances.	Cardiac disorders: hypertension, palpitations, tachycardia, arrhythmias, chest pain Gastrointestinal disorders: ischaemic colitis, anorexia, nausea, vomiting Nervous system disorders: restlessness, insomnia, tremor, dizziness, agitation, excitability, nervousness, sleep disturbances, irritability Psychiatric disorders: anxiety, hallucinations (particularly in children), psychotic disorders, paranoid delusion Skin and subcutaneous tissue disorders: rash, allergic dermatitis Urinary and renal disorders: dysuria, urinary retention in men (especially by prostatic hypertrophy) General disorders: dry mouth, dizziness, headache A fixed drug eruption, in the form of erythematous nodular patches, has been rarely associated with pseudoephedrine. Rare cases of psychosis have occurred following misuse of pseudoephedrine. Hypersensitivity reactions/ cross-sensitivity may occur with other sympathomimetics.

Ephedra alkaloid Posology (oral use)	Therapeutic indication[(a)]	Contraindication/warnings[(a)]	Adverse effects[(a)]
(+)-norpseudoephedrine (cathine)[(b)]:			
adults and adolescents: 10-20 mg at morning and midday before meals or 20-30 mg once daily after breakfast with plenty of liquid	Supportive treatment in overweight patients with a body-mass-index of at least 30, which failed to respond to alone adequate weight-reducing measures. Treatment should not exceed 3 months.	**contraindications:** hypersensitivity to norpseudoephedrine pulmonary and severe arterial hypertension current or anamnesis known cardiovascular or cerebrovascular disorders severe forms of angina pectoris current or previous mental illness incl. anorexia nervosa and depressions tendency to alcohol abuse, pre-existing alcoholism phaeochromocytoma hyperthyroidism angle-closure glaucoma concomitant therapy with other centrally acting appetite suppressants (due to the risk of a possibly fatal pulmonary hypertension) pregnancy and lactation Children <12 years **warnings:** Use with caution in patients with heart disease, diabetes, epilepsy or prostatic enlargement. Administration at the evening should be avoided due to possibly caused nervousness and insomnia. Duration of treatment 4-6 weeks and should not exceed 3 months. Treatment should be stopped if no weight loss has been determined within 3-4 weeks.	Cardiovascular disorders: tachycardia, palpitations, hypertension, chest pain, cardiovascular or cerebrovascular events, particularly stroke, angina pectoris, myocardial infarction, myocardial insufficiency and cardiac arrest. Central nervous system: psychic reactions, psychoses, depressions, nervousness, restlessness, sleeping disorders, dizziness, seizures Treatment over a longer period may lead to habituation, dependency and withdrawal symptoms. Cases of severe, pulmonary arterial hypertension, often with fatal outcome, have been reported.

(a): As reported in the Summary of Product Characteristics of the authorised medicinal products
(b): According to Fachinformation, 2013

2.8. Exposure

2.8.1. Exposure via food supplements

Dietary exposure from food supplements to total and to five individual ephedra alkaloids (ephedrine, norpseudoephedrine, pseudoephedrine, methylephedrine and norephedrine) was estimated by the Panel by multiplying the maximum number of servings per day (as suggested on the label of each individual food supplement) by the ephedra alkaloid content per serving, as analysed and reported in Table 4.

The maximum number of recommended daily servings of food supplements, as reported on the label of the analysed products, is shown in Table 7 and exposure estimates are reported in Table 8.

The maximum number of suggested servings per day was available only for 95 samples analysed by Lake et al. (2001). In the case of capsules and tablets, the maximum number of servings per day ranged from one capsule every three days to 4 capsules every 8 hours (equal to 12 per day) (Table 7). Consequently, only data from this study were used for the assessment of exposure. It is also important to note that this study also presented the highest values for ephedra alkaloid and ephedrine content in food supplements and is the only one carried out in Europe. As suggested by the Scientific Committee of EFSA (2012), a body weight of 70 kg was used to express the exposure results in mg/day per kg body weight.

Exposure to total ephedra alkaloids and ephedrine were both estimated to be $\leq$ 394.8 mg/day (5.6 mg/kg bw/day). This result was obtained in the case of a subject consuming 6 tablets per day containing a total of 65.8 mg of total ephedra alkaloids (100 % ephedrine).

Table 7: Recommended maximum number of daily servings of food supplements as reported on the label of the analysed products.

Maximum number of servings per day	Supplement type							
	Capsule / Tablet		Chewing gum		Liquid		Powder	
	N	%	N	%	N	%	N	%
< 1 serving per day	4	4.5	.	.	.	.	.	.
≥ 1 and < 2 servings per day	6	6.7	1	100	.	.	.	.
≥ 2 to < 5 servings per day	63	70.8	.	.	3	75	1	100
≥ 5 to < 10 servings per day	13	14.6	.	.	.	.	.	.
≥ 10 servings per day	3	3.4	.	.	1	25	.	.

Table 8: Exposure levels to different ephedra alkaloids in mg per day (and mg/kg bw/day) according to different type of servings

Type of serving		Number of food supplements	Minimum		Mean		Standard Deviation		Median		Maximum	
Capsules or Tablets	**Total alkaloids** in mg/day[(a)]	89	3.0	(0.043)	92.6	(1.322)	75.6	(1.080)	74.4	(1.063)	394.8	(5.640)
	Ephedrine in mg/day[(a)]	89	1.5	(0.021)	64.8	(0.926)	58.7	(0.838)	54.3	(0.775)	394.8	(5.640)
	Pseudoephedrine in mg/day[(a)]	74	0.8	(0.011)	24.4	(0.349)	25.6	(0.366)	14.5	(0.207)	117.5	(1.679)
	Methylephedrine in mg/day[(a)]	20	0.1	(0.001)	11.5	(0.165)	10.6	(0.151)	6.7	(0.096)	35.2	(0.503)
	Norephedrine in mg/day[(a)]	5	0.1	(0.001)	0.3	(0.005)	0.2	(0.003)	0.3	(0.004)	0.6	(0.009)
	Norpseudoephedrine in mg/day[(a)]	10	0.1	(0.001)	2.4	(0.034)	3.5	(0.050)	1.1	(0.016)	10.7	(0.152)
Chewing gums	**Total alkaloids** in mg/day[(a)]	1	0.2	(0.003)	0.2	(0.003)	-		0.2	(0.003)	0.2	(0.003)
	Ephedrine in mg/day[(a)]	1	0.2	(0.003)	0.2	(0.003)	-		0.2	(0.003)	0.2	(0.003)
Liquid	**Total alkaloids** in mg/day[(a)]	4	17.6	(0.251)	59.2	(0.846)	49.2	(0.703)	44.4	(0.634)	130.5	(1.864)
	Ephedrine in mg/day[(a)]	4	4.0	(0.057)	24.4	(0.349)	18.3	(0.261)	24.0	(0.342)	45.8	(0.654)
	Pseudoephedrine in mg/day[(a)]	4	1.5	(0.022)	29.8	(0.426)	31.6	(0.452)	22.2	(0.318)	73.3	(1.048)
	Methylephedrine in mg/day[(a)]	1	6.0	(0.086)	6.0	(0.086)	-		6.0	(0.086)	6.0	(0.086)
Powder	**Total alkaloids** in mg/day[(a)]	1	5.0	(0.071)	5.0	(0.071)	-		5.0	(0.071)	5.0	(0.071)
	Ephedrine in mg/day[(a)]	1	5.0	(0.071)	5.0	(0.071)	-		5.0	(0.071)	5.0	(0.071)

a): in parenthesis the exposure levels to different ephedra alkaloids is reported in mg / per kg body weight/ day by assuming a standard body weight of 70 kg

2.8.2. Other sources of exposure

All the information in this section refers to the use of ephedra alkaloids as medicinal products for adults, with normal release formulations.

As shown in Table 6, for (-)-ephedrine the single dose ranges from 15-60 mg/person and the daily dose ranges from 40-200 mg/person.

(±)-Norephedrine (phenylpropanolamine) is used in single doses of 50 mg/person, for a maximum daily dose of 100 mg/person. The doses refer to the racemate which contains only 50 % of the active naturally-occurring enantiomer.

For (+)-pseudoephedrine, the single dose is 60 mg with a maximum daily dose of 240 mg/person.

For (+)-norpseudoephedrine (cathine) the single dose is 10-20 mg/person, while the maximum daily dose from use as a medicinal product is reported to be up to 40 mg/person.

2.9. Information on existing authorisations, evaluations and regulations

Ephedra spp. is listed in the EFSA Compendium of Botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements (EFSA, 2012).

No records could be found for *Ephedra* species in the "EU Register on Nutrition and Health Claims" for health claims falling under Article 13.5 (health claim applications based on newly developed scientific evidence and/or proprietary data) or Article 14 (claims referring to children's development and, health and disease risk reduction claim) of Regulation (EC) No 1924/2006[20] (EC, online). An Article 13.1 (function claim) on Ephedra (*Ephedra sinica*)/Ma huang and weight loss is still pending (EC, online).

The EMA confirmed that no monographs or assessment reports on safety/efficacy are available or currently expected from the Committee on Herbal Medicinal Products (HMPC) and that Ephedra is not on the HMPC priority list. It is important to note that one of the reasons for the lack of priority is due to concerns about the safety and known risks associated with the content and use of these substances. The HMPC does not normally prioritise substances with a predictable negative outcome of the benefit-risk assessment in line with provisions of Directive 2001/83/EC[21] for traditional herbal medicinal products, which includes the provision to establish a plausible and safe medicinal use under specified conditions suitable for self-medication without medical supervision. (Letter from EMA to EFSA, personal communication, April 2013).

Ephedra sinica Stapf appeared on the former European Commission's Committee for Proprietary Medicinal Products list of Herbal drugs with serious risks, dated 1992[22] as "*Drugs with toxic principles, where a more detailed discussion concerning the benefit/risk ratio is necessary"* (alkaloid containing plants requiring a benefit risk assessment during the revision)". In a statement from 2005, the HMPC provided clarification on how this list should be read today (EMA, 2005).

A response of 16 European Economic Area (EEA) Member States to EMA's request for information indicated that there are currently no medicinal products containing Ephedra herb or extracts thereof registered in these countries. Several MS have registered medicinal products containing pseudoephedrine and ephedrine for oral use (tablets, syrup, capsules, soluble powder or granules, oral

[20] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.
[21] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2011, p.67-128.
[22] http://www.ema.europa.eu/docs/en_GB/document_library/Other/2011/09/WC500111303.pdf

liquids or drops). There was only one registered medicinal product containing norpseudoephedrine (cathine) (drops, Germany).

The Austrian food codex (Codex Alimentarius Austriacus, Österreichisches Lebensmittelbuch) includes the species *Ephedra vulgaris* in a list of plants not to be used for the production of herbal teas.[23]

The use of *Ephedra* and its alkaloids is not authorised as food or food supplement in Belgium[24] (Royal Decree of 29 August 1997).

The use of the aerial parts of any *Ephedra* species is prohibited in the production of foods in the Czech Republic.[25]

The Danish Drogelisten lists *Ephedra* species as plants that are unacceptable as food regardless of amount. (DTU, online).

In France, because of adverse case reports in humans, the import, medical prescription, release and administration of the plant *Ephedra* (Ma huang) are forbidden since October 2003 (AFSSAPS, 2003).

In Ireland, the use of *Ephedra* species is not permitted in Traditional Herbal Medicinal Products, as it would be controlled as prescription medicines under the terms of the Medicinal Products Regulations.

In Italy, *Ephedra* species are not included in the list of botanical substances and botanical preparations permitted for use in food supplements[26].

Food supplements with *Ephedra* species herbal substance/herbal preparations are not allowed on the Dutch market based on the 2001 Warenwetbesluit Kruidenpreparaten.

In Spain, aerial parts of any *Ephedra* species are prohibited to be sold to the final consumer because of their toxicity. A list of toxic plants is published by means of Orden SCO/190/2004, which is currently being redrafted[27].

In Sweden, all products that contain ephedrine or *Ephedra* extracts are covered by the Medicines Act. All products that contain ephedrine are classified as medicinal products (as of Sep 1, 2005, LV FS 2005:08, changing LVFS 1995:9 concerning implementation of the Medicines Act 1992:859). Thus products containing ephedrine (regardless of the source of the substance, whether synthetic or produced from natural sources) may not be sold as food supplements.

The United Kingdom does not consider any products containing *Ephedra* or ephedrine to be classified as foods. In the UK the sale and supply of botanicals containing *Ephedra* are controlled under the Medicines (Retail Sale or Supply of Herbal Plants Remedies)[28].

[23] Österreichisches Lebensmittelbuch, Codexkapitel B 31 – Tee und teeähnliche Erzeugnisse, Anhang II – Liste der für die Herstellung teeähnlicher Erzeugnisse nicht verwendeter Pflanzen bzw. Pflanzenteile. Available online: http://www.bmg.gv.at/cms/home/attachments/4/9/6/CH1252/CMS1167207128242/b_31_tee.pdf

[24] Arrete Royal du 29 Aout 1997 relatif à la fabrication et au commerce de denrées alimentaires composées ou contenant des plantes ou préparations de plantes Annexe Liste 1: Plantes dangereuses qui ne peuvent être utilisées en tant que ou dans les denrées alimentaires. Available online: http://www.health.belgium.be/filestore/19077559_FR/20120320%20Consolidated%20version%20KB%2029%20Augustus%201997_2_FR.pdf

[25] 225. Vyhláška, kterou se stanoví požadavky na doplňky stravy a na obohacování potravin. Sbírka Zákonů Česká Republika 30.06.2008, Částka 71, p. 3230-3242. Available online:. www.mvcr.cz/soubor/sb071-08-pdf.aspx

[26] Ministero della Salute, Decreto 09 luglio 2012, Disciplina dell'impiego negli integratori alimentari di sostanze e preparati vegetali. (12A07895), GazzettaUfficiale Serie Generale, n. 169 del 21 luglio 2012

[27] Ministerio de Sanidad y Consumo 2225 ORDEN SCO/190/2004, de 28 de enero, por la que se establece la lista de plantas cuya venta al público queda prohibida o restringida por razón de su toxicidad. (Boletín Oficial del Estado). Available online: http://www.boe.es/boe/dias/2004/02/06/pdfs/A05061-05065.pdf

Due to concern that pseudoephedrine and ephedrine can be extracted from over-the-counter (OTC) medicines and used in the illegal manufacture of the Class A controlled drug methylamphetamine (crystal meth) the UK introduced restrictions. These medicines were reclassified as "Pharmacy" medicines, sold under the supervision of a pharmacist, rather than OTC. It became illegal to sell or supply any product that contains > 720 mg pseudoephedrine or 180 mg ephedrine without a prescription. It is also illegal to sell a combination of products that between them add up to > 720 mg pseudoephedrine or 180 mg ephedrine without a prescription. It also became illegal to sell or supply a product that contains pseudoephedrine and a product that contains ephedrine in one transaction.

The regulation limiting a maximum amount of pseudoephedrine that can be dispensed at one time to 720 mg is also applied in Slovakia (e.g. maximum 24 tablets with 30 mg each).

The FDA decided in 2004[29] by a final rule that dietary supplements containing ephedrine alkaloids are listed as "dietary supplements that present a significant or unreasonable risk" in the Federal Food, Drug, and Cosmetic Act and established a regulation declaring dietary supplements containing ephedrine alkaloids adulterated, reasoning that "dietary supplements containing ephedrine alkaloids present an unreasonable risk of illness or injury under conditions of use recommended or suggested in the labelling, or if no conditions of use are recommended or suggested in the labelling, under ordinary conditions of use".

In 2005, the FDA issued a notice of proposed rulemaking for over-the-counter nasal decongestant and weight-control products containing phenylpropanolamine preparations. This proposed rule reclassifies phenylpropanolamine as nonmonograph (Category II) not generally recognized as safe and effective.[30]

The annex to the Natural Health Product Compliance and Enforcement Policy of Health Canada lists *Ephedra sinica* and (-)-ephedrine in the Appendix B (Ingredients subject to a stop sale and recall if found in natural health products) stating that they may pose a serious risk to health (Health Canada, 2010).

Health Canada's drugs directorate policy on herbals used as non-medicinal ingredients in nonprescription drugs in human use includes *Ephedra* (Ma huang, *E. sinica*, *E. equinestina*, *E. gerardiana*) in the list of herbs unacceptable as nonmedicinal ingredients in oral use products (Health Canada, 1995).

Health Canada's guidance document Drugs Currently Regulated as New Drugs includes in The New Drugs List several combinations of ephedra alkaloids: acetaminophen (paracetamol) with pseudoephedrine (for the treatment of earache); caffeine with ephedrine; loratadine with pseudoephedrine (Health Canada, 2012, online).

The Australia New Zealand Food Standards Code lists in the standard 1.4.4 (Prohibited and Restricted Plants and Fungi) the species *Ephedra sinica* (Ma huang) in the schedule of prohibited plants and fungi, stating that these plants or fungi, or a part or a derivative of a plant or fungus, or any substance derived therefrom, must not be intentionally added to food or offered for sale as food.[31]

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[28] Order 1977 SI 2130

[29] Final Rule Declaring Dietary Supplements Containing Ephedrine Alkaloids Adulterated Because They Present an Unreasonable Risk. 21 CFR Part 119. Federal Register, 69, 28, pp. 6788- 6854.

[30] Phenylpropanolamine-Containing Drug Products for Over-the-Counter Human Use; Tentative Final Monographs. 21 CFR Parts 310, 341 and 357. Federal Register,70, 245, p. 75988. Available online: http://www.gpo.gov/fdsys/pkg/FR-2005-12-22/html/E5-7646.htm

[31] Australia New Zealand Food Standards Code. Standard 1.4.4 Prohibited and Restricted Plants and Fungi. Federal Register of Legislative Instruments F2011C00580. Issue 124. Prepared on 11.07.2011. Available online: http://www.comlaw.gov.au/Details/F2011C00580/2088f85d-011f-41e3-a20b-b60c9dec04d3

Ephedrine and pseudoephedrine are listed in a schedule of drugs the importation of which without a licence or permission is prohibited into Australia by the Customs (Prohibited Imports) Regulations 1956 of Australia.[32]

The World Anti-Doping Agency (WADA) prohibits all stimulants in the 2013 Prohibited List (as well as in preceding years' lists) *in-competition*, and lists ephedrine, methylephedrine, and pseudoephedrine and cathine, including all optical isomers, stating that each of ephedrine and methylephedrine is prohibited when its concentration in urine is greater than 10 µg/mL (similarly: pseudoephedrine when > 150 µg/mL, cathine when > 5 µg/mL). Further rules apply to the use of these substances in conjunction with a diuretic or other masking agent (WADA, 2012).

Ephedrine and pseudoephedrine are monitored worldwide as precursors (chemical substances having licit uses, but also used for the illicit manufacture of drugs) of the illicit drug methamphetamine. The EU legislation on drug precursors is based on the 1988 United Nations Convention against the Illicit Traffic in Narcotic Drugs and Psychotropic Substances[33], part of an international framework. Due to their legitimate uses, trade in drug precursors cannot be prohibited. Therefore, drug precursors are controlled through monitoring their licit trade. The EU drug precursor legislation requires a systematic reporting from EU Member States on seizures and stopped shipments of drug precursors. Both raw material and pharmaceutical preparations containing ephedrine or pseudoephedrine are monitored. About 1.5 kg of ephedrine or pseudoephedrine is needed to produce 1 kg of methamphetamine. The quantities stopped/seized vary between years (recent peaks: 1 206 kg ephedrine in 2010, 3 052 kg pseudoephedrine in 2011).

[32] Customs (Prohibited Imports) Regulations 1956. Statutory Rules 1956 No. 90 as amended made under the Customs Act 1901. Available online: http://www.comlaw.gov.au/Details/F2013C00003/b99e9b0b-1df6-45b3-a1b5-0770a2fe1d4b

[33] United Nations Convention against illicit traffic in narcotic drugs and psychotropic substances, 1988. Available online: http://www.incb.org/documents/PRECURSORS/1988_CONVENTION/1988Convention_E.pdf

3. Biological and toxicological data

In the following sections, priority was given to the toxicological studies investigating Ephedra herb and its preparations. Studies with the single ephedra alkaloids found in the different *Ephedra* species (see Table 1) and in particular with (-)-ephedrine and (+)-pseudoephedrine being the main alkaloids measured in food supplements containing Ephedra herbs were also considered.

The Panel noted that compounds other than ephedra alkaloids of the ephedrine-type occurring in the herbs of *Ephedra* species may have biological activity. For example, certain glycans (ephedran A, B, C, D and E) which have been isolated from *Ephedra distachya* herbs have been shown to significantly reduce blood glucose levels in normal and alloxan-induced diabetic mice (Konno et al., 1985; Abourashed et al., 2003). Ephedroxane may potentiate the action of noradrenaline in the catecholaminergic nervous system as concluded by Hikino et al. (1985) from studies in which ephedroxane was injected intraperitoneally to mice (Hikino et al., 1985). However, since no analytical data were available on their occurrence in food supplements based on Ephedra herbs, they were not considered in this section.

3.1. Influence of stereochemistry on biological activity

The individual ephedra alkaloids of the ephedrine-type occur in nature in only one of the two possible enantiomeric forms (see Table 1). By synthesis these alkaloids may also be obtained in the forms of the racemates, which may qualitatively and/or quantitatively differ in their pharmacological and toxicological potencies from the enantiomers found in nature. As indicated in the pharmaceutical literature, norephedrine is only used therapeutically in the form of the racemate (±)-norephedrine, while ephedrine may be used either in the form of the natural occurring enantiomer (-)-ephedrine or the racemate (±)-ephedrine for medical purposes.

3.1.1. Ephedrine

In older literature it is described that the average ratio of the increase in blood pressure of (±)-ephedrine to that of (-)-ephedrine in cats is 1:1.33 and that the average ratio of the mydriatic action of synthetic (±)-ephedrine to that of (-)-ephedrine in Caucasians is 1:1.29. Furthermore it is said that (±)-ephedrine on local application contracts the congested nasal mucous membranes and hypertrophied turbinates not unlike the action of (-)-ephedrine. In the treatment of bronchial asthma, (±)-ephedrine appears to have a weaker action than (-)-ephedrine (Chen, 1928).

In *in vitro* experiments with homogenates of cerebral cortex, (-)-ephedrine exhibited a 4-fold higher relative affinity for (-)-noradrenaline synaptosomal uptake than (+)-ephedrine (Snyder, 1974).

The comparative metabolism of ephedrine stereoisomers was investigated *in vitro* in rabbit hepatic preparations (Jenner and Testa, 1973). (-)-Ephedrine was N-demethylated at rates 15-31 % greater than that observed for (+)-ephedrine. The authors concluded that the stereoisomer of 2S-configuration is apparently N-demethylated more rapidly than the corresponding isomers of 2R-configuration.

The relative *in vitro* potency of (-)-ephedrine as inhibitor of (-)-noradrenaline-induced lipolysis was 31-fold higher than that of its enantiomer (+)-ephedrine and 50-fold higher than that of its diastereomer (+)-pseudoephedrine (Fauley et al., 1974).

In rabbit liver microsomal preparations, (-)-ephedrine was metabolised at a faster rate than (+)-ephedrine (Feller and Malspeis, 1977) (see section 3.2.2.1.).

Using rats trained to discriminate 1 mg/kg of (+)-amphetamine from a saline vehicle in a two-lever drug discrimination test procedure, it was shown that (-)-ephedrine, but not (+)-ephedrine, substitutes for the (+)-amphetamine stimulus (Young et al., 1998).

3.1.2. Pseudoephedrine

In homogenates of cerebral cortex (+)-pseudoephedrine exhibited a 3-fold higher affinity for noradrenaline synaptosomal uptake than (-)-pseudoephedrine (Snyder, 1974).

In rabbit hepatic preparations (+)-pseudoephedrine was N-demethylated at rates 15-31 % greater than that observed for (-)-pseudoephedrine (see analogue results for ephedrine). The authors concluded that the stereoisomers of 2S-configuration are apparently N-demethylated more rapidly than the corresponding isomers of 2R-configuration (Jenner and Testa, 1973).

3.1.3. Norephedrine

The blood pressure responses to the norephedrine enantiomers were determined after separate oral administration of racemic (±)-norephedrine (75 mg), (-)-norephedrine (37.5 mg), and (+)-norephedrine (37.5 mg) to six healthy volunteers. Significant increases from baseline were observed in systolic and diastolic blood pressure for racemic (±)- and (-)-norephedrine, whereas (+)-norephedrine had no effect on blood pressure. The effects of (±)-norephedrine (75 mg), (-)-norephedrine (37.5 mg) on blood pressure were not significantly different. Thus it could be concluded that the effect of the racemate on blood pressure was essentially attributable to (-)-norephedrine. Additional investigations from this study showed that pharmacokinetic factors did not contribute to the differences in the cardiovascular effects of norephedrine enantiomers (Stockley et al., 1994).

Moya-Huff et al. (1987) assessed the effect of (±)-norephedrine, (-)-norephedrine, (+)-norephedrine, (+)-norpseudoephedrine, and (-)-norpseudoephedrine on mean arterial blood pressure (MAP) in normal and reserpinised rats after repetitive intravenous administration. (-)-Norephedrine increased MAP significantly, followed by (±)-norephedrine, while (+)-norephedrine and the two enantiomers of norpseudoephedrine were without significant effect at the doses tested (0.31-10 mg/kg bw) (Moya-Huff et al., 1987).

Using the cardiovascular system of pithed rats, Moya-Huff and Maher (1988) examined the α_1-, α_2- and β_2-adrenoceptor mediated effects of (±)-norephedrine, (-)-norephedrine and (+)-norephedrine. On all types of adrenoceptors tested, the potency was (-)-norephedrine > (±)-norephedrine > (+)-norephedrine (Moya-Huff and Maher, 1988).

3.1.4. Ephedrine in comparison with other phenylethylamines

Patil et al. (1965) conducted studies with the two ephedrine enantiomers, (-)-ephedrine and (+)-ephedrine, and the two ephedrine diastereomers, (+)-pseudoepherine and (-)-pseudoephedrine in anaesthetised dogs. All the isomers except (-)-pseudoephedrine increased blood pressure. (+)-Ephedrine and (+)-pseudoephedrine showed only 33 and 20 % of the pressor potency of (-)-ephedrine, respectively. All four isomers caused an increase in heart rate (potencies: (-)-ephedrine > (+)-ephedrine > (+)-pseudoepherine > (-)-pseudoephedrine). In additional studies in the isolated perfused rabbit heart (+)-ephedrine and (+)-pseudoephedrine exhibited 20 and 25 % of the chronotropic potency of (-)-ephedrine. (-)-Pseudoephedrine caused only a minimal increase in rate being the least active (Patil et al., 1965).

0.33 mg of (-)-ephedrine HCl, (+)-ephedrine HCl, and (+)-pseudoephedrine, (-)-pseudoephedrine, calculated as base, were injected slowly into the isolated hearts of male albino rabbits at a concentration of 1 mg/mL. (-)-ephedrine appeared to release approximately three times as much noradrenaline from the rabbit heart as any of the other isomers, which did not differ significantly from one another in amount of noradrenaline release (Abdallah et al., 1967).

The relative *in vitro* potency of (-)-ephedrine as inhibitor of (-)-noradrenaline-induced lipolysis was 50-fold higher than that of its diastereomer (+)-pseudoephedrine (Fauley et al., 1974).

Effects of optical isomers of ephedrine and methylephedrine on the spontaneous beating rate of isolated right atrium of normal and reserpinised rats were investigated to assess actions on β_1-

adrenoceptors. (-)-Ephedrine and (+)-ephedrine markedly increased the beating rate of the rat right atrium. (-)-Methylephedrine showed slight increase in heart rate. The potency to induce positive chronotropic effect is (-)-ephedrine > (+)-ephedrine > > (-)-methylephedrine. (-)-Ephedrine was about 3 times as potent as (+)-ephedrine. In addition (+)-methylephedrine caused a decrease in heart rate (Kawasuji et al., 1996).

Fairchild and Alles (1967) administered (+)-amphetamine, (-)-ephedrine, (-)-norephedrine, (+)-pseudoephedrine, (+)-norpseudoephedrine and their enantiomers intraperitoneally to mice and investigated their potency to induce central locomotor stimulation. (+)-Norpseudoephedrine produced 10 %, (-)-ephedrine 4 % and (-)-norpseudoephedrine 2 % of the central stimulating activity of (+)-amphetamine. (-)-Norephedrine, (+)-norephedrine, (-)-pseudoephedrine, (+)-pseudoephedrine and (+)-ephedrine were clearly less effective and exhibited central locomotor stimulation only at doses approaching lethal amounts (Fairchild and Alles, 1967).

The potencies of a number of phenylethylamines to inhibit food intake in rats when given by gavage were compared by determining the dose required to produce 50 % decrease (RD_{50}) in food consumption. Individual enantiomers of (+)-amphetamine, (-)-ephedrine, (-)-norephedrine, (+)-pseudoephedrine and (+)-norpseudoephedrine all inhibited food intake with a two to five-fold greater potency than that of their respective optical isomers. The resulting RD_{50}-values of (+)-amphetamine and the natural occurring ephedra alkaloids (-)-ephedrine, (-)-norephedrine, (+)-pseudoephedrine and (+)-norpseudoephedrine were 16.5, 99.4, 80.9, 164.6 and 60.3 µmol/kg bw, respectively. Comparing the naturally-occurring ephedra alkaloids, (+)-norpseudoephedrine showed the lowest RD_{50} and thus was 2.7 times more effective than (+)-pseudoephedrine exhibiting the highest RD_{50} (Blosser et al., 1987).

A comparison of the relative pharmacological activity of naturally occurring ephedra alkaloids and their enantiomers is presented in Table 9.

Table 9: Comparative pharmacological activity of naturally occurring ephedra alkaloids and their enantiomers

	Blood pressure activity		**Chronotropic action heart model**	**Noradrenaline release rabbit heart**	**Inhibition of noradrenaline-induced lipolysis**	**CNS activity**	
References	(a)	(b)	(b)	(c)	(d)	(e)	(f)
(-)-ephedrine	1	1	1	1	1	1	1
(+)-ephedrine	no data	0.33	0.2	0.3	0.03	< 0.1	no data
(-)-pseudoephedrine	no data	no activity	no activity	0.04	0.02.	< 0.1	no data
(+)-pseudoephedrine	no data	0.2	0.25	0.04	0.02	< 0.1	no data
(-)-noradrenaline	0.01	no data	no data	no data	no data	0.7	no data
(+)-noradrenaline	0.01	no data	no data	no data	no data	< 0.1	no data
(-)-norpseudoephedrine	no data	no data	no data	no data	no data	< 0.1	0.5
(+)-norpseudoephedrine	no data	no data	no data	no data	no data	1.2	2.5

a): Stockley et al., 1994
b): Patil et al., 1965
c): Abdallah et al., 1967
d): Fauley et al., 1974;
e): Blosser et al., 1987
f): Fairchild and Alles, 1967

3.2. Absorption, distribution, metabolism and excretion

Similarly to their biological activities, the fate and more particularly the metabolism of ephedra alkaloids is influenced by the stereochemistry of these compounds.

3.2.1. Ephedra herb and its preparations

3.2.1.1. Pharmacokinetic studies of Ephedra herb preparations in animals

Ephedrine levels in rat plasma samples were measured by a rapid and sensitive ultra performance liquid chromatography (UPLC) tandem mass spectrometry method with electrospray ionization (Zheng et al. 2012). The method was validated and was used to determine the pharmacokinetic behaviour of this sympathomimetic compound in Sprague-Dawley rats. Ephedrine hydrochloride, Herba Ephedrae single-herb or Wu tou tang decoctions were orally administered (10 mg/kg bw ephedrine equivalent) whereas ephedrine hydrochloride (4 mg/kg bw) was also administered by intravenous injection and blood samples were collected over 24 hours. Ephedrine was measured in plasma and pharmacokinetic parameters were determined. The absolute bioavailability values of ephedrine in the rat after oral administration of ephedrine hydrochloride, Herba Ephedrae single herb decoction and Wu tou tang decoction were 99.7, 85.4 and 32.1 %, respectively, suggesting that some components in the Wu tou tang decoction may reduce the bioavailability of ephedrine.

3.2.1.2. Pharmacokinetic studies of Ephedra herb preparations or dietary supplements in humans

White et al., (1997) determined the pharmacokinetic properties of a commercially available source of Ma-huang, a natural source of ephedrine, and evaluated heart rate and blood pressure responses to the product in normotensive, healthy adults. On day 1, twelve normotensive volunteers (6 women and 6 men) were monitored with an ambulatory blood pressure device between hours 7 and 20. On day 2, they ingested four capsules of powdered Ma huang (375 mg of Ma huang containing 19.4 mg of ephedrine, 4.9 mg of pseudoephedrine and 1.2 mg methylephedrine for a four-capsule dose) at hours 8 and 17, while again wearing the monitor between hours 7 and 20. The ephedrine alkaloid content of each capsule was determined by HPLC. Serial plasma samples were obtained and concentrations of ephedrine were also analysed by HPLC and the pharmacokinetic parameters of ephedrine were determined from plasma concentration time profiles. Six participants experienced a statistically significant increase in heart rate, but the effects on blood pressure were variable. The elimination half-life (5.2 hours), volume of distribution (182 L), clearance (24.3 L/hour) and maximum concentration in plasma (81 ng/mL) of ephedrine in the ma-huang product were similar to values previously reported for a 20-mg, immediate-release ephedrine tablet (Pickup et al., 1976). Absorption rate was considerably lower (0.49 $hours^{-1}$ compared to 1.73-2.35 $hours^{-1}$ pure ephedrine) and time to reach maximum ephedrine concentration was longer for the capsules, compared with the standard tablet (T_{max} of almost 4 hours compared to only 2 hours for pure ephedrine). According to the authors, Ma huang had variable effects on blood pressure and increased heart rate in healthy normotensive adults. Pharmacokinetic parameters for ephedrine such as area under the time-concentration curves, apparent volume of distribution and biological half-life were in agreement with those previously reported in other studies using only pure ephedrine. The particle size of the herb preparation was not specified but may have an effect on the intestinal dissolution and subsequent absorption of ephedrine.

Gurley et al. (1998b) examined the pharmacokinetics in humans after the ingestion of a dietary supplement containing Ephedra herb extract. The pharmacokinetics of ephedrine after the ingestion of three commercially available Ma huang products (containing 27.0, 25.6 or 23.6 mg ephedrine) were compared with a 25 mg racemic ephedrine capsule in ten subjects (5 adult males and 5 adult females) enrolled in a randomized, crossover study. Eligibility was determined by the results of a medical history, brief physical examination, medication history and a pregnancy test for female subjects. The dietary supplements contained one or more of the following ingredients; bee pollen, caffeine, *Astragalus*, *Camellia sinensis*, *Centella asiatica*, *Cola nitida*, d-alpha tocopherol, *Ginkgo biloba*, *Glycyrrhiza glabra*, *Panax ginseng*, *Paullinia cupana*, *Spirulina pratensis*, and *Triticum aestivum*. On Day 1, after an 8-hour fast, an oral dose of medication and 8 oz (equivalent to 237 mL) of water was administered at 7 am. Subjects were then asked to refrain from eating for an additional 4 hours. There was a 1-week washout phase between ingestion of each of the 4 products. Pharmacokinetic parameters for ephedrine from botanically derived products were similar to those for synthetic ephedrine hydrochloride. All subjects experienced minor effects typical of ephedrine alkaloids, including tachycardia, anxiety, headache, irritability, insomnia, and loss of appetite (frequency not reported).

When compared with the results of previously published studies, it appeared that the administration of Ephedra herb extract, in combination with other botanicals and stimulants, had little bearing on the distribution and elimination of ephedrine. According to the authors, these results indicated that when ephedrine was supplied as an Ephedra herb extract in combination with other botanicals, the absorption and disposition characteristics were similar to those observed for a single-ingredient ephedrine capsule.

Csajka et al. (2005) developed a mechanistic model describing ephedrine, norephedrine, and caffeine pharmacokinetics and their interactions in healthy subjects. This model was based on the simultaneous modelling using plasma concentrations from blood samples collected from two clinical trials. The first study involved eight subjects receiving a single oral dose of a commercial dietary supplement consisting of 17.3 mg ephedrine, 0.2 mg norephedrine, 5.3 mg pseudoephedrine, 0.42 mg norpseudoephedrine and 175 mg caffeine. In the second study, single oral doses of either 25 mg ephedrine sulphate and/or 200 mg caffeine sulphate were administered alone and together to 16 subjects. To investigate the sources of inter-individual variability, a mixed-effect statistical model implemented in the program NONMEM was used. From these 24 subjects, 379 ephedrine, 352 norephedrine, 417 caffeine plasma concentrations and 40 ephedrine urine concentrations were obtained. Caffeine data were described by a one-compartment model with first-order absorption whereas a four-compartment model described the pharmacokinetics of ephedrine and norephedrine. Ephedrine was eliminated mostly in the urine, with a clearance of 0.34 l/min and a volume of distribution of 181 l. Nonlinearity in the conversion of ephedrine to norephedrine was observed. A 32 % greater relative bioavailability of ephedrine was observed from herbal supplement compared with pharmaceutical ephedrine sulphate administration. This could indicate that other constituents of the herbal formulation would increase the amount of ephedrine absorbed. Moreover different models showed that the simultaneous administration of caffeine, or the amount of caffeine in the absorption compartment, was associated with a slower rate of absorption of ephedrine. However, according to the authors, the effect of concomitant caffeine on the concentration-time profile of pharmaceutical and herbal ephedrine was "small and probably of little clinical relevance".

3.2.2. Ephedrine and other ephedra alkaloids

3.2.2.1. Pharmacokinetic studies of ephedrine in animals

The metabolism of ephedrine in humans, dogs and several species of rodents proceeds primarily by three reactions; aromatic hydroxylation, N-demethylation and oxidative deamination. The extent to which ephedrine is metabolised and the major metabolites vary quantitatively between species (Axelrod, 1953; Williams et al., 1973). The extent of aromatic hydroxylation is greatest in rats, followed by rabbits, guinea-pigs, and dogs, with no aromatic hydroxylation observed in humans. N-demethylation of ephedrine is greatest in rabbits followed by dogs, guinea-pigs, rats, and humans. Deamination is greatest in rabbits, followed by humans and rats.

The comparative metabolism of ephedrine stereoisomers was investigated *in vitro* in rabbit hepatic preparations (Jenner and Testa, 1973). The rate of N-demethylation observed for (-)-ephedrine was 15-31 % greater than that observed for (+)-ephedrine. The authors concluded that the stereoisomer with 2S-configuration is apparently N-demethylated more rapidly than the corresponding isomers with 2R-configuration.

Investigations were carried out with [carbinol-^{14}C]-radiolabelled (-)-ephedrine and (+)-ephedrine to establish whether differences exist in their metabolic fate in the rabbit, *in vivo* and *in vitro* (Feller and Malspeis, 1977). In liver microsomal preparations, (-)-ephedrine was metabolised at a faster rate than (+)-ephedrine, benzoic acid was formed from (-)ephedrine at a rate about three times greater than from the (+)-isomer and the relative amounts of norephedrine and 1-phenyl-1,2-propanediol formed from both ephedrine isomers were nearly identical throughout the entire incubation period.

In rabbits receiving an intraperitoneal dose of 3 mg/kg of ^{14}C-ephedrine, both ephedrine isomers were extensively metabolised and the majority of total radioactivity (71-91 %) was excreted within 24 hours. A greater ^{14}C-excretion rate was observed for (+)-ephedrine. From an analysis of 0 to 24-hr urine, it was found that 47-50 % of the urinary radioactivity was attributable to acidic metabolites (hippuric acid and benzoic acid) from (+)- and (-)-ephedrine, and 4 to 16 % of the total ^{14}C obtained with both isomers was accountable as 1-phenyl-1,2-propanediol, either free or as a glucuronide conjugate. No appreciable quantities of sulphate or glucuronide conjugates of p-hydroxylated metabolites of ephedrine or norephedrine were detectable and small amounts (< 4 % of metabolites corresponding to unchanged ephedrine, norephedrine, or 1-hydroxy-1-phenyl-2-propanone) were found in urine of animals given either isomer. These experiments indicated that the major pathway for the biotransformation of (-)-ephedrine and (+)-ephedrine involved N-demethylation and oxidative deamination of the side chain.

After oral administration of ephedrine hydrochloride (33 mg/kg bw), ephedrine was well absorbed from the gastrointestinal tract of rodents and was rapidly excreted in the urine (Marvola and Kivirinta, 1978). Direct measurement of ephedrine concentration in plasma showed that the peak blood level was reached within 5 to 6 minutes after oral administration of ephedrine hydrochloride to mice. The intravenous administration of the same dose was also investigated and the oral bioavailability was calculated to be 76 % (ratio of oral to iv area under the curve (AUC)). Elimination from plasma was reported to be mono-exponential, with a half-life of 30.6 minutes. In the same study, the authors also investigated the relationship between stimulating effects on the CNS and blood levels of the drug, following intravenous or oral administration of ephedrine in mice. It was concluded that after intravenous injection of ephedrine the blood concentration data fitted best with a two-compartment open model but after oral administration of the drug, with a one-compartment model. The locomotor activity stimulating effect of the drug did not have a linear relationship to the drug levels in the central or in the tissue compartment. According to the authors, this suggested that the mechanism of the locomotor activity increasing effect of ephedrine was indirect.

The differences in metabolic fates of the optical isomers of ephedrine were investigated in rats given an oral dose (20 mg/kg bw) of an equimolar mixture of the isomers. The study showed that (-)-ephedrine was more easily p-hydroxylated than (+)-ephedrine. A stereoselective reaction in the formation of the glucuronides of ephedrine and its metabolites norephedrine and p-hydroxyephedrine was observed. The (-)-enantiomers were more easily subjected to glucuronide formation than the (+)-enantiomers (Baba et al., 1986).

3.2.2.2. Pharmacokinetic studies of norephedrine in animals

The urinary excretion of ^{14}C-norephedrine (radiolabelled on the phenyl[1-^{14}C]propan-1-ol part) was identified and measured in orally administered (12 mg/kg bw) rats and rabbits (Sinsheimer et al., 1973). Pronounced species differences in the metabolism of the drug were found. In rats, almost 80 % of the ^{14}C administered was excreted in the first day. The major metabolites in the urine of rats were the unchanged drug (48 % of the dose), 4-hydroxynorephedrine (28 %) and trace amounts of side-chain degradation products. Rabbits excreted 85-95 % of the radioactive dose in the urine in the first 24 hours after dosing. The major metabolites in rabbits urine were conjugates of 1,2-dihydroxy-1-phenylpropane (31 % of the dose) and of 1-hydroxy-1-phenylpropan-2-one (27 %) and hippuric acid (20 %). The unchanged norephedrine was excreted in relatively small amounts (8 %).

3.2.2.3. Pharmacokinetic studies of pseudoephedrine in animals

The comparative metabolism of pseudoephedrine stereoisomers was investigated *in vitro* in rabbit hepatic preparations (Jenner and Testa, 1973). Rate of N-demethylation of (+)-pseudoephedrine was 15-31 % greater than that observed for (-)-pseudoephedrine (see analogue results for ephedrine). The authors concluded that the stereoisomers of 2S-configuration are apparently N-demethylated more rapidly than the corresponding isomers of 2R-configuration (Jenner and Testa, 1973).

Palamanda et al. (2010) characterised the pharmacokinetics of pseudoephedrine in rats, dogs, and monkeys, and evaluated its lower gastrointestinal tract regional bioavailability in rats. Plasma levels of pseudoephedrine were determined by an original HPLC-MS/MS assay with a lower limit of quantification of 0.4 ng/mL. The total body clearance was the highest in rats (78 mL/min/kg), lowest in monkeys (15 mL/min/kg) and the dog averaged in between (33 mL/min/kg). The volume of distribution at steady state (Vdss) ranged from 3-5 L/kg in all species. In rats and dogs, the mean half-life ($t_{1/2}$) was about 1.5 hours, while in monkeys it was 4.6 hours, comparable to that observed in adult humans (4-8 hours). The oral bioavailability was 38, 58 and 87 % in rats, dogs and monkeys, respectively. In rats, the bioavailability following intra-ileum or intra-colonic administration was higher than that following oral dosing (66 % and 78 %, respectively). According to the authors, this would suggest that colonic absorption may be compensating for the short half-life, thus enabling successful once-a-day sustained release formulations of pseudoephedrine. The pharmacokinetic/pharmacodynamic relationship (PK/PD) of pseudoephedrine was also investigated in a model of nasal congestion in cats and an EC_{50} (plasma concentration that elicits 50 % of the maximum response) of 0.32 μM was determined which was consistent with human plasma concentrations required for efficacy.

3.2.2.4. Pharmacokinetic studies of ephedrine, norephedrine, pseudoephedrine and methylephedrine in humans

Wilkinson and Beckett (1968a) demonstrated that absorption of oral ephedrine was complete within 2 to 2.5 hours following oral administration of 25 mg ephedrine in three subjects. In this study, the kinetic parameters of absorption, metabolism and excretion of (-)-ephedrine, (-)-norephedrine and (-)-methylephedrine were calculated using pharmacokinetic compartment models.

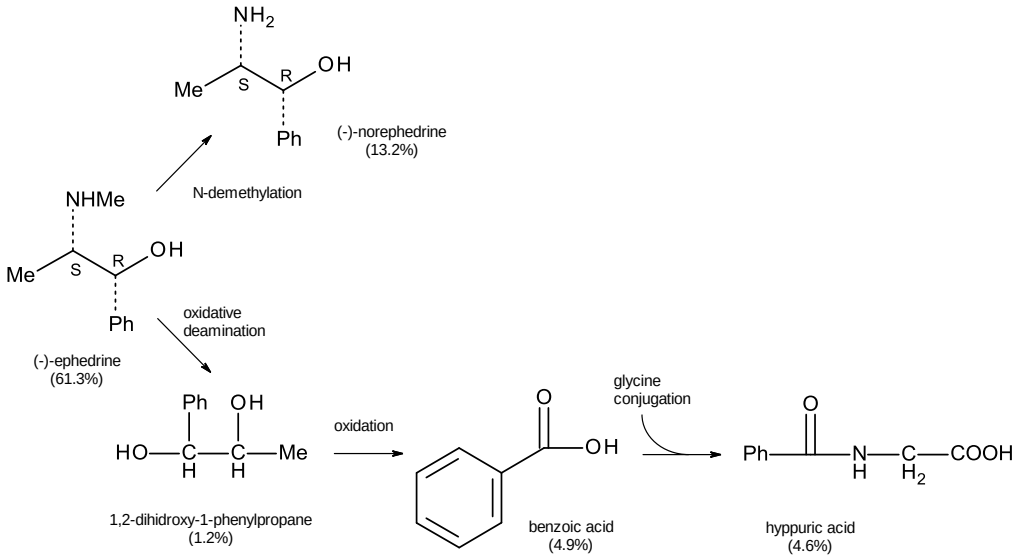
In a further study in three male adults, Wilkinson and Beckett (1968b) investigated the urinary excretion of orally administered (-)-ephedrine (25 mg), (-)-norephedrine (22.9 mg) and (-)-methylephedrine (27.1 mg) under extreme acidic and alkaline urinary conditions. The urinary excretion of ephedrine was pH-dependent, due to the presence of an ionizable group in the ephedrine molecule, and was increased in acidic urine (73 to 93 % of the dose). In alkaline urine, ephedrine excretion was reduced to 20 to 35 % of the dose. The result suggested that at high urine pH, ephedrine, as a weak base, is non-ionized and is easily reabsorbed from the renal tubules, whereas at low urine pH, ephedrine is charged and is thus cleared faster. Within the ephedrine series, (-)-methylephedrine was extensively metabolised, in part to ephedrine and then to norephedrine. By contrast, norephedrine and ephedrine were excreted mainly as unchanged drugs.

The urinary excretion of ephedrine was studied in 3 male adults given three commercial ephedrine sulphate preparations orally (25 mg/person) either as syrup or capsules (Welling et al., 1971); the urine pH was not controlled in this study. There was an insufficient number of subjects to draw conclusions on the differences between dosage forms. However in all cases 70 to 80 % of the dose was excreted as ephedrine in the urine in 48 hours and the average elimination half-life of ephedrine was 6 hours, regardless of the dosage form.

Sinsheimer et al., (1973) investigated the fate of orally administered ^{14}C-norephedrine (radiolabelled on the phenyl[1-^{14}C]propan-1-ol part) in man. Three male subjects each received 25 mg of ^{14}C-norephedrine hydrochloride and excreted over 90 % of the ^{14}C in the first day. The main metabolite was unchanged norephedrine (86 % of the dose); minor metabolites were hippuric acid and 4-hydroxynorephedrine.

Williams et al. (1973) reviewed the comparative hepatic metabolism of some amphetamines, including ephedrine and norephedrine in various animal species and man. In humans, aromatic hydroxylation of ephedrine and norephedrine was found to be negligible (0-1 % of the dose) and N-dealkylation of ephedrine and deamination of ephedrine and norephedrine were considered to be relatively minor reactions (3 to 10 %). When compared to other amphetamines, the least lipid-soluble drugs, ephedrine and norephedrine were those most extensively excreted unchanged (70 – 80 % of the dose).

Sever et al. (1975) investigated the urinary excretion of orally administered (-)-^{14}C-ephedrine (radiolabelled on 1-propanol) in three human subjects. The metabolites were determined quantitatively by solvent extraction, paper chromatography and reverse isotope dilution procedures. Following an oral dose of (-)-ephedrine (0.35 mg/kg, 1.6 μCi), 97 % of the radioactive dose was excreted in the urine within 48 hours, 88 % in the first 24 hours. Unchanged ephedrine was the major urinary excretory product (53-74 %), with N-demethylation occurring to a variable extent (8-20 %), although there was little inter-individual variation in urine pH. Oxidative deamination was also variable (4-13 %); the main identified products of this reaction were benzoic acid (free and conjugated) and 1,2-dihydroxy-1-phenylpropane (free and conjugated). Since no phenolic metabolites were detected in the urine, the authors considered that such compounds would not be involved in the acquisition of tolerance to ephedrine, which can occur following repeated dosage. The metabolic pathways of (-)-ephedrine as proposed by Sever et al. (1975) are illustrated in Figure 3.



Legend: % excreted in urine (% dose excreted in 24 hours).

Figure 3: Metabolic pathways of (-)-ephedrine adapted from Sever et al. (1975)

Pickup et al. (1976) studied the pharmacokinetics of (-)-ephedrine (oral doses of 22 mg (-)-ephedrine hydrochloride/person administered at Day 1 and Day 16) in ten asthmatic volunteers. Ephedrine plasma levels were measured on Day 1 and after two weeks of treatment with 33 mg (-)-ephedrine/person/day, alone or in combination with theophylline and phenobarbitone. The comparison of pharmacokinetics indicated no significant intraindividual changes in kinetic parameters (biological half lives and volume of distribution around 6.7 hours and 215 L, respectively) before or after repeated treatment with (-)-ephedrine hydrochloride. In addition, no change was observed between (-)-ephedrine hydrochloride given alone or in combination with theophylline or phenobarbitone.

Dickerson et al. (1978) assessed dose tolerance and pharmacokinetics of (+)-pseudoephedrine in a double-blind parallel study. Thirty-four healthy male volunteers were randomly assigned to groups receiving either a dose of 120 or 150 mg (+)-pseudoephedrine/person as sustained action capsule, taken every 12 hours for seven test days. Blood samples were taken pre-medication and twice daily on days 1, 3, 5, and 7 of the study and at 12, 14, 16, 18 and 20 hours after the last dose of the drug. Pseudoephedrine plasma concentrations varied considerably between individuals. Steady state plasma concentrations were achieved after the third dose. Biological half-lives were similar among most subjects and were not dependent on the small variations in urine pH. Mean biological half-life was 5.9 hours and mean steady state plasma concentration of pseudoephedrine 447 ng/mL for the 120 mg group. These values were respectively 6.9 hours and 510 ng/mL for the 150 mg group. According to

the authors, there was no correlation between the elimination half-life of (+)-pseudoephedrine and the severity or number of symptoms experienced by the subjects.

In patients undergoing caesarean section, no change in the disposition kinetics of ephedrine after intramuscular dosing (25-50 mg) was observed (Hughes et al., 1985). This study demonstrated that placental transfer of ephedrine occurred because fetal blood levels were approximately 70 % those of the mother. Ephedrine was also excreted in breast milk. Excretion patterns may be much more rapid in children, and a greater dosage is usually required to achieve therapeutic effects.

The pharmacokinetics of ephedrine was investigated in six healthy female volunteers before and after exercise (Strömberg et al., 1992). Before entering the study, the subjects were ascertained to be healthy by a clinical examination, including resting ECG and maximal exercise test on a treadmill. All subjects were regularly engaged in physical activity, but none of them participated in competitive sports. The subjects received single oral doses of 50 mg ephedrine or placebo. All treatments were given twice; before exercise (control session) and during exercise. Relevant safety/tolerability assessments included heart rate and blood pressure, critical flicker fusion frequency, which measures sedative effects of psychotropic drugs, Maddox wing test, used to measure external eye muscle balance; visual analog scales relating to mood and feelings of tiredness were included in the sessions as pharmacodynamic measures. According to the authors, the pharmacokinetics of ephedrine was not altered by exercise whereas ephedrine increased heart rate and systolic blood pressure 2 hours after administration. It was reported that the heart rate response was even higher after exercise, whereas exercise abolished the blood pressure response.

Vanakoski et al. (1993) investigated the effects of a sauna (3 x 10 min; temperature 80-100 °C; relative humidity 30-50 %) on the pharmacokinetics and pharmacodynamics of ephedrine. Six young healthy women in a placebo-controlled, double-blind study received single oral doses of ephedrine (50 mg/person) and midazolam (15 mg/person). In the sauna, ephedrine was more rapidly absorbed and the maximum plasma concentration occurred earlier than in the control sessions. Changes in the pharmacodynamics due to the sauna were consistent with the pharmacokinetic findings because ephedrine made the volunteers subjectively more alert at that time. According to the authors, this would indicate an influence of a sauna on drug pharmacodynamics in the post-sauna adaptive phase and an exposure to a sauna may alter both ephedrine pharmacokinetics and pharmacodynamics.

Berlin et al. (2001) determined the pharmacokinetics and the cardiovascular, subjective effects and potential of abuse liability of single oral doses of (-)-ephedrine (50 mg) or intranasal doses (10 mg and 5 mg). Sixteen healthy Caucasian men with no history of drug/alcohol/nicotine abuse or dependency received single intranasal doses of 5 or 10 mg (-)-ephedrine, oral doses of 50 mg (-)-ephedrine and placebo in a double-blind and crossover study. Maximal plasma concentration (C_{max}) and AUC for up to 8 hours were proportional to the dose of (-)-ephedrine. Following oral administration (50 mg/person), the pharmacokinetic parameters were as follows, T_{max} = 2 hours; C_{max} = 138 ± 33 ng/mL, AUC (0-8 hours) = 778 ± 201 ng/mL·hour and $t_{1/2}$el = 7.1 ± 1.9 hours. A clockwise hysteresis was observed for systolic blood pressure in all but one subject with ephedrine 50 mg by the oral route. In conclusion, (-)-ephedrine even at low doses and by the nasal route can decrease tiredness in healthy persons; this is accompanied by a substantial increase in blood pressure and orthostatic hypotension exposing individuals in case of intensive physical exercise to cardiovascular risks. No clear evidence of abuse liability in healthy drug naive subjects was observed.

3.2.3. Summary of ADME

In humans, when ephedrine is supplied as Ephedra herb extract in combination with other botanicals (commercially available Ma huang products), the absorption and disposition characteristics were found to be similar to those observed for an equivalent single-ingredient ephedrine oral dose. When administered as a single ingredient, oral ephedrine is completely absorbed and pharmacokinetic studies revealed a large volume of distribution including a transfer of the unchanged compound in placenta and maternal milk. The major pathway for the biotransformation of ephedrine involves N-

demethylation and oxidative deamination of the side-chain, however, unchanged ephedrine was the major urinary excretory product (53-74 %). Regarding the other alkaloids, the orally administered methylephedrine is extensively metabolised whereas in a norephedrine pharmacokinetic study, the main metabolite was the unchanged norephedrine (86 % of the dose).

Regarding stereochemical aspects, both *in vitro* and *in vivo* metabolic animal studies demonstrated that (-)-ephedrine is more rapidly and more intensely N-demethylated, hydroxylated and conjugated than the (+)-ephedrine isomer.

3.3. Toxicological data

3.3.1. Acute oral toxicity

3.3.1.1. Ephedra herb and its preparations

Mice

Gavage studies in ICR mice gave LD_{50} values of 5.3 g/kg bw ('ephedrine extract'; not further specified) and 24.0 g/kg bw ('crude ephedra'; not further specified) (Minamatsu et al., 1991 as referred to in Cantox, 2000). Another gavage study in mice (strain not specified) reported LD_{50} values of 4.35 g/kg bw (males) or 5.0 g/kg bw (females) Ephedra herb extract ([Japanese authors], 1978, as referred to in Cantox, 2000).

Rats

Dunnick et al. (2007) administered Ma huang, containing approximately 4 % of ephedrine in a crude extract, as a single oral dose to Fischer 344 rats (20 animals/group), either alone or in combination with caffeine. The Ma huang (ephedrine equivalents)/caffeine dosages used were: 0/0; 312.5 (12.5)/0; 625 (25)/0; 1 250 (50)/0; 0 (0)/15; 312.5 (12.5)/15; 625 (25)/12; 1 250 (50)/15; 0 (0)/30; 312.5 (12.5)/30; 625 (25)/30; 1 250 (50)/30; 0(0)/45; 3 312.5 (12.5)/45 mg/kg bw) (NTP, 2004). In the group given 1 250 mg/kg bw Ma huang (equivalent to 50 mg ephedrine /kg bw) alone, most of the animals showed increased salivation 2 hours after treatment and 6/20 animals were lethargic and were sacrificed as moribund. Ephedra herb in combination with caffeine (both 15 and 30 mg/kg bw) caused rapid clinical signs of toxicity including salivation, hyperactivity, ataxia and eventually lethargy, and failure to respond to stimuli. The clinical signs (except the hyperactivity) showed a slight increase in incidence with increasing doses of Ma huang and caffeine. Mortality was increased in the groups treated with 625 or 1 250 mg/kg Ma huang (equivalent to 25 or 50 mg/kg bw ephedrine, respectively) in combination with 15 or 30 mg/kg bw caffeine in comparison with the groups treated with Ma Huang alone. Treatment-related cardiotoxicity was observed in most of the animals sacrificed as moribund, and consisted of haemorrhage, degeneration, and necrosis (Nyska et al., 2005). Cardiotoxicity was significantly increased in animals treated with 1 250 mg/kg bw Ma huang in combination with 15 or 30 mg/kg bw caffeine in comparison to animals treated with 1 250 mg/kg bw Ma huang alone.

Dogs

Accidental ingestion of herbal supplements containing mostly guarana (*Paullinia cupana*), a natural source of caffeine, and Ma huang (*Ephedra sinica*) (concentration of ephedra alkaloids not specified) was examined in dogs. Clinical signs of toxicity included tremors, seizures, vomiting, tachycardia, hyperthermia and nonspecific behavioural changes. Doses of guarana and Ma huang at which signs of intoxication were reported, ranged between 4.4 and 296 mg/kg bw and 1.3 and 89 mg/kg bw, respectively. Clinical signs developed within 8 hours after ingestion in 80 % of the dogs; the duration ranged from 10 to 48 hours (Ooms et al., 2001).

3.3.1.2. Ephedrine

Mice

NTP (1986) reported an LD_{50} of 812 mg/kg bw in male mice and 1 072 mg/kg bw in female mice of the B6C3F1 strain for ephedrine sulphate administered by gavage.

Rats

An LD_{50} of ephedrine sulphate was not determined in Fischer344 rats dosed by gavage at 75-1 200 mg/kg bw because deaths occurred in all dose groups (NTP, 1986).

Dunnick et al. (2007) administered ephedrine hydrochloride as a single oral dose to Fischer344 rats (20 animals/group) alone and in combination with caffeine. The ephedrine/caffeine dosages (in mg/kg bw) were: 0/0; 6.25/0; 12.5/0; 25/0; 12.5/7.25; 25/7.25; 0/15; 12.5/15; 0/30; 25/15; 0/30; 6.25/30; 12.5/30; 25/30. In the group treated with 25 mg /kg bw ephedrine alone, almost half of the animals showed increased salivation and 2/20 rats had to be sacrificed as moribund. Clinical signs of toxicity were also seen in rats receiving a combination of ephedrine and caffeine and included salivation, hyperactivity, ataxia and eventually lethargy, and failure to respond to stimuli, but the incidence was not significantly increased in comparison to the group treated with ephedrine alone. Mortality and cardiotoxicity, characterised by haemorrhage, degeneration and necrosis (Nyska et al., 2005), were increased in the groups treated with 25 mg/kg bw ephedrine in combination with caffeine at 7.25, 15 or 30 mg/kg bw in comparison to the group treated with 25 mg/kg bw ephedrine alone. There was no statistical significant difference among the caffeine groups combined with 25 mg/kg bw ephedrine.

Using rats which were trained to discriminate 1 mg/kg bw of (+)-amphetamine (ED_{50} =0.4 mg/kg bw) from saline vehicle in a two-lever drug discrimination procedure, it was shown that (-)-ephedrine (ED_{50}=4.5 mg/kg bw) substitutes for the (+)-amphetamine stimulus. The authors also showed that caffeine (ED_{50}=12.9 mg/kg bw) can substitute for (+)-amphetamine in a dose-related way. Doses of (-)-ephedrine and caffeine, which produced < 1 % drug-appropriate response when administered alone, were able to enhance the stimulus effects of the other, when administered in combination. According to the authors low doses of (-)-ephedrine and caffeine may mutually potentiate one another's stimulus effects in (+)-amphetamine-trained rats (Young et al., 1998).

3.3.2. Short-term and subchronic toxicity

3.3.2.1. Ephedra herb and its preparations

Mice

The safety of a Ma huang (*E. sinica*) herbal preparation containing 9 % (-)-ephedrine (concentrations of ephedra alkaloids besides ephedrine not specified) was addressed in a repeated-dose study in BALB/c mice. Male and female mice (5/sex/dose) were dosed twice a day at 20-4 000 mg/kg bw for 2 weeks. The highest daily dose (8 000 mg/kg bw/day) was lethal to 50 % of the mice, with gastrointestinal bleeding observed at necropsy. Similar findings were observed to a lesser degree in the 4 000 mg/kg bw/day groups. From these data, the LD_{50} for (-)-ephedrine in this herbal preparation was determined to be 360 mg/kg bw twice daily (720 mg/kg bw/day). Based on body surface area corrections, this corresponds, for a 70 kg human, to 2.1 g twice daily of (-)-ephedrine and 23.3 g twice daily of Ma huang (Law et al., 1996).

3.3.2.2. Ephedrine

Mice

$B6C3F_1$ mice (10 animals/sex/group) were exposed to ephedrine sulphate via the feed at doses of 310-5 000 mg/kg diet (47-750 mg/kg bw/day) for thirteen weeks (NTP, 1986). Hyperactivity and excitability were observed with the highest incidence in animals of the 1 000 mg/kg diet (150 mg/kg bw/day) group or higher. No compound-related histopathological changes were found. Compound-

related reduced weight gain was observed in each sex from 500 mg/kg diet (75 mg/kg bw/day) ephedrine sulphate onwards.

Rats

Fischer 344 rats were exposed to ephedrine sulphate via the feed at doses of 125-2 000 mg/kg diet (12.5-200 mg/kg bw/day) for thirteen weeks (NTP, 1986). Hyperactivity and excitability were observed with the highest incidence in animals of the 1 000 mg/kg diet (100 mg/kg bw/day) group or higher. No compound-related histopathological changes were found. Compound-related reduced weight gain was observed in each sex from 500 mg/kg diet ephedrine sulphate onwards.

3.3.2.3. Pseudoephedrine

No relevant data are available.

3.3.3. Genotoxicity

3.3.3.1. Ephedra herb and its preparations

Yin et al. (1991) reported that Ma huang (*Ephedra sinica* Stapf) extract (concentrations of ephedra alkaloids besides ephedrine not specified) was not mutagenic in *Salmonella typhimurium* TA98 or TA100 either in the absence or presence of S9 at doses up to 40 mg/plate. The Ephedra herb extract was also administered i.p. to mice at doses described to be equivalent to 1-40 times that used in traditional medicine. No changes were observed in the ratio of polychromatic erythrocytes to total erythrocytes or in the frequency of micronucleated polychromatic erythrocytes in the bone marrow micronucleus assay. The Panel noted several shortcomings in the experimental design of this study and in data reporting: the limited set of bacterial tester strains, the uncertainty on the dose administered and sacrifice time, the lack of any raw data concerning the experimental results obtained. Moreover, the Panel noted that the material tested in this study was obtained by extraction from plant powder in boiling water for 90 min, in order to simulate the traditional use of plant medications. It is conceivable, and acknowledged by the study authors, that any labile genotoxic components of the plant, if present, will be extensively degraded under such harsh extraction conditions. Therefore, the Panel considered that no conclusion could be drawn from this study.

Herba Ephedrae (*Ephedra* species and concentrations of ephedra alkaloids not specified) was screened for genotoxic activity by the rec-assay with *Bacillus subtilis* as well as the Ames test with *S. typhimurium* TA98 and TA100 with and without S9. Plant extracts were prepared by water and methanol extraction at 40 °C, dried and dissolved in DMSO. Under the condition of this study, both extracts were negative when tested up to 10 mg/plate (Morimoto et al., 1982). The Panel noted the limited protocol of the *S. typhimurium* reversion test (Ames test), with only two tester strains and no confirmatory experiment, and the insufficient validation of the rec assay in *B. subtilis* as a test for genotoxic hazard identification.

Considering the limitations of the available mutagenicity studies in bacteria, and the absence of any information on genotoxicity in mammalian cells, the Panel concluded that the genotoxicity of Ephedra herb could not be assessed.

3.3.3.2. Ephedrine

Ephedrine sulphate at 100-10 000 µg/plate was negative in *S. typhimurium* TA97, TA98, TA100, TA1535 with and without metabolic activation (NTP, 1986).

3.3.3.3. Pseudoephedrine

No relevant data are available.

3.3.4. Chronic toxicity and carcinogenicity

3.3.4.1. Ephedra herb and its preparations

Mice

Ray et al. (2005), investigated the effects of short-term and long-term (up to 12 months) exposure of female B6C3F$_1$ mice to an ephedra and caffeine-containing multinutrient and botanical extract supplement (Metabolic Nutrition System Orange; MNSO) at five dietary concentrations, on serum biochemistry and histopathology of the hearts. The MNSO contained a total of 1 g fat (in a serving size of 13.26 g. Overall, MNSO has 40 mg of ephedrine group alkaloids per serving in the form of herbal extracts, as well as 150 mg of caffeine alkaloids per serving. The mice were fed control (without MNSO) or MNSO diets (Chow premixed with one time, two times, three times, six times or ten times the human equivalent of MNSO). The MNSO exposure did not significantly affect any of the cardiosensitive enzymes (creatine kinase; lactate dehydrogenase and aspartate aminotransferase) or result in any histopathological change of the heart.

3.3.4.2. Ephedrine

Mice

Ephedrine was tested for carcinogenic activity in a 103-week feeding study in B6C3F$_1$ mice (NTP, 1986). The compound was administered at 0, 125 or 250 mg/kg diet (corresponding to 0, 19 or 37.5 mg/kg bw/day) to 50 mice/sex/group. Throughout most of the 2-year study, mean body weights of male and female treated mice were lower than those of controls. No evidence of a tumourigenic effect was observed. The Panel noted that the reduction in body weights was related to a decrease in the feed intake.

Rats

Ephedrine was tested for carcinogenic activity in a 103-week feeding study in Fischer344 rats (NTP, 1986). The compound was administered at 0, 125 or 250 mg/kg diet (corresponding to 0, 6, 25 or 12.5 mg/kg bw/day) to 50 rats/sex/group. Throughout most of the 2-year study, mean body weights of male and female treated rats were lower than those of controls. There was no evidence of a tumourigenic effect, however, a significant decrease in the incidence of mammary gland fibroadenomas observed in ephedrine sulphate-treated female rats was considered by the authors to be associated with reduced weight gain (NTP, 1986). The Panel agreed with this suggestion.

3.3.4.3. Pseudoephedrine

No relevant data are available.

3.3.5. Reproduction and developmental studies

3.3.5.1. Ephedra herb and its preparations

No relevant data are available.

3.3.5.2. Ephedrine

Chick embryos

Ephedrine was administered to 3-day chick embryos (Hamburger-Hamilton developmental stage 19) either alone or together with caffeine at doses where each compound alone caused minimal embryotoxicity (Nishikawa et al., 1985). Embryos were examined for malformations on day 14 of incubation. Ephedrine at doses of 0.5 and 5 µmol (corresponding to approximately 1 mg/egg and 10 mg/egg, respectively) appears to be teratogenic to the chick cardiovascular system and induced cardiac malformations in 8 % of the embryos treated with 0.5 µmol. The dose of 5 µmol ephedrine caused malformations in 26 % of the embryos, which are all characterized by small ventricular septal

defects. Caffeine at a dose as low as 0.5 µmol (0.1 mg/egg; 2 mg/kg egg) significantly potentiated the teratogenicity of ephedrine.

The Panel noted that these data are inadequate to assess the possible reproductive and developmental effects of ephedrine in humans.

3.3.5.3. Pseudoephedrine

No relevant data are available.

3.3.6. Human data

3.3.6.1. Ephedra herb and its preparations

Case reports associated with intake of dietary supplements containing preparations of Ephedra/ephedra alkaloids including combinations with caffeine

In the U.S.A. reports on adverse events, such as heart attacks, seizures, stroke and death, as well as nausea, vomiting, anxiety, hypertension, tremors, and palpitations, seen in association with the intake of Ephedra herb containing food supplements, had raised concerns and finally lead to a ban of food supplements containing ephedrine-type alkaloids in 2004 (Schulman, 2003; FDA, 2004). According to Schulman (2003), from February 1993 through July 2003, FDA's adverse event reporting system documented 2 277 adverse events among "Ephedra"[34] users. The data from the American Association of Poison Control Centers, which in 2002 alone received reports of 1 428 adverse events among "Ephedra" users, were deemed to reflect the situation in a more accurate manner. In addition, an investigation of a major manufacturer of products containing "Ephedra", found records of 14 684 reports of "Ephedra"-related adverse events from May 1997 through July 2002. The entirety of case reports was seen as a limited but the major source of safety information to draw inferences and to support the analysis of a causal relationship (Schulman, 2003).

Selected studies, investigating the presence of an association between the intake of Ephedra herb containing food supplements and adverse events based on the available observational data, are summarised briefly below.

One hundred and forty reports of adverse events which were observed in conjunction with the intake of dietary supplements containing preparations of "Ephedra"/ephedra alkaloids (and which were submitted to FDA between June 1, 1997 and March 31, 1999 were evaluated. The reasons for the use of the food supplements include weight loss, athletic performance, increase energy and intentional misuse. The reported side-effects were assessed using the causality assessment scheme by Blanc et al. (1979). Among 87 events which were definitively, probably, or possibly related to the use of dietary supplement preparations containing ephedra alkaloids 10 resulted in death and 13 in permanent impairment, 8 in ongoing treatment at the time of the evaluation in 14 events the outcome is unknown, and 42 fully recovered. Under cardiovascular events, hypertension was the most frequently observed symptom, followed by palpitation, cardiac arrest/sudden death. Under central nervous system events, stroke and seizures were among the frequently observed events. Nine serious adverse events, partly leading to death occurred in persons who were taking relatively low doses of ephedra alkaloids (range, 12 to 36 mg/day, no indication of food supplement composition and concomitant caffeine intake) and who had no important medical risk factors. According to Haller and Benowitz (2000), the dose of ephedrine that was associated with an adverse event was often less than a typical dose of ephedrine used for bronchodilation (25 to 50 mg/day), which in experimental studies showed only moderate effects on heart rate and blood pressure. The authors assumed that the discrepancy between such data and the findings of serious adverse events reported with the use of dietary supplements containing

[34] Reports in the open literature often refer to use of Ephedra without further specification of species, plant parts and preparations, covering partly the use in combination with other substances such as caffeine. In this case the term "Ephedra" in quotation marks is used in this opinion.

ephedra alkaloids may be due to individual susceptibility, the additive stimulant effects of caffeine, the variability in the contents of pharmacologically active chemicals in the products, or pre-existing medical conditions. The authors considered that caffeine was likely to enhance the cardiovascular and central nervous system effects of ephedrine (see section 3.3.7.2) (Haller and Benowitz, 2000).

Samenuk et al. (2002) evaluated possible cardiovascular toxic effects associated with the use of dietary supplements containing Ma huang (*Ephedra* species and preparations not reported, no indication of combination with other substances such as caffeine) using the comprehensive database Adverse Reaction Monitoring System of the FDA, which included clinical records, investigative reports, and autopsy reports. Reviewing 926 cases of *Ephedra* toxicity reported from January 1995 to January 1997, the authors found a time correlation between Ephedra use and 37 reported cases of stroke (16 cases from which 12 had an ischaemic haemorrhagic stroke), myocardial infarction (10 cases), or sudden death (11 cases). Autopsies performed in 7 of the 11 patients who experienced sudden death showed a normal heart in 1, coronary atherosclerosis in 3, and cardiomyopathies in 3. In 36 of the 37 patients, use of Ma huang was reported to be within the manufacturers' dosing guidelines. The authors concluded that (i) Ma huang use was temporally related to stroke, myocardial infarction, and sudden death; (ii) underlying heart or vascular disease was not a prerequisite for Ma huang–related adverse events and (iii) the cardiovascular toxic effects associated with Ma huang were not limited to massive doses. The authors also noted that, although the pathogenesis of the cardiac toxic effects of Ma huang remained incompletely defined, available observational and circumstantial evidence indicated that use of the substance may be associated with serious medical complications (Samenuk et al., 2002).

In a meta-analysis assessing the safety of "Ephedra" and ephedrine containing products for weight loss and athletic performance, the results of 50 controlled trials were reviewed. Clinically similar adverse events were subsumed in 7 categories: (i) psychiatric symptoms (e.g. irritability and anxiety), (ii) autonomic hyperactivity symptoms (e.g. insomnia), (iii) upper gastrointestinal symptoms (e.g. nausea, vomiting), (iv) palpitations, (v) tachycardia, (vi) hypertension and (vii) headache. The authors found sufficient evidence to conclude that treatment with ephedrine or "Ephedra" resulted in a 2 to 3 times increased risk of psychiatric symptoms, autonomic symptoms, upper gastrointestinal symptoms, and heart palpitations compared to the placebo treatment. There was a trend toward an increase of risk of similar magnitude regarding hypertension, but this increase was not statistically significant. It was not possible to estimate the degree to which caffeine contributed to this increase in risks, because there were too few trials of ephedrine alone or "Ephedra" alone to support stratified analyses. Furthermore the authors assessed all case reports for ephedrine and "Ephedra" in the FDA MedWatch files as of September 30, 2001, all case reports they identified in published literature, and a very large file of symptoms reported to a manufacturer of ephedra-containing dietary supplements. All case reports were searched by the authors for documentation that (i) an adverse event had occurred; (ii) the subject had consumed Ephedra or ephedrine within 24 hours prior to the adverse event or that a toxicological examination had revealed ephedrine or one of its associated products in the blood or urine; and (iii) an adequate investigation had evaluated for and excluded other potential causes. Cases meeting all these criteria were labelled "sentinel events". Cases meeting the first 2 criteria that had other possible causes of the event were labelled "possible sentinel events". Concomitant caffeine intake was not recorded. 284 case reports concerned serious adverse events after intake of Ephedra and ephedrine containing products. The authors identified 2 deaths, 3 myocardial infarctions, 9 cerebrovascular accidents, 3 seizures, and 5 psychiatric cases as "sentinel events" with prior Ephedra consumption; and 3 deaths, 2 myocardial infarctions, 2 cerebrovascular accidents, 1 seizure, and 3 psychiatric cases as "sentinel events" with prior ephedrine consumption. They identified additional 43 and 7 cases as possible sentinel events with prior Ephedra and ephedrine consumption, respectively. About half of sentinel events occurred in persons aged 30 years or younger (Shekelle et al., 2003).

Sixty-five cases of dietary supplement-associated seizures reported to MedWatch from 1993 to 1999 were analysed. In the 20 probably related cases, 19 involved "Ephedra" preparations (no further details, no doses are given). "Ephedra" preparations were also associated with 7 of the 13 possibly

related cases, making "Ephedra" containing dietary supplements the most noted cause of seizures in the MedWatch reports (Haller et al., 2005a).

Complete listings of all suspected adverse drug reactions or side-effects are contained in the Drug Analysis Prints maintained by the UK Medicines and Healthcare products Regulatory Authority (MHRA). For "Ephedra", 7 cases have been reported in the UK between 01 July 1963 and 12 May 2012 (the earliest reaction being reported in October 2000), one of which was fatal. The fatal case was associated with general disorders only (death), whereas for all the remaining six cases psychiatric disorders (agitation, anxiety, paranoia, delusion, restlessness and visual hallucinations) were reported (MHRA, 2012, online).

Human studies on Ephedra herbal preparations plus caffeine

In an efficacy trial, (Boozer et al., 2001), 35 overweight patients received a commercial herbal mixture containing Ma Huang and Guarana as the main active ingredients and corresponding to 72 mg 'ephedrine alkaloids' plus 240 mg caffeine for eight weeks. The study was completed by 24 patients, whilst 11 participants (31.4 %) did not complete it because of the side-effects encountered: elevated blood pressure (2 patients = 6 %), palpitation and chest pain (1 patient = 3 %) and palpitations (4 patients = 11.5 %). In the treatment group, the heart rate was statistically significantly elevated and there was a trend for increased systolic blood pressure. The 24 patients reported, in total, 60 symptoms and insomnia (13 x mentioned) was the most reported symptom.

In a second efficacy trial reported by Boozer et al. (2002), 83 overweight patients received 90 mg 'ephedrine alkaloids' plus 192 mg caffeine for six months. The trial was completed by 46 patients, whilst 37 (44.6 %) withdrew from the study. For 16 (19.3 %) of them elevated blood pressure, palpitation, insomnia, irritability were reported as side-effects. In the treatment group, the heart rate was elevated and there was a trend for increased systolic and diastolic blood pressure. The lack of statistical significance was not an absence of side-effects but rather due to the small number of participants reducing the statistical power of the trial.

Haemodynamic effects of ephedra alkaloids in combination with caffeine were investigated by Haller et al. (2002). The study was a single-arm open study conducted in five female and three male healthy volunteers. The pharmacokinetics and pharmacodynamics of a commercial product containing a measured total ephedra alkaloid content of 11.85 mg (from Ma huang extract defined as the extract of the dried above ground parts of *Ephedra* spp., most commonly *E. sinica*) and 87.5 mg caffeine (from guarana seed extract) were investigated. The subjects received 23.7 mg ephedra alkaloids (17.3 mg ephedrine, 5.3 mg pseudoephedrine, 0.4 mg norpseudoephedrine, 0.5 mg methylephedrine and 0.2 mg norephedrine), and 175 mg caffeine once. Plasma concentration time profile of four alkaloids and of caffeine were measured and increase in systolic (+ 15 mm Hg) and diastolic (+ 6 mm Hg) blood pressure and heart rate (+ 15 beats/min) were noted. The subjects reported restlessness, shakiness and heart pounding.

Overweight and obesity were the endpoints of the study of Coffey et al. (2004). Fifty two overweight patients received a product which contained ephedra alkaloids in the form of Ma huang, caffeine from Kola nut and other herbal components. The producer declared that the content of the three most prominent constituents was: 125 mg Ma huang (10 mg ephedra at 8 %), 250 mg Kola nut (60 mg caffeine at 25 %), and 100 mg White willow bark (15 mg salicin at 15 %). However, tests of the pill at two independent laboratories revealed that they contained approximately half the amount declared. Whilst no difference between active treatment and control group was observed for blood pressure changes and heart rate changes, concerning the assessment of adverse reactions after ephedra alkaloid intake this study has severe limitations given by the fact that the amount declared by the producer did not match the amount measured by analysis of ephedra alkaloids and of caffeine.

The study of Hioki et al. (2004) was performed in 41 obese women who received an oriental herbal medicine which among other herbal extracts contained ephedrine, and caffeine. Since Ephedra herb

was not the only botanical component of this product, no conclusions for the risk assessment of Ephedra herb can be drawn from this study.

In a cross-over study, 15 volunteers were given a commercial product containing 19 ingredients (among which a "proprietary blend" containing Ma huang extract aerial part 12 mg and guarana seeds 'caffeine alkaloids' 40 mg) or matching placebo for 7 days (McBride, 2004). Mean maximal QTc was increased from 396 to 419 milliseconds during the treatment with the commercial product. Systolic blood pressure was statistically significantly increased (118.9 vs 123.5 mm Hg). No conclusions can be drawn from this study, since there are a number of active substances included in the preparation used.

In their 2005 paper, Haller et al. (2005b) looked into the short-term metabolic and hemodynamic effects of "Ephedra" extract and guarana combinations. In this cross-over study, 16 healthy subjects received 2 doses of 'Ephedra" extract-guarana' (composition is given as follows "Ephedra" extract 325 mg: total alkaloids 23.2 mg; ephedrine 20 mg, pseudoephedrine 1.9 mg, noradrenaline 0.3 mg, norpseudoephedrine 0.2 mg, methylephedrine 0.8 mg, methylpseudoephedrine 0.04 mg; guarana containing 83.3 mg caffeine) as well as a product containing 25 mg ephedra alkaloids and 200 mg caffeine. Plasma concentration time profiles of ephedrine and of caffeine were measured. Increases in systolic (+ 11.5 mm Hg) and diastolic (+ 7.3 mm Hg) blood pressure and heart rate (+ 9.4 beats/min) were noted for both preparations.

In a cross-over study 13 healthy subjects received a product with more than 30 ingredients including 15 mg Ma huang extract, containing ephedra alkaloids, and 60 mg guarana seeds, containing caffeine, three times for 7 days in one period and matching placebo in the other period (Caron et al., 2006). The content of the tested preparations was for preparation (1) Ma huang extract 12 mg, Guarana seed (caffeine) 40 mg, zinc chelate 5 mg, chromium picolinate 75 µg, magnesium chelate 75 mg, vitamin E (di-α-tocopherylacetate) 6 IU, proprietary blend unspecified, and for preparation (2) Ma huang extract 15 mg, guarana seed (caffeine) 60 mg, zinc 2.5 µg, chromium 100 mg, vanadium 34.5 µg, *Gymnema sylvestre* (leaf and leaf extract) 83.4 µg, glucosamine sulphate 100 mg, vitamin B6 3.4 mg, manganese 1 mg, proprietary blend unspecified. No significant differences were noted for ECG or blood pressure variables. The study has several limitations, among them the low number of participants, which makes it impossible to draw any conclusion.

Hackman et al. (2006) investigated 29 patients who received a preparation 2 times daily which contained 55 ingredients among them 40 mg ephedrine group alkaloids (not further specified) plus 100 mg of caffeine (1 time prior to breakfast, 1 time prior to lunch) for 36 weeks. Ten patients (34.5 %) from the treatment group did not complete the trial, seven of them (24 %) because of side-effects among them insomnia, nervousness, dizziness, headache. From the patients in the treatment group 123 events were reported during the study period: headache, dry mouth, nervousness and insomnia were among the most mentioned adverse symptoms. Blood pressure changes or changes in heart rate were not observed.

A study of Greenway et al. (2004) used a complicated study model to evaluate the effect of a dietary supplement containing herbal caffeine (70 mg/dose) and "Ephedra" (24 mg/dose) on metabolic rate, weight loss, body composition and safety parameters. Concerning side-effects the second phase of the study can be used to gain information whereas it was not the aim of the first and the third phase of the study to obtain information on side-effects. The second phase was a study with 20 overweight subjects in the treatment group as compared to 20 subjects in the placebo control group. Treatment was by two times daily a pill containing among other constituents "Ephedra*"* (12 mg) and caffeine 35 mg. Treatment was for 12 weeks. No difference was observed between the treatment group and placebo group in the number of reported side-effects (e.g., respiratory, pain, headache, pinched nerve, hair loss, skin problem, neuropsychiatric symptomps, arrhythmia) (n = 20 under treatment; n = 27 in the control group) and no serious adverse effects were reported in the treatment group.

3.3.6.2. Ephedrine

Human studies with ephedrine alone

Adrenergic reactions were seen in the study of Astrup et al. (1992). One hundred and eighty overweight patients took part in a clinical trial of 24 weeks duration. Four groups of patients were treated with ephedrine/caffeine combination (20 mg/200 mg), caffeine (200 mg), ephedrine (20 mg) and placebo. Dosing was three times a day. In every group 10 patients did not complete the trial (the number in the caffeine alone group was 9). Side-effects were mentioned by three patients in the ephedrine/caffeine combination, two in the caffeine and one in the ephedrine group as the underlying cause for withdrawal. Insomnia was the most often occurring symptom in the ephedrine (8 patients = 23 %) and in the ephedrine/caffeine group (9 patients = 26 %) followed by tremor (4 and 5 patients in the ephedrine and in the ephedrine/caffeine group, respectively). Systolic and diastolic blood pressure and heart rate were not significantly changed.

In the cross-over study of Berlin et al. (2001), 50 mg ephedrine was given orally and 5 mg and 10 mg ephedrine were given intranasally to 16 volunteers. As compared to placebo ephedrine increased supine systolic blood pressure in a dose dependent manner as well as supine diastolic blood pressure and heart rate. 5 mg ephedrine did not have an effect which was clearly discernible form the effect seen after placebo.

Concentration-effect modeling was done in healthy humans by Persky et al. (2004). The study was in 8 subjects (five males, three females) who were given oral doses of 0.25, 0.5 and 1.0 mg/kg ephedrine sulphate. Blood pressure and heart rate were measured as was the plasma ephedrine concentration. The authors showed that after initial increase in systolic blood pressure a tolerance mechanism developed within 75 minutes which abolished the blood pressure increase. In contrast increase in heart rate did not show a tolerance phenomenon and the percentage change in heart rate was linearly related to the plasma ephedrine concentration within the observed range of concentrations (between 0 and 200 µg/L).

Human studies with ephedrine in combination with caffeine

The study of Molnár et al. (2000) was performed in 16 overweight children under treatment and 16 children under placebo for 20 weeks. Treatment consists of three times 100 mg ephedrine plus 10 mg of caffeine daily if below 80 kg and three times 200 mg ephedrine and 20 mg of caffeine if above 80 kg. No differences in heart rate, blood pressure or subjective side-effects were noted in this study.

3.3.6.3. Other ephedra alkaloids

Pseudoephedrine

As a dose-finding study, two men and two women received (-)-ephedrine and (+)-pseudoephedrine *per os* to investigate the lowest amount needed to raise the diastolic blood pressure (BP) over 90 mm Hg. Placebo was used as control. (-)-Ephedrine 60-90 mg (0,9 – 1.5 mg/kg bw) rendered a diastolic BP of 90-110 mm Hg, whereas for (+)-pseudoephedrine 210-240 mg (3.2 – 4.0 mg/kg bw) was needed to render a diastolic BP of 90-100 mm Hg. Resting and highest BP after receiving placebo was 70 – 80 mm Hg. The doses of 60 mg (-)-ephedrine and 210 mg (+)-pseudoephedrine were considered to have approximately the same pressor activity, raising BP over 90 mm Hg, and were therefore chosen for the following study of bronchodilation in 9 subjects (6 men and 3 women) with reversible airway obstruction. Using these doses, that had approximately the same pressor effect, the bronchodilatory effect of pseudoephedrine was less than half of that of ephedrine, when measured by spirometry.

The authors discussed that the differences in effect on bronchodilation as well as in pressor effect, was due to the fact that (+)-pseudoephedrine had less β-adrenoreceptor stimulant action than (-)-ephedrine. Relief of nasal congestion, however, may occur due to stimulation of α-adrenoreceptors of the blood vessels in mucous membranes, and in earlier studies pseudoephedrine was shown to be as effective as

(-)-ephedrine to attain this effect. It was suggested that (+)-pseudoephedrine has much less effect on β-adrenoreceptors than (-)-ephedrine, but equal effect on the α-adrenoreceptors of the nasal blood vessels (Drew et al., 1978) (salt of (-)-ephedrine and (+)-pseudoephedrine not stated).

In a cross-over study the effect of the antihistamine acrivistine (8 mg) alone was compared with pseudoephedrine (60 mg alone, chemical and physical form of pseudoephedrine not stated), the combination of acrivistine and pseudoephedrine (8 mg + 60 mg) and placebo to alleviate symptoms of seasonal allergic rhinitis. Medication was taken 3 times daily. Forty patients, both male and female, were admitted and 35 completed the study. The combination of acrivistine and pseudoephedrine was shown to be most effective to alleviate all recorded symptoms: sneezing, itchy nose/throat, running and blocked nose, watery and itchy eyes. Acrivistine had a better effect than pseudoephedrine alone, which in turn had a better effect than placebo. An additive rather than synergistic effect was observed with the combination of acrivistine and pseudoephedrine. The most commonly reported adverse effects were insomnia and dryness of mucous membranes and these were mainly associated with the use of pseudoephedrine (Meran et al., 1990).

The object of the study by Empey et al. (1980) was to determine the optimal oral dose of pseudoephedrine on nasal airway resistance. The authors described (+)-pseudoephedrine primarily as an α-adrenergic stimulant, acting similarly to (-)-ephedrine, but with less vasopressor activity. Eighteen subjects were challenged with histamine diphosphate after being pretreated in a double blind randomised design with doses of 15, 30, 60, 120 or 180 mg pseudoephedrine hydrochloride or placebo. Nasal airway resistance was measured at different time points after pretreatment and challenge. Subjects were studied on 6 occasions, one week apart, one dose per occasion. One subject developed sinus tachycardia after the dose of 180 mg pseudoephedrine, and therefore did not receive the dose of 120 mg on the following occasion. This was the only missed dose during the study, except for one other subject who missed the dosage of 30 mg due to an infective illness. The result of the study showed that the doses 60, 120 and 180 mg pseudoephedrine significantly reduced the effect of histamine on nasal airway resistance to the same degree, compared to placebo. The doses 15 and 30 mg did not show any effect. Pulse and systolic blood pressure increased significantly with the doses 120 and 180 mg, but not with the doses 15, 30 or 60 mg pseudoephedrine. None of the doses had effects on diastolic blood pressure, altered mood or showed excess of other unwanted subjective effects compared to placebo. The conclusion of the study was that 60 mg pseudoephedrine hydrochloride *per os* achieved maximal nasal decongestion without cardiovascular or other unwanted effects (Empey et al., 1980).

A stroke registry since 1988 was used in the study by Cantu et al. (2003). Twenty two of 2 500 stroke patients had a stroke associated with an OTC cough and cold sympathomimetic drug within 24 hours before the onset of the stroke. Stroke was considered to be related to the drug when there was (a) a close relationship between ingestion of drug and onset of stroke and (b) other known causes of stroke were excluded. Of the 22 patients 21 had a haemorrhagic stroke and one an ischaemic stroke. Traditional vascular risks were observed in 8 of the cases: alcohol use (4), tobacco use (2) and systemic hypertension (2). One event occurred from a suicide attempt. In sixteen of the cases stroke was associated to use of phenylpropanolamine and in four of the cases to pseudoephedrine (oral doses 60 – 300 mg). In the remaining two cases other sympathomimetics were used, and these were administered by the nasal route. In the study blood pressure, CT/MRI-findings and angiographic findings were recorded. The conclusion drawn by the authors was that stroke associated with over-the-counter sympathomimetics was associated with hypertensive crisis and/or vasculitis-like mechanisms. Stroke occurred when higher than recommended doses were used, but also with recommended doses. (Cantu et al, 2003).

A double-blind parallel study design was used to assess tolerability and pharmacokinetics of (+)-pseudoephedrine (Dickerson et al., 1978). Thirty three healthy male volunteers were included in the study, and were randomly assigned to either a dose of 120 or 150 mg pseudoephedrine as sustained action capsule, taken every 12 hours for seven test days. Blood samples were taken pre-medication and twice daily on days 1, 3, 5, and 7 of the study. Plasma samples were taken at 12, 14, 16, 18 and

20 hours after the last dose of the drug. Pulse and blood pressure were measured before each blood sample was drawn. Subjects were evaluated for presence or absence of side-effects by an indirect questionnaire. One subject in the 150 mg-group was excluded from the study after the eighth dose, due to excitatory-type effects (dry mouth, insomnia, tension, restlessness, dysuria, palpitation, anxiety, and vertigo, occurring in that order) of increasing severity and a raised diastolic blood pressure above 90 on two occasions and above 100 on one occasion and a pulse rate greater than 25 % above baseline on three occasions. The symptoms disappeared 20 hours after the last dose. His elimination half-life was not significantly different from the mean half-life of the 150 mg treatment group. This subject had previously self-medicated with 60 mg (+)-pseudoephedrine as acute single doses during the recent 2-3 years, without noting side-effects. Both treatment groups showed a significant and persistent increase in pulse rate over baseline values during the treatment period, but there was no difference between the groups. The 150 mg group had a significant increase in systolic blood pressure between 4 and 60 hours of treatment, and both groups had a significant decline in systolic blood pressure from 170 – 176 hours of the treatment period, i.e. the last hours of the treatment period. The most common subjective side-effects that were reported by both groups were insomnia, anxiety, tachycardia and anorexia, of which tachycardia and anorexia along with dry mouth were the most persistent. The total number of reports was higher in the high-dose group. Only one subject in the 150 mg group did not experience any side-effects, his elimination half-life was lower than the group mean, but 6 other subjects in the group with shorter half-lives experienced side-effects. (+)-Pseudoephedrine plasma concentrations varied considerably between individuals. Steady state plasma concentrations were achieved after the third dose. Biological half-lives were similar among most subjects, a few persons had considerably longer half-lives than the mean values. Biological half-life was not dependent on the small variations in urine pH, between 5.3-6.0 for 27 of 31 subjects. Mean biological half-life was 5.9 ± 2.2 hours and mean steady state plasma concentration of (+)-pseudoephedrine 447 ± 116 ng/mL in the 120 mg group, these values were 6.9 ± 3.9 hours and 510 ± 133 ng/mL respectively in the 150 mg group. There was no correlation between the elimination half-life of (+)-pseudoephedrine and the severity or number of symptoms experienced by the subjects (Dickerson et al., 1978).

The relief of symptoms of seasonal allergic rhinitis was studied in 874 subjects in a double-blind, placebo-controlled multicenter study (Bronsky et al., 1995). The patients were treated orally for 2 weeks with either a combination of 10 mg loratadine (a non-sedating antihistamine) and 240 mg pseudoephedrine sulphate once daily, 10 mg loratadine once daily, 120 mg pseudoephedrine sulphate every 12 hours or placebo. The combination of loratidine and pseudoephedrine was most effective in relieving symptoms of rhinitis. No serious or unusual adverse events were recorded, insomnia and nervousness were significantly more common with the combination treatment (5 %, 5 % respectively) and pseudoephedrine alone (9 %, 4 %), than with loratadine alone (< 1 %, < 1 %) or placebo (0 %, < 1 %). Hyperkinesia was reported significantly more by patients in the pseudoephedrine group (4 %), than by the loratadine (< 1 %) or placebo (< 1 %) groups. Twelve patients in the combination group, 1 in the loratadine group, and 8 in the pseudoephedrine group discontinued the study due to adverse effects. Safety of the treatments was evaluated by physical examination including measurement of pulse and blood pressure, blood tests (blood count, blood chemistry) and urinanalysis at the beginning and end of the study period. Evaluation of safety and efficacy was also performed by a physician at days 4, 8, and 15 of the study. Patients in the combination group and the pseudoephedrine group showed greater mean increases in pulse rates than the loratadine and placebo groups throughout the study. These differences were significant for the combination group (3.3-4.5 beats per minute) compared to loratadine and placebo groups, but numerically higher but not statistically significant for the pseudoephedrine group (2.5-3.0 beats per minute). The conclusion drawn from the study was that the combination treatment was safe and more effective than the other treatments and placebo (Bronsky et al., 1995).

Norpseudoephedrine (cathine)

Cathine is formed from cathinone, the main active ingredient contained in khat leaves, when leaves are dried. Cathine is also a metabolite of cathinone in humans (Guantai and Maitai, 1983). Cathine

possesses psychostimulant activity which is less pronounced than that of cathinone (Szendrei, 1980; Aktories at al., 2009).

Khat ingestion produces increased motor stimulation, euphoria, and a sense of excitement and energy (Widler et al., 1994; Kalix, 1994). Nencini et al. (1998) investigated the action of cathine specifically comparing its action to morphine. It also results in decreased appetite and increased blood pressure and heart rate. The mode of action is described by an increase of the activity of the dopaminergic and noradrenergic transmission (Pehek and Schechter, 1990; Pehek et al.1990; Patel, 2000). It is not well documented whether cathinone and its metabolite cathine are producing classical drug dependency. Withdrawal symptoms, such as inertia, nightmares, trembling, depression, sedation and hypotension have been described (Cox and Rampes, 2003).

Cases of severe, pulmonary arterial hypertension, often with fatal outcome, have been reported associated with the intake of cathine for a supportive treatment in overweight patients (see Table 6, no details available).

Norephedrine

Norephedrine is used therapeutically only in the form of the racemate (±)-norephedrine (Phenylpropanolamine Hydrochloride Ph. Eur., CAS-No. 154-41-6) (EDQM, 2011; Sweetman, 2011). While a variety of clinical studies describe unwanted side-effects for the racemate (±)-norephedrine and its intake as a component of appetite suppressants has been associated with an increased risk of haemorrhagic stroke for women (e.g. Kernan et al., 2000; Salerno et al., 2005; Horwitz et al., 2007; Sweetman, 2011), no relevant case reports and studies on adverse effects referring to the isolated intake of (-)-norephedrine were available (see also section 2.7.3.2).

Differences in the α-adrenergic potency of (-)- and (+)-norephedrine have been investigated by Stockley et al. (1994). The pharmacokinetics and blood pressure responses of the norephedrine enantiomers were determined after the separate oral administration of racemic (±)-norephedrine (75 mg), (-)-norephedrine (37.5 mg), and (+)-norephedrine (37.5 mg) to six healthy volunteers. No significant differences were observed between any of the pharmacokinetic parameters of (-)- and (+)-norephedrine when the enantiomers were administered individually or as the racemate. There was also no difference in the ex vivo plasma protein binding of (-)- and (+)-norephedrine, determined individually or as the racemate. Significant increases from baseline in systolic and diastolic blood pressure (supine and standing) were observed for racemic (±)- and (-)-norephedrine, whereas (+)-norephedrine had no effect on blood pressure. The effects of (±)-norephedrine (75 mg) and (-)-norephedrine (37.5 mg) on blood pressure were not significantly different. Thus it could be concluded that the effect of the racemate on blood pressure was essentially attributable to (-)-norephedrine and that pharmacokinetic factors do not contribute to the differences in the cardiovascular effects of norephedrine enantiomers (Stockley et al., 1994).

3.3.6.4. Summary of the data from human studies

The Panel noted that reviews of human case reports described adverse cardiovascular and cerebrovascular events, including hypertension, palpitations, tachycardia, arrhythmias, myocardial infarction, cardiac sudden death, stroke and intracerebral haemorrhage, after intake of Ephedra herb containing food supplements (Haller an Benowitz, 2000; Samenuk et al., 2002; Shekelle et al., 2003). These are consistent with the adverse effects associated with the therapeutic use of medicinal products containing isolated ephedra alkaloids as single active ingredients (see Table 6).

For Ephedra herb preparations only studies with concomitant caffeine administration were available. The Panel noted that in most of these studies cardiovascular effects (increased heart rate, increased blood pressure) were reported. The lowest dose with an effect on cardiovascular and central nervous system was Ephedra herb extract corresponding to 23.7 mg/day ephedrine in combination with caffeine (Haller et al., 2002). In the studies with ephedrine effects were seen at oral doses of roughly 20 mg/person/day alone and with caffeine in a dose of about 200 mg and higher (Astrup et al., 1992;

Persky et al., 2004). Thus in human studies, the lowest dose of ephedrine tested, with and without caffeine, which produced an effect was 20 mg/person/day. An exception is the study of Molnár (2000) in which 100 mg ephedrine combined with 10 mg caffeine did not influence haemodynamic parameters in children. In the study of Persky et al., 2004 the plasma concentration of ephedrine of 25 µg/L was a concentration without increased heart rate.

(+)-Pseudoephedrine acts directly on α-adrenoreceptors, and to a lesser degree on β-adrenoreceptors, and releases noradrenaline from storage sites.

Clinical data for (+)-pseudoephedrine indicate that it was less potent than (-)-ephedrine at increasing blood pressure and inducing bronchodilation.

In the study by Drew et al (1968) the dosage of 210 mg (+)-pseudoephedrine (3.2 mg/kg bw) had approximately the same pressor effect as the dosage of 60 mg (-)-ephedrine (0.9 mg/kg bw). As an oral medical agent, (+)-pseudoephedrine is mainly used as a nasal decongestant in rhinitis and similar conditions, due to its vasoconstrictor properties.

Similar detailed information as for (-)-ephedrine and (+)-pseudoephedrine was not available for other ephedra alkaloids. From the study of Stockley et al. (1994) the Panel concluded that (+)-norephedrine has no influence on blood pressure whereas (-)-norephedrine did have an effect at a dose of 37.5 mg. Thus the LOAEL of (-)-norephedrine for cardiovascular effects seems to be near the LOAEL for (-)-ephedrine.

3.3.7. Possible pharmacological mechanisms

Ephedra alkaloids are indirectly acting sympathomimetic amines which act by evoking noradrenaline release in the absence of nerve terminal depolarization. The principal mechanism of action relies on their indirect stimulation of the adrenergic receptor system by increasing the concentration of noradrenaline at the post-synaptic α-receptors and at the β-receptors. Direct receptor activation is contributing to the sympathomimetic effects of ephedra alkaloids. They are able to cross the blood-brain barrier and they are CNS stimulants by releasing noradrenaline and dopamine in the substantia nigra. Therefore, the effects of ephedra alkaloids, such as blood pressure elevation, anxiety, restlessness, and aggravation of psychiatric disorders are evoked by the released noradrenaline.

Experimental data show that the naturally occurring ephedra alkaloids differ quantitatively in their peripheral and central sympathomimetic effects (see section 3.1.4 and Table 9).

In animal and *in vitro* studies (-)-ephedrine showed higher potencies to increase blood pressure and heart rate and to act as an inhibitor of (-)-noradrenaline-induced lipolysis compared to its diastereomer (+)-pseudoephedrine. In an isolated organ system (-)-ephedrine also exhibited a higher potency to induce a positive chronotropic effect than (-)-methylephedrine. Furthermore (-)-norephedrine was more effective in rats to increase arterial blood pressure than (+)-norpseudoephedrine (Patil et al., 1965; Fauley et al., 1974; Moya-Huff et al., 1987; Kawasuji et al., 1996).

From a study in rats administered by gavage, measuring inhibition of food intake, a surrogate measure for central sympathomimetic effect, (Blosser et al., 1987) it can be concluded that (+)-norpseudoephedrine was 1.7-fold more potent than (-)-ephedrine and 1.4-fold more active than (-)-norephedrine. Compared to (-) ephedrine, (+)-pseudoephedrine showed a 0.2-fold activity (Blosser et al., 1987). The activity ranking is (+)-norpseudo-ephedrine > (-)-ephedrine > (-)-norephedrine > (+)-pseudoephedrine > (+)-ephedrine > (-)-norpseudo-ephedrine > (+)-norephedrine > (-)-pseudoephedrine. However, it is unclear how the findings translate to the human situation.

In a study by Fairchild and Alles (1967) in mice with intraperitoneal administration, clear central locomotor stimulation was produced by (+)-norpseudoephedrine, (-)-ephedrine, and (-)-norpseudoephedrine whereas (-)-norephedrine, (+)-norephedrine, (-)-pseudoephedrine, (+)-pseudoephedrine, and (+)-ephedrine were active in this paradigm only in doses approaching lethal

amounts. The most active compound was (+)-norpseudoephedrine, exhibiting 10 % of the activity of (+)-amphetamine, followed by (-)-ephedrine (4 %) and (-)-norpseudoephedrine (2 %). (Fairchild and Alles, 1967).

From the studies described above it can be concluded that most of the compounds have an effect on the cardiovascular system although their activities differ markedly. This occurs by direct and indirect action on α-receptors. However, also agonistic activity on ß-receptors has been demonstrated. However, the CNS activity of the range of substances is somewhat different in the two paradigms used to test (+)-amphetamine like effects. Only for (-)-ephedrine a factor of about 0.5 can be calculated compared to (+)-norpseudoephedrine because both types of tests resulted in the same order of activity. Because of the differences in activity in the two tests for the components other than (-)-ephedrine, the studies did not provide the basis to calculate a common factor which could be used to account for the different activity.

From the evidence reviewed, it appeared that in food supplements containing Ephedra herb preparations, caffeine is also present in combination.

According to current knowledge most of the pharmacological effects of caffeine are due to competitive antagonism for adenosine receptors. Other proposed mechanisms of action, such as inhibition of phosphodiesterase activity and induction of mobilisation of calcium, require higher serum concentrations than may be obtained via normal human consumption of caffeine-containing beverages and foods. Caffeine may interact with A_1 and A_2A adenosine receptors at plasma levels of 4-100 μM, which corresponds to an intake of 50–1 500 mg caffeine for an adult. These receptors partly mediate opposing reactions at the cellular level. Activation of the high affinity receptor A_1 leads to inhibition of adenyl cyclase via a guanyl nucleoside binding protein, Gi. Activation of A_2A stimulates adenyl cyclase via a protein Gs.

Adenosine receptor antagonism has been implicated in the indirect action of caffeine on dopamine receptors. Adenosine receptors are co-localised and interact with dopamine receptors, adenosine and dopamine receptors exert opposite effects on the same nerve cells in the brain.

Tolerance may develop to some of the pharmacological effects of caffeine. Tolerance for cardiovascular effects develops rapidly, within hours or days when there is a regular intake of caffeine, whereas tolerance to the CNS effects of caffeine only appears slowly and to a varying extent (Fredholm et al., 1999, 2005; Andersson et al., 2004).

Caffeine as well as ephedra alkaloids act on the cardiovascular and on the central nervous system. No interaction of the substances at the level of receptors is to be anticipated as they act on different receptor systems. However, even if working by independent receptor and signaling systems it is to be expected that their effects on heart-rate, blood pressure and the central nervous system will be additive.

By inhibiting adenosine-mediated dilatation of blood vessels, caffeine constricts blood vessels and may increase blood pressure in persons prone to hypertension. Caffeine also augments the release of catecholamines, an effect that, when combined with that of ephedrine, could lead to increased stimulation of the central nervous system and cardiovascular system (Robertson et al. 1978; Haller and Benowitz, 2000; Aktories et al., 2009).

4. Discussion

EFSA was asked to assess the safety in use of *Ephedra* species and its preparations when consumed as a component of food, e.g. in food supplements. The risk assessment is carried out according to the EFSA Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009).

Concerns had been raised by an EU Member State Authority regarding possible severe health risks especially associated with the consumption of food supplements, containing herbs of *Ephedra* species, their preparations or their alkaloids, for weight loss, to enhance athletic performance and in bodybuilding. (-)-Ephedrine and its congeners occurring in the herbs of *Ephedra* species act as sympathomimetics with pronounced cardiovascular effects as well as stimulating effects on the CNS (Sweetman, 2011). In combination products, containing *Ephedra* species/ephedra alkaloids together with the stimulant caffeine, the effects of ephedra alkaloids in the body might even be increased. Consequently, the Commission has initiated the procedure under Article 8 (2) of Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods for *Ephedra* species. Therefore EFSA has been asked to review the relevant existing scientific data on the possible link between the intake of *Ephedra* species and a harmful effect on health and to provide advice on a tolerable upper intake level (UL) for *Ephedra* species, for the general population, and as appropriate, for vulnerable subgroups of the population. In the absence of an UL, advice on a daily intake of *Ephedra* species that does not give rise to concerns about harmful effects to health should be provided.

In addressing the Terms of Reference, the Panel noted that the term "tolerable upper intake level" was used by EFSA so far only for nutrients, such as vitamins or minerals, to describe the maximum level of total chronic daily intake judged to be unlikely to pose a risk of adverse health effects to humans. The Panel considered that the term "tolerable upper intake level" for botanicals and botanical preparations could lead to the misinterpretation that they could play a similar role in human nutrition as minerals and vitamins, which differ from them *inter alia* in being in general constituents of the normal diet and in being in many cases essential. This is particularly so in this specific case, where the botanicals/botanical preparations or their main components have known medical uses based on scientifically established pharmacological properties and/or where the botanicals/botanical preparations cannot be regarded as a component of the normal diet. Therefore, the Panel considered that the use of the term "tolerable upper intake level" would not be appropriate for botanicals and botanical preparations being constituents of food supplements or other food products.

Food supplements containing Ephedra herb or its preparations are commercially available and are promoted for weight loss and to enhance athletic performance. According to the information provided by the relevant food industry associations for this sector, in general these products are not marketed in Europe. The Panel however noted that they are offered for sale via the internet, often in combination with other substances (e.g. caffeine).

Pharmacologically active constituents of the Ephedra herb which are of toxicological relevance are ephedra alkaloids, which exhibit sympathomimetic action. There are 20-66 *Ephedra* species with a number of subspecies and varieties (The Plant List, online). Some studies reported the lack of occurrence of ephedra alkaloids in certain *Ephedra* species, albeit these claims are not supported by detailed investigations using state of the art methods of analysis and including a variation of samples within each of the species in question. The relative composition of the alkaloids in ephedra alkaloid-containing Ephedra herb varies considerably between the *Ephedra* species and within individual species (Lake et al., 2001; Kitani et al., 2009; Wang et al., 2010; Hong et al., 2011). The total alkaloid content, depending on the species, origin, conditions and time of harvest has been reported to vary between 0.5 and 49 mg/g (WHO, 1999; Cui et al., 1991, Lake et al., 2001; Kitani et al., 2009). The two main alkaloids (ephedrine and pseudoephedrine) account usually for 70-99 % of the total alkaloid content in Ephedra herb (Cui et al., 1991; White et al., 1997; Trujillo and Sorenson, 2003; Long et al., 2005; Kitani et al., 2009). The ephedrine content may be between 0 and 90 % and the pseudoephedrine content may be between 0.1 and 99 % of the total alkaloid content (WHO, 1999; Cui et al., 1991; Long et al., 2005; Blaschek et al., 2008; Kitani et al., 2009). The concentrations of (+)-norpseudoephedrine in the herb vary as well among different species and may amount up to 7.3 mg/g (see Table 2, Section 2.2). The occurrence of other alkaloids and amino compounds which are not structurally related to (-)-ephedrine, such as ephedroxane, ephedradine A, B, C and D, cyclopropyl-α-amino acids, 6-methoxykynurenic acid, N-methylbenzylamine and tetramethylpyrazine, and of glycans, such as ephedrans A, B, C, D and E, have been reported in the aerial parts of *Ephedra*

species. Other reported components of the twigs and barks of *Ephedra* species are e.g. flavanols (Abourashed et al., 2003).

Specifications for Ephedra herb are available in the European Pharmacopoeia 7.0 (EDQM, 2011) and in the WHO monographs on selected plants (WHO, 1999).

Two inter-laboratory validated methods have been published for the determination of ephedra alkaloids in botanicals and food supplements. The AOAC official method 2003.13 uses HPLC with UV detection. This method determines ephedrine and pseudoephedrine in the presence of norephedrine, norpseudoephedrine, methylephedrine and methylpseudoephedrine in botanicals and food supplements (Roman et al., 2004), while the other inter-laboratory validated method is a liquid chromatography-tandem mass spectrometry method for the determination of the above mentioned six ephedrine-type alkaloids in dietary supplements and botanicals (Trujillo and Sorenson, 2003).

No records for *Ephedra* species were found in the "EU Register on Nutrition and Health Claims" for health claims falling under Article 13.5 (health claim applications based on newly developed scientific evidence and/or proprietary data) or Article 14 (claims referring to children's development and, health and disease risk reduction claim) of Regulation (EC) No 1924/2006 (EC, online). An Article 13.1 (function claim) on Ephedra (*Ephedra sinica*)/Ma huang and weight loss is still pending (EC, online).

The FDA banned food supplements containing ephedra alkaloids of the ephedrine-type in 2004, stating they present a significant or unreasonable risk for the consumers.

As for other national regulations the use of Ephedra herb and/or ephedra alkaloids is specifically prohibited in foods and food supplements, in several European countries (e.g. United Kingdom, Ireland, France, Netherlands, Belgium, Denmark, Sweden, Austria, Czech Republic, Italy and Spain), as well as in Canada, Australia and New Zealand.

Several ephedra alkaloids (ephedrine, methylephedrine, pseudoephedrine, and cathine) are prohibited as stimulants by the World Anti-Doping Agency (WADA).

Ephedrine and pseudoephedrine are monitored worldwide as precursors of the illicit drug methamphetamine.

The pharmaceutical use of Ephedra herb and preparations thereof is described in older literature. According to information made available by the EMA, no assessment report on safety and efficacy is available or currently expected from the HMPC since Ephedra herb is not on the HMPC priority list. It is important to note that this lack of priority is a consequence of the known risks associated with the use of Ephedra herb and its preparations as herbal medicinal product.

Authorised medicinal products exist for four ephedra alkaloids as oral formulations in the EU Member States, with the following therapeutic indications:

In some Member States, ephedrine is authorised for use in adults in oral doses of 15-60 mg (daily doses 40-200 mg) for the treatment of airflow obstruction where bronchial mucosal oedema is thought to be contributing factors, hypotension, collapse, imminent paralysis of the respiratory centre, chronic bronchitis, allergic diseases, narcolepsia and kinetosis in case of lack of other treatment options and for the treatment or prevention of attacks of bronchospasm in asthma. The authorised dose for children is 15-30 mg (daily doses 45-90 mg) depending on the age group. It should only be used periodically, 4-5 days at a time.

In similar indications (allergic swelling of the nasal mucosa or in vasomotoric rhinitis for which nasal congestion is the main symptom) racemic norephedrine (phenylpropanolamine) is used orally. The

authorised dose for adults is 50 mg two times daily, while children between 6 and 12 years of age take 25 mg two times daily.

For pseudoephedrine (for the symptomatic relief of conditions such as allergic rhinitis, vasomotor rhinitis, nasal, sinus and upper respiratory congestion, common cold and influenza) the single dose is 60 mg (up to 4 times daily). The posology for children is 15-30 mg (daily doses 45-120 mg) depending on the age group.

Norpseudoephedrine (20-30 mg once per day or 10-20 mg twice a day) and norephedrine (50 mg/day) are indicated for the supportive, short-term treatment of nutrition related overweight.

The contraindications/warnings as listed in the summary of product characteristics for all four ephedra alkaloids are similar: hypersensitivity; (cardio) vascular disorders, e.g. hypertension; tachycardia, cardiac insufficiency; cerebrovascular disorders; enlargement of the prostate with residual urine; phaeochromocytoma; diabetes; thyroid disorders; renal disorders; angle-closure glaucoma; concomitant treatment with MAO inhibitors and within 10-14 days following termination of MAO-inhibitor; patients receiving other sympathomimetics (such as decongestants, appetite suppressants and amphetamine-like psychostimulants); hyperexcitability; administration before the use of halogenated anaesthetics; current or previous mental illness incl. anorexia nervosa and depressions; pregnancy and lactation; children (depending from indication). The treatment period is always restricted.

Also the adverse effects listed in the summary of product characteristics are similar: cardiovascular effects (e.g. palpitations, hyper or hypotension, tachycardia, arrhythmia, precordial pain, myocardial infarction); vascular effects (e.g. cerebral haemorrhage, impaired circulation to the extremities); gastrointestinal effects (e.g. nausea, vomiting, ischaemic colitis, anorexia); effects on kidneys and urinary tract (e.g. micturition problems, urinary retention), effects on nervous system/psyche (e.g. tremor, seizures, restlessness/nervousness, increased irritability, sleeping disorders, dizziness, anxiety, psychotic disorders, aggression, hallucinations; paranoid delusion, depressions); effects on respiratory tract (e.g. pulmonary oedema, dyspnoea, cases of severe, pulmonary arterial hypertension, often with fatal outcome); general effects (e.g. muscle weakness, asthenia, dry nose, dry mouth, thirst, dizziness, headache, increased perspiration, salivation). Tolerance, dependence and withdrawal symptoms have been reported with prolonged administration.

Medicinal products containing phenylpropanolamine have been removed from the market by the US FDA and Health Canada due to the risk of haemorrhagic stroke.

It was not possible to assess exposure to Ephedra herb and its preparations as such due to lack of data. Instead an estimation of the exposure to total alkaloids and to five individual ephedra alkaloids (ephedrine, norpseudoephedrine, pseudoephedrine, methylephedrine and norephedrine) from food supplements containing Ephedra herb was carried out, using data available from the literature.

In comparing estimated intake figures of ephedra alkaloids from food supplements with the approved single/daily doses of individual ephedra alkaloids from use as medicinal products, it has to be taken into account that based on structural considerations and their common mode of action, exposure to a mixture of ephedra alkaloids, as found in extracts, may lead to combination effects, such as additive or synergistic effects. Different potencies of individual ephedra alkaloids for various biological endpoints could not be taken into account due to the lack of data on the ephedra alkaloid composition of individual products. Thus, for the purpose of this scientific opinion, single servings and daily exposure to total ephedra alkaloids from food supplements were compared to doses used in the medicinal products. In each comparison it was assumed that the potency of the total ephedra alkaloids in food supplements was equivalent to that of the specific alkaloid in the medicinal product with which it was being compared.

For total ephedra alkaloids from food supplements (capsules and tablets), the mean single dose per serving ranged from 11.5 to 26.8 mg and the maximum single doses per serving (see Table 4 and

Table 7) ranged from 23.5 to 75.5 mg. Mean daily exposure to total ephedra alkaloids from food supplements was estimated to be 93 mg/person and maximum daily exposure was estimated to be 395 mg/person. Therefore, it was concluded that exposure to ephedra alkaloids from food supplements falls within or exceeds the therapeutic dose ranges for the individual ephedra alkaloids ephedrine, norephedrine, pseudoephedrine and norpseudoephedrine in single active ingredients medicinal products (see Table 6).

A similar estimation was done for (-)-ephedrine, being the major component of Ephedra herb containing food supplements. The mean single dose per serving from food supplements (capsules and tablets) ranged from 8.4 to 18.3 mg (-)-ephedrine and the maximum single doses per serving reported in the studies ranged from 15.3 mg to 65.8 mg (-)-ephedrine. Mean daily exposure from food supplements was estimated to be 65 mg/person (-)-ephedrine and maximum daily exposure was estimated to be 395 mg/person (-)-ephedrine. Therefore, the Panel considered that exposure to (-)-ephedrine from food supplements is within the range or may exceed the therapeutic dose for ephedrine in medicinal products (single dose: 50-60 mg; daily dose: 50-200 mg/person).

Regarding the ephedra alkaloids norpseudoephedrine, pseudoephedrine and norephedrine, individual exposure from food supplements lies below that of the single active ingredients in medicinal products (see Table 4, Table 6 and Table 8).

When comparing estimated exposure to single ephedra alkaloids from food supplements to the therapeutic doses of the same alkaloid when used in medicinal products, the Panel did not take into account combination effects by other ephedra alkaloids present in food supplements. The Panel was aware that this procedure underestimated the pharmacological effects which could occur following ingestion of a food supplement containing ephedra alkaloids.

The Panel noted that qualitative and quantitative data for other biologically active components than ephedrine-type ephedra alkaloids in Ephedra herb preparations in food supplements are not available but are needed. This is the case for the glycans, ephedran A, B, C, D and E, which have been shown to significantly reduce blood glucose levels in diabetic mice, and for ephedroxane which may potentiate the action of noradrenaline in the catecholaminergic nervous system.

In general, botanicals and botanical preparations for use in food supplements should be evaluated based on existing data on the chemical specifications and existing toxicological data, including read-across where appropriate, for the individual botanical and botanical preparations. Therefore, the Panel gave priority to the toxicological studies investigating Ephedra herb and its preparations. Studies with the single ephedra alkaloids found in the different *Ephedra* species (see Table 1) and in particular with (-)-ephedrine and (+)-pseudoephedrine, being the main alkaloids measured in food supplements containing Ephedra herbs were also considered.

The Panel noted that the (-)- and (+)-enantiomers of the individual ephedra alkaloids differ in their pharmacological and toxicological potencies (see section 3.1.) and that only limited data are available on the ratios of potencies for different target organs. Therefore only results obtained with the enantiomeric form of the individual ephedra alkaloid found in nature as presented in Table 1 are considered relevant in this opinion and results derived from studies with the racemates are considered not to be of sufficient relevance.

In humans, when (-)-ephedrine was supplied as Ephedra herb extract in combination with other botanicals (as the commercially available Ma huang products), the absorption and disposition characteristics were found to be similar to those observed for an equivalent single-ingredient (-)-ephedrine oral dose (Gurley et al. 1998b). In another study (Csajka et al. 2005), a slightly higher relative bioavailability of (-)-ephedrine from herbal extracts (increased by 32 %) was observed when compared with a similar dose of pharmaceutical (-)-ephedrine. In the same study, no significant interaction between (-)-ephedrine and caffeine was observed. When considered as a pure compound, the absorption of oral ephedrine is reported to be complete in humans (Wilkinson and Beckett 1968a)

and pharmacokinetic studies revealed a large volume of distribution including transfer of ephedrine across the placenta and into maternal milk (Hughes et al., 1985). Furthermore, Berlin et al. (2001) demonstrated that the maximal plasma ephedrine concentration (Cmax) and areas under the curves for up to 8 hours were proportional to the doses. Ephedrine was found to be moderately metabolised in humans through hepatic N-demethylation and oxidative deamination of the side-chain (Feller and Malspeis, 1977). In humans, 70 to 80 % of the dose was excreted as unchanged ephedrine in urine in 48 hours and this urinary excretion was shown to be pH-dependent (Welling et al., 1971).

Regarding stereochemical aspects, both *in vitro* and *in vivo* metabolic animal studies demonstrated that (-)-ephedrine is more rapidly and more efficiently N-demethylated, hydroxylated and conjugated than the (+)-ephedrine isomer (Jenner and Testa, 1974; Feller and Malspeis, 1977; Baba et al., 1986).

When norephedrine was orally administered alone in humans (Sinsheimer et al., 1973), unchanged norephedrine (86 % of the dose) was excreted along with the minor metabolites hippuric acid and 4-hydroxynorephedrine. By contrast, orally administered (-)-methylephedrine was extensively metabolised, in part to ephedrine and then to norephedrine (Wilkinson and Beckett 1968b).

From the ADME studies considered, the Panel noted that in humans, ephedrine from herbal extracts would be of a similar or slightly higher bioavailability than in a single ingredient formulation and that pharmacokinetic studies demonstrated widespread distribution of unchanged ephedrine within the body. Considering the major urinary excretion of ephedrine and norephedrine, the Panel also noted a possible accumulation of these alkaloids in renal insufficiency and/or repeated high doses of Ma huang.

Accidental ingestion of herbal supplements containing Ma huang (*Ephedra sinica*) and mostly guarana (*Paullinia cupana*), a natural source of caffeine, was examined in dogs. Clinical signs of toxicity included tremors, seizures, vomiting, tachycardia, hyperthermia and nonspecific behavioural changes. Doses of guarana and Ma huang at which signs of intoxication were reported, ranged between 4.4 and 296 mg/kg bw and 1.3 and 89 mg/kg bw, respectively. Clinical signs developed within 8 hours after ingestion in 80 % of the dogs; duration ranged from 10 to 48 hours (Ooms et al., 2001).

The acute toxicity (mortality and cardiotoxicity) of Ma huang (containing 4 % ephedrine crude extract) and ephedrine was increased when rats were treated in combination with caffeine.

A Ma huang herbal preparation (*Ephedra sinica*) containing 9 % (-)-ephedrine was lethal to 50 % of Balb/c mice when dosed twice daily at 4 000 mg/kg bw (total of 8 000 mg/kg bw/day) for 2 weeks. From these data the LD_{50} for (-)-ephedrine in this herbal preparation was determined to be 720 mg/kg bw/day. Based on surface area corrections, this corresponds to 4 g ephedrine/day or 46 g Ma huang/day for a 70 kg human (Law et al., 1996).

Given the limits of the mutagenicity studies in bacteria available and the absence of any reliable information on genotoxicity in mammalian cells, the Panel concluded that the genotoxicity of Ephedra herb could not be assessed.

Ephedrine sulphate was negative for mutagenicity in *S. typhimurium* with and without S_9 (NTP, 1986).

Ephedrine sulphate was tested for carcinogenic activity in a 103-week feeding study in $B6C3F_1$ mice and Fischer344 rats (NTP, 1986). The compound was administered at 0, 125 or 250 mg/kg diet (corresponding to 0, 19 or 37.5 mg/kg bw/day in mice and 0, 6.25 or 12.5 mg/kg bw/day in rats) to 50 animals/sex/group. No evidence of a tumourigenic effect was observed.

Ephedrine was administered to chick embryos (Hamburger-Hamilton developmental stage 19) either alone or together with caffeine at doses where each compound alone caused minimal embryotoxicity (Nishikawa et al., 1985). The Panel concluded that this type of study was inadequate for the assessment of possible reproductive and developmental effects in humans.

Relevant experimental data on genotoxicity, short-term, long-term and reproductive and developmental toxicity for pseudoephedrine were not available.

Reviews of human case reports described adverse cardiovascular and cerebrovascular events, including hypertension, palpitations, tachycardia, arrhythmias, myocardial infarction, cardiac sudden death, stroke and intracerebral haemorrhage, after intake of Ephedra herb containing food supplements (Haller and Benowitz, 2000; Samenuk et al., 2002; Shekelle et al., 2003). These are consistent with the adverse effects associated with the therapeutic use of medicinal products containing isolated ephedra alkaloids as single active ingredients (see Table 6).

The Panel noted that in most of the clinical studies in which preparations of Ephedra herb were investigated, cardiovascular effects (increased heart rate, increased blood pressure) were reported. For preparations of Ephedra herb, no studies without concomitant caffeine administration were identified. The lowest dose with an effect on cardiovascular and central nervous system was *Ephedra* herb extract corresponding to 23.7 mg/day ephedrine in combination with caffeine (Haller et al., 2002). In the studies with ephedrine itself, effects were seen at oral doses of approximately 20 mg/person/day without and with caffeine in a dose of about 200 mg and higher (Astrup et al., 1992, Persky et al., 2004). Thus in human studies, the lowest dose of ephedrine tested, with and without caffeine, which produced an effect was 20 mg/person/day. An exception is the study of Molnár (2000) in which 100 mg ephedrine (origin unknown) combined with 10 mg caffeine did not influence haemodynamic parameters in children.

(+)-Pseudoephedrine acts directly on α-adrenoreceptors, and to a lesser degree on β-adrenoreceptors, and releases noradrenaline from storage sites.

Data for (+)-pseudoephedrine indicated that it was less potent than (-)-ephedrine at increasing blood pressure and inducing bronchodilation.

In the study by Drew et al. (1968) the dosage of 210 mg (+)-pseudoephedrine (3.2 mg/kg bw) had approximately the same pressor effect as the dosage of 60 mg (-)-ephedrine (0.9 mg/kg bw).

Norephedrine is used therapeutically only in the form of the racemate (±)-norephedrine (Phenylpropanolamine Hydrochloride Ph. Eur., CAS-No. 154-41-6) (EDQM, 2011; Sweetman, 2011). While a variety of clinical studies described unwanted side-effects for the racemate (±)-norephedrine and its intake as a component of appetite suppressants has been associated with an increased risk of haemorrhagic stroke for women (e.g. Kernan et al., 2000; Salerno et al., 2005; Horwitz et al., 2007; Sweetman, 2011), no relevant case reports and studies on adverse effects referring to the isolated intake of (-)-norephedrine which is the naturally occurring stereoisomer were available. But from investigations of Stockley et al. (1994) it can be concluded that the effect of the racemate on blood pressure is essentially attributable to (-)-norephedrine and that pharmacokinetic factors do not contribute to the differences in the cardiovascular effects of norephedrine enantiomers.

Cases of severe pulmonary arterial hypertension, often with fatal outcome, have been reported associated with the intake of (+)-norpseudoephedrine (cathine) for a supportive treatment in overweight patients (no details available).

CNS effects seen with (+)-norpseudoephedrine are less expressed with (-)-ephedrine (Kalix, 1991).

Overall the Panel noted the following:

GENERAL:

- Botanicals and botanical preparations, for use in food supplements should be evaluated based on origin (e.g. species), chemical specifications and existing toxicological data.

- The term "Ephedra herb" comprises herbs which may derive from up to 66 different *Ephedra* species showing widely varying total and individual alkaloid contents.

- There is a lack of standardisation of the Ephedra herb preparations used with regard to the ratio of extracted material to starting material, alkaloid content or content of other biologically active ingredients.

- While the sympathomimetic effects of the single ephedra alkaloids are based on the same mode of action, there are quantitative differences in their peripheral and central effects.

- Dose effect relationships for different target organs are difficult to predict for individual Ephedra herb preparations, due to the very limited data available.

CHEMICAL DATA:

- Analytical data from the literature show that ephedrine is the major ephedra alkaloid representing more than 50 % of total alkaloids in food supplements containing Ephedra herb. Pseudoephedrine mainly ranged between 10 and 50 % of total alkaloids whereas more than 60 % of the samples did not contain detectable levels of methylephedrine, methylpseudoephedrine, norephedrine and norpseudoephedrine. Among these substances only methylephedrine (in 20 samples) resulted to contribute to total alkaloids more than 20 %.

- Some studies reported the lack of occurrence of ephedra alkaloids in certain *Ephedra* species, however detailed investigations using state of the art methods of analysis and including a variety of samples within each species were not available to support these claims.

- Qualitative and quantitative data for other biologically active components in Ephedra herb preparations in food supplements were not available but would be required for the evaluation, e.g. for certain glycans (ephedran A, B, C, D and E) which have been shown to significantly reduce blood glucose levels in diabetic mice, and for ephedroxane which may potentiate the action of noradrenaline in the catecholaminergic nervous system.

MEDICINAL USE:

- According to EMA no single ingredient (traditional) herbal medicinal products containing Ephedra herb or its preparations appear to be authorised or registered within the EU. No assessment report on safety and efficacy was available or currently expected from the Committee on Herbal Medicinal Products (HMPC) since Ephedra herb is not on the HMPC priority list. This lack of priority was a consequence of the known risks associated with the use of Ephedra herb and its preparations as herbal medicinal product.

- In European Member States authorised medicinal products for oral use exist containing as single active ingredients:

  (-)-ephedrine (single doses: 15-60 mg, daily doses: 40-200 mg),

  (+)-pseudoephedrine (single dose: 60 mg; in delayed release formulations: 120 mg; maximal daily dose: 240 mg), or

  (+)-norpseudoephedrine (single doses: 10-30 mg, daily doses: 30-40 mg).

  Duration of treatment with these medicinal products is generally restricted.

  Ephedrine may also be used as the racemate ((±)-ephedrine), and norephedrine is only used in the form of the racemate ((±)-norephedrine) therapeutically.

- The (+)- and (-)-enantiomers of the individual ephedra alkaloids differ in their pharmacological and toxicological potencies and only limited data are available on their relative potencies for different target organs. Therefore only results obtained with the enantiomeric forms of the individual ephedra alkaloids found in nature and not results obtained with the racemates should be used to describe dose response relationships of the naturally occurring ephedra alkaloids.

FOOD USE AND EXPOSURE ESTIMATES

- In the published studies considered for the exposure assessment, the mean content of total ephedra alkaloids in food supplements (capsules and tablets) ranged from 11.5 to 26.8 mg per serving, and the maximum content amounted to 75.5 mg total ephedra alkaloids per serving.

- The mean content of the main alkaloid (-)-ephedrine in food supplements (capsules and tablets) ranged from 8.4 to 18.3 mg per serving, and the maximum content amounted to 65.8 mg per serving.

- Estimated exposure of total ephedra alkaloids resulting from food supplement use of Ephedra herb and its preparations would fall within or may exceed the therapeutic dose range for the individual ephedra alkaloids in medicinal products.

- Estimated exposure of (-)-ephedrine alone resulting from food supplement use of Ephedra herb and its preparations would fall within or may exceed the therapeutic dose range of ephedrine when used as a single active ingredient in medicinal products.

ADME:

- When ephedrine was administered to humans in the form of an Ephedra extract (Ma huang) in combination with other botanicals the absorption and disposition characteristics were similar to those observed for an equivalent oral dose of ephedrine given as a single substance.

- When orally administered as a single substance, ephedrine was completely absorbed, widely distributed in the body and metabolised by N-demethylation, oxidative deamination of the side-chain and conjugation. Unchanged ephedrine was the major urinary excretory product (53-74 % of the dose).

TOXICOLOGICAL DATA:

- Several *in vivo* and *in vitro* toxicity studies with Ephedra herbs or their preparations were of limited relevance due to the lack of an indication of the *Ephedra* species from which the botanical/botanical preparation being tested originate and the lack of specification of the individual ephedra alkaloid contents.

- No data were available on subchronic or chronic toxicity and carcinogenicity of Ephedra herb or its preparations and of pseudoephedrine.

- Mice and rats exposed to ephedrine sulphate via the feed at doses up to 750 mg/kg bw/day (mice) or 200 mg/kg bw/day (rats) for 13 weeks exhibited hyperactivity and excitability with the highest incidence in mice at doses of 150 mg/kg bw/day and higher and in rats at doses of 100 mg/kg bw/day and higher. In both species, no compound-related histopathological changes were found. Compound-related reduced weight gain was observed in each sex from 75 mg/kg bw/day (mice) or 50 mg/kg bw/day (rats) onwards.

- No adequate data on the genotoxicity of Ephedra herb, its preparations or pseudoephedrine were available. Ephedrine sulphate was negative in a limited Ames test with and without metabolic activation.

- Regarding the evaluation of possible reproductive and developmental effects, the only data available were derived from a chick embryo study with ephedrine, which is insufficient by itself for risk assessment in humans.

- Ephedrine sulphate did not demonstrate a carcinogenic activity in 103-week feeding studies in mice and rats.

HUMAN CASE REPORTS AND CLINICAL STUDIES:

- Eighty-seven adverse events reported to FDA between June 1997 and March 1999 were interpreted to be definitively, probably, or possibly related to the use of dietary supplement preparations containing ephedra alkaloids (including combination products containing caffeine). Ten among them resulted in death and 13 in permanent impairment. The reported adverse events were considered indicative of severe side-effects of ephedra alkaloids-containing food supplements. Hypertension was the most frequently observed cardiovascular symptom, followed by palpitation and cardiac arrest/sudden death. Under the cerebral events, stroke and seizures were among the frequently observed events.

- There were limited data from clinical studies on Ephedra herbs and their extracts, and no data on individual ephedra alkaloids other than ephedrine and pseudoephedrine.

- The Panel noted that the cardiovascular and cerebrovascular events, reported as adverse effects after intake of Ephedra herb containing food supplements were consistent with those described for the therapeutic use of single ingredient medicinal products containing isolated ephedra alkaloids.

- Cardiovascular effects, such as increased heart rate and increased blood pressure, were noted in most clinical studies of oral ephedrine with or without caffeine or Ephedra herb preparations with caffeine (no studies without concomitant caffeine administration were identified). Clinical data demonstrated that pseudoephedrine was less potent at increasing blood pressure than ephedrine.

- Individuals with underlying cardiovascular diseases, that might be unrecognised, or other conditions as described as contraindications for medical uses of ephedra alkaloids might be vulnerable subgroups of the population.

- Caffeine, which is often used in combination with Ephedra herbs or its preparations in food supplements could enhance the cardiovascular and central nervous system effects of ephedrine and other ephedra alkaloids.

CONCLUSIONS

The present opinion deals with the safety of the herbs from *Ephedra* species and preparations made from them when used in food, e.g. in the form of food supplements.

There is wide variation in the concentrations of the individual ephedra alkaloids within *Ephedra* species, and in preparations made from them for use in food. There were no adequately specified individual preparations of Ephedra herbs for which sufficient toxicological data were available for hazard characterisation. In particular there is an absence of data on genotoxicity, subchronic toxicity, carcinogenicity, and reproductive and developmental toxicity.

The Panel concluded that according to the Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA SC, 2009) Ephedra herb and its preparations belong to the category of botanicals and botanical preparations for which the available data are not sufficient to conclude that there is no safety concern (Level A safety assessment based on available knowledge).

Overall, the absence of adequate data on genotoxicity, short-term, long-term and reproductive and developmental toxicity does not enable the Panel to provide advice on a daily intake of *Ephedra* species or preparations made thereof that does not give rise to concerns about harmful effects to health.

The Panel considered that intake of Ephedra herb or its preparations via food supplements, could lead to estimated exposure to ephedra alkaloids within or above the range of therapeutic doses. Such an exposure could lead to severe adverse effects on cardiovascular and central nervous systems, which may be enhanced in combination with caffeine. The Panel further noted that for medicinal products a potential for tolerance to and dependency on ephedra alkaloids was identified which would be applicable to food supplements and could lead to significant potential for misuse. The Panel also noted that a series of severe cases of adverse effects including fatalities was reported to have a definitive, probable, or possible relationship with the intake of ephedra alkaloids containing food supplements.

Moreover, despite the data gaps identified in the toxicity database, the available information on the pharmacological and toxicological profile and the severe adverse events reported in humans were judged by the Panel to be sufficient to conclude that Ephedra herb and its preparations containing ephedra alkaloids used as food supplements were of significant safety concern at the estimated use levels.

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ABBREVIATIONS

ANS:	Scientific Panel on Food Additives and Nutrient Sources added to Food
AUC:	area under the curve
BfR:	German Federal Institute for Risk Assessment
C_{max}:	Maximal plasma concentration
CNS:	central nervous system
EEa:	European Economic Area
EFSA:	European Food Safety Authority
EHIA:	European Herbal Infusions Association
ERNA:	European Responsible Nutrition Alliance
EMA:	European Medicines Agency
FDA:	US Food and Drug Administration
GC-MS:	gas chromatography-mass spectrometry
HMPC:	Committee on Herbal Medicinal Products of the EMA
HPCE:	high performance capillary electrophoresis
HPLC/PDA:	high performance liquid chromatography with photodiode array detection
HPLC-UV:	high performance liquid chromatography with UV detection
LC-MS/MS:	liquid chromatography-tandem mass spectrometry
MAO:	mono amino oxidase
MAP:	mean arterial blood pressure
OTC:	over-the-counter
PCR:	polymerase chain reaction
PK/PD:	pharmacokinetic/pharmacodynamic
RD_{50}:	response-dose 50
RSD_r:	repeatibility standard deviation
RSD_R:	reproducibility standard deviation
UL:	tolerable upper intake level
UPLC:	ultra performance liquid chromatography (
WADA:	World Anti-Doping Agency