The Director General

Maisons-Alfort, 14 March 2014

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the risks associated with the presence in food supplements of *p*-synephrine or ingredients obtained from *Citrus* spp. fruits containing this substance

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 14 March 2014 shall prevail.

On 6 August 2012, ANSES issued an internal request to conduct the following expert appraisal: Risks associated with the presence in food supplements of *p*-synephrine or ingredients obtained from *Citrus* spp. fruits containing this substance.

1. BACKGROUND AND PURPOSE OF THE REQUEST

The emergence in the general population of real or perceived overweight and obesity has led to a considerable increase in the consumption of food supplements claiming to reduce body fat and correct body composition. Some of these contain *p*-synephrine as well as other ingredients obtained from *Citrus* spp. fruits. In addition, given the recommendations to take regular physical exercise in order to combat overweight, some overweight subjects may combine consumption of these food supplements with physical exercise.

P-synephrine is found in the peel (epicarp and mesocarp) of bitter oranges (*Citrus aurantium* ssp. *aurantium)* and other species of *Citrus*.

Forty reports of adverse effects possibly related to the consumption of food supplements containing an ingredient obtained from *Citrus* spp. fruits that are sources of *p*-synephrine have been brought to ANSES's attention since its nutrivigilance scheme was set up in 2009. Only 18 of these reports were deemed admissible and contained enough information to be analysed.
In this context, on 6 August 2012, ANSES issued an internal request to investigate the risks associated with the presence in food supplements of *p*-synephrine or ingredients obtained from *Citrus* spp. fruits containing this substance. This expert appraisal was based on an analysis of the literature and of the cases reported within the nutrivigilance scheme.
In the analysis carried out, the effect of synephrine and *Citrus* spp. fruits on weight loss was not assessed.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French standard NF X 50-110 “Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)”.

ANSES entrusted the expert appraisal to the permanent Working Group on Nutrivigilance and to external rapporteurs. The methodological and scientific aspects of the work were discussed by the Working Group at meetings on 13 December 2012, 8 January, 11 February, 8 October and 10 December 2013. The conclusions were adopted by the Working Group on Nutrivigilance on 10 December 2013. The Working Group's conclusions were presented for discussion to the Expert Committee (CES) on Human Nutrition at its meetings on 21 February, 21 March and 19 December 2013, then validated on 30 January 2014. The composition of the expert groups involved is provided in Annex 1.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts’ declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. *Citrus* spp. and synephrine

3.1.1. Synephrine

Current use of the fruit *Citrus aurantium* var. *aurantium* L. or bitter orange (Neroli/bitter/sour/Seville/bigarade orange; syn. *Citrus aurantium* L. ssp. *amara* Engl.) as a source of ingredients for food supplements is due to the presence of synephrine. This proto-alkaloid is found in several species of the genus *Citrus* in combination with other tyrosine derivatives, mainly tyramine, *N*-methyltyramine and octopamine (Wheaton et Stewart 1969). It falls within the more general class of sympathomimetics, which also include octopamine, hordenine and phenylephrine (*m*-synephrine).

There are three isomers of synephrine, according to the position of the hydroxyl group on the benzene ring: in the *ortho* (*o*-synephrine), *meta* (*m*-synephrine) or *para* (*p*-synephrine) position (Figure 1). The compound isolated from *Citrus* species is *p*-synephrine (also called oxedrine).

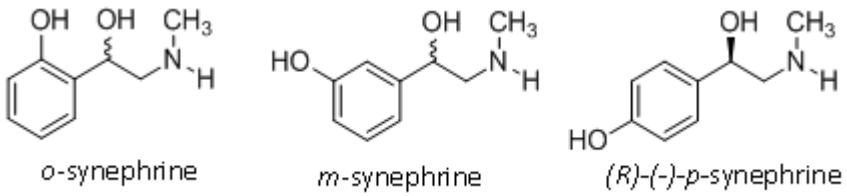

Figure 1: Structures of the synephrines

M-synephrine (also called phenylephrine or neosynephrine) has never been detected in the genus *Citrus.* Its presence has nevertheless been demonstrated in food supplements containing *C. aurantium* extracts (Allison *et al.* 2005). This may be the result of a fraudulent addition.
There is no *o*-synephrine of natural origin.
When this information was available in the literature articles analysed for this expert appraisal, the isomer is specified. The term "synephrine" is used when the article did not state the nature of the isomer. By default, when a publication did not state the isomer but used a *Citrus* extract, the CES assumed that it was *p*-synephrine.
There are two enantiomers of *p*-synephrine. The natural *p*-synephrine isolated from *Citrus* fruits is (*R*)-(-)-*p*-synephrine. Epimerisation of natural *p*-synephrine can be observed during extraction processes combining a high temperature (80°C) with alkaline or acidic pH conditions (Pellati et Benvenuti 2007; Pellati *et al.* 2010). The two forms have also been identified in urine after ingestion of products containing *p*-synephrine (Kusu *et*

al. 1996). Synthetic *p*-synephrine is a racemic mixture. The two enantiomers of *p*-synephrine do not have the same affinity for adrenergic receptors (Jordan *et al.* 1987).

3.1.2. Species of *Citrus* that are sources of *p*-synephrine

P-synephrine has been quantified in a large number of species and hybrids of the genus *Citrus* (Rutaceae): *Citrus aurantium* var. *aurantium* L. (bitter orange); *C. sinensis* L. Osbeck (sweet orange); *C. deliciosa* Ten. (tangerine); *C. limon* L. Burm. f. (lemon); *C. reticulata* Blanco (mandarin); *C. reshni* hort. ex Tanaka (Cleopatra mandarin); *C. unshiu* (Satsuma mandarin); *C. hassaku* hort. ex Tanaka; *Poncitrus trifoliata* (trifoliate orange); *Fortunella* spp.; *Trovia* × *C. unshiu* Marc. × *C. reticulata* Blanco; *C. unshiu* Marc. × *C. clementina* Hort. (ex Tanaka) × Xizixiang; *C. sinensis* L. × *C. unshiu* Marc.; *C. unshiu* Marc. × *C. clementina* Hort. ex Tanaka; (Trovia × (*C. unshiu* Marc. × *C. unshiu* Marc.) × Pécs orange) (Arbo *et al.* 2008; Avula *et al.* 2005; He *et al.* 2011; Kusu *et al.* 1992; Pellati et Benvenuti 2007; Pellati *et al.* 2004; Pellati *et al.* 2005; Pellati *et al.* 2002; Wheaton et Stewart 1969; Wheaton et Stewart 1970).
It has also been found in some Amaryllidaceae (Wheaton et Stewart 1970).
However, some *Citrus* such as grapefruits (*C. paradisi*), citrons (*C. medica*) and pomelos (*C. maxima*) do not produce *p*-synephrine (Avula *et al.* 2005; Bartley *et al.* 2010).
The part of the fruit used is commonly referred to by the term "peel". Botanically speaking, this part of the fruit can be likened to the epicarp and at least part of the mesocarp, with the term pericarp designating the whole fruit excluding the seeds.

3.1.2.1. *P*-synephrine levels in *Citrus* spp.

- *Levels in fresh fruit and leaves*

Depending on the species, the regions of origin and the maturity of the fruits, the *p*-synephrine levels reported in the literature vary considerably (Annex 2).
In whole fresh fruit the levels measured vary from 0.019 to 1.97 mg/g (Arbo *et al.* 2008; Wheaton et Stewart 1969; Wheaton et Stewart 1970). A recent study reported *p*-synephrine levels that varied from 0.01 to 1.02 mg/g in the peel and from 0.007 to 0.054 mg/g in the pulp of different hybrids of the genus *Citrus* (He *et al.* 2011). Lastly, Wheaton and Steward showed that the *p*-synephrine levels in the leaves of different *Citrus* can reach 2.2 mg/g (Wheaton et Stewart 1969; Wheaton et Stewart 1970).

- *Levels in dried fruit*

Several studies have reported *p*-synephrine levels in dried fruit from several species of *Citrus* (Annex 3). Concentrations vary from 0.11 to 6.9 mg/g depending on the species. The highest concentrations have been measured in immature fruit (Avula *et al.* 2005; Kusu *et al.* 1992; Pellati et Benvenuti 2007; Pellati *et al.* 2004; Pellati *et al.* 2005; Pellati *et al.* 2002; Vieira *et al.* 2007).

- *Levels in fruit juices and jams from* Citrus *spp.*

Synephrine intake via the juices of certain *Citrus* spp. fruits is non-negligible (Annex 4). Indeed, studies have reported *p*-synephrine levels that can reach 160 mg/L in Satsuma mandarin juice (*C. unshiu* Markovich) (Dragull *et al.* 2008) and 280 mg/L for Cleopatra mandarin (*C. reshni* hort. ex Tanaka) (Wheaton et Stewart 1965). Other related amines may be detected in variable but minority concentrations, especially tyramine and octopamine, in orange, lemon and mandarin juices (Mattoli *et al.* 2005; Uckoo *et al.* 2011; Vieira *et al.* 2007; Wheaton et Stewart 1965). Concentrations in jams range from 0.9 to 121 mg/kg (Avula *et al.* 2007; Kusu *et al.* 1996).

- *Levels in dried extracts*

Some studies mention the levels of *p*-synephrine and other proto-alkaloids contained in certain *Citrus aurantium* L extracts (Annex 5). The *p*-synephrine levels vary from 25 to 65 mg/g and the presence of tyramine and octopamine is specified in some samples (Pellati et Benvenuti 2007; Pellati *et al.* 2004; Pellati *et al.* 2002).

3.1.2.2. Other compounds found in *Citrus*

- *Coumarins*

Chuang *et al.* (2007) report the presence of several coumarins in the dried fruit of *C. aurantium*: umbelliferone (0.28-0.56 mg/g) and imperatorin (0.12-0.88 mg/g). The presence of 6',7'-dihydroxybergamottin, an inhibitor of cytochrome P450 3A4, has also been reported (Edwards *et al.* 1999).

- *Flavonoids*

Naringin and neohesperidin are the two most abundant flavonoids isolated from *C. aurantium.* Their levels vary from 17.3 to 43.6 mg/g of dried fruit for naringin, and from 15.6 to 52 mg/g for neohesperidin (Avula *et al.* 2005; Chuang *et al.* 2007). The fruit of *Citrus aurantium* L. also contains polymethoxylated flavones (Avula *et al.* 2005).

- *Volatile compounds found in the peel of Citrus spp.*

The volatile fraction of the essential oil obtained by cold pressing the peel of *Citrus* spp. mainly contains limonene (93.5 to 93.6% of commercial essential oils). The low-volatile compounds detected in essential oil are coumarins (meranzin, isomeranzin, bergapten), psoralens (8-geranyloxypsoralen) and polymethoxylated flavones (Dugo et Mondello 2010). *P*-synephrine has not been detected in essential oil, whether using gas chromatography or other techniques (Dugo *et al.* 1996).

3.1.3.*P*-synephrine levels in food supplements

The *p*-synephrine content in food supplements is highly variable and often not documented. Thus, the food supplements involved in the nutrivigilance cases detailed in Section 3.5.1.1 provide between 1 and 72 mg of *p*-synephrine per day at the doses recommended by the manufacturer. The clinical studies conducted with food supplements have identified daily *p*-synephrine doses that do not generally exceed 100 mg/d (Annex 7). Some studies state the levels of *p*-synephrine and other proto-alkaloids in certain food supplements available on the market (Annex 6). The *p*-synephrine levels in these studies vary between 0.49 and 27.41 mg/g. The presence of tyramine and octopamine has been shown in some samples. Nevertheless, these articles do not specify the daily doses recommended by the manufacturers for the food supplements studied, which prevents a daily dose of *p*-synephrine from being determined (Avula *et al.* 2005; Pellati et Benvenuti 2007; Pellati *et al.* 2004; Pellati *et al.* 2005; Pellati *et al.* 2002).

3.1.4.Presence of synephrine in medicinal products

Currently, only *m*-synephrine is found in some medicinal products in France, such as mydriatic eye-drops or OTC nasal decongestants (Vidal 2012).
In Germany and Switzerland, a formulation (oral solution) containing *p*-synephrine tartrate (Sympalept®) is available on medical prescription as a cardiovascular tonic, with a dosage in adults of 100-150 mg three times a day, and in children of 15-25 mg three times a day (children 3 months old), 50-75 mg three times a day (children 1 to 4 years old) and 75-100 mg three times a day (children 4 to 10 years old).

3.2. Pharmacology and pharmacological interactions

3.2.1. *P*-synephrine

P-synephrine, octopamine and tyramine are regarded as endogenous trace amines as their concentrations in animals are very low. The physiological production of *p*-synephrine in animals, from tyramine, follows the same biosynthetic pathway as in *Citrus* spp. (Rossato *et al.* 2011b).
P-synephrine is an adrenergic receptor agonist. There are several types and subtypes of these receptors:

- α1 receptors control the contraction of arterial smooth muscle;
- α2 receptors control arterial pressure centrally and at a peripheral level control energy metabolism, including lipolysis;
- β1 receptors regulate cardiac tropism and β2 receptors regulate vascular and bronchial tropism;
- β3 receptors control energy metabolism, including lipolysis and thermogenesis.

Hibino *et al.* (2009) assessed contraction of isolated rat aorta by *p*-synephrine at concentrations of 10^{-7} and 3.10^{-5} µMr. They concluded that this effect was mainly related to binding with α1 adrenergic receptors. Nevertheless, *p*-synephrine generally has a much lower affinity (10 to 10^4 lower) than noradrenaline for all the adrenergic receptors (Ma *et al.* 2010). Consequently, a significant vasoconstrictor effect related to direct stimulation of these receptors is unlikely.

In addition, a 2011 study demonstrated a difference in activity between the *para* and *meta* isomers, expressed as a decrease in intracellular glutathione, in reduced and total form, induced by exposure of rat cardiomyocytes to a concentration of 1 mM of m-synephrine for three hours, whereas exposure to the same concentration of *p*-synephrine caused no change to glutathione stock (Rossato *et al.* 2011a) cited by Rossato *et al.* (2011b).

3.2.2. Other constituents of the peel of *Citrus* spp.

Naringenin (resulting from the intestinal hydrolysis of naringin, a majority flavanone glycoside of the peel of *Citrus aurantium* L. fruit) is a cardiac hERG (human *Ether-à-go-go* Related Gene) channel blocker. This effect has been associated with a lengthening of the QT interval observed during the administration of grapefruit juice in healthy subjects (Scholz *et al.* 2005).

3.2.3. Interaction with caffeine

P-synephrine may alter caffeine's inhibitory effect on baroreflex sensitivity (Mosqueda-Garcia *et al.* 1990). In a study on a small number of subjects (n=10), a comparison was made between a *C. aurantium* extract containing 46.9 mg of *p*-synephrine and a mixture containing, among others, 5.5 mg of *p*-synephrine and 239.2 mg of caffeine. An increase in blood pressure (systolic and diastolic) was observed with the mixture but not with the extract, despite it containing *p*-synephrine doses that were eight times higher. This effect can be explained by the presence of caffeine and other components in the mixture (tyramine, theobromine, 2-dimethylaminoethanol, *L*-tyrosine, plant sources of caffeine: maté, green tea, etc.) (Haller *et al.* 2005). Lastly, in a 28-day study in rats, Hansen *et al.* (2012) showed that the weak cardiovascular effects of *p*-synephrine alone are exacerbated during concomitant caffeine administration.

3.3. Pharmacokinetics and pharmacokinetic interactions

3.3.1. Pharmacokinetics

P-synephrine is poorly absorbed in the digestive tract. Haller *et al.* (2005) report that the oral administration of a food supplement containing only a *C. aurantium* extract and corresponding to 46.9 mg of *p*-synephrine leads to a peak plasma concentration of 2.85 ng/mL reached in 90-120 minutes with a half life of 2.5 to 3 hours. In a second study, this team stated that ingestion of a dose of 21 mg of *p*-synephrine in adults, engaging in moderate physical activity leads to plasma levels below 2 ng/mL (Haller *et al.* 2008).
Suzuki *et al.* (1979) showed that *p*-synephrine and *p*-octopamine are substrates of types A and B monoamine oxidases (MAO). They are metabolised in humans and rats into *p*-hydroxymandelic acid then into *p*-hydroxyphenylglycol in free, glucuronide- or sulfo-conjugated forms, which can be detected in urine (Crowley *et al.* 1982; Ibrahim *et al.* 1983).
No information is available on the extent to which it is secreted in human milk.

3.3.2. Interaction with medicinal products

Interactions can be observed between *Citrus* spp. extracts and drugs belonging to various pharmacological classes (beta blockers, antidepressants, calcium-channel blockers, antiarrhythmic agents, oestradiol, angiotensin II antagonists, cyclosporin, etc.) that may have their plasma concentrations increased significantly with a risk of overdose (Di Marco *et al.* 2002; Hou *et al.* 2000; Malhotra *et al.* 2001; Penzak *et al.* 2002). This effect may be related to the ability of *Citrus* spp. extracts to inhibit the CYP450 3A4/*P*-gP pair, inducing a risk of interactions between *Citrus* spp. extracts and the drugs metabolised or transported by these proteins. Some flavonoids (naringin and naringenin) and furanocoumarins (bergamottin) are indeed described as possibly having such inhibitory properties (Edwards *et al.* 1999; Gurley *et al.* 2004; Saito *et al.* 2005). However, synephrine has no action on *P*-glycoprotein (*P*-gP) nor on the Multidrug Resistance Protein 1 (MRP1) (Nabekura *et al.* 2008).

Metabolisation of synephrine by monoamine oxidases (MAO) leads to a risk of hypertensive crisis when combined with an MAO inhibitor (MAOI). A case of interaction between a food supplement containing *p*-synephrine and an MAOI (phenelzine) was indeed described by Simmons et Schneir (2010). Moreover, this metabolisation by MAOs causes a decrease in the catabolism of other monoamines and therefore an increase in their circulating levels.
Conversely, although less frequently, in some cases *Citrus* spp. extracts reduce the plasma concentrations of drugs such as certain antifungals (itraconazole) or antihistamines (fexofenadine), thereby diminishing their efficacy. This effect can last up to 24 hours (Bressler 2006; Lim *et al.* 2003).

3.4. Toxicology

3.4.1. Acute toxicity

Lagarto Parra *et al.* (2001) calculated an LD_{50} of 477 mg/kg for a *C. aurantium* extract administered orally to mice, but the type of extract used was not specified, nor its *p*-synephrine content. A study conducted in male CF1 mice showed that oral administration of C. *aurantium* extract (containing 2.5% of *p*-synephrine) led to a decrease in locomotor activity in animals treated with the extract starting at a dose of 1000 mg/kg (expressed in mg of extract) and in animals treated with *p*-synephrine starting at a dose of 300 mg/kg (expressed in mg of *p*-synephrine). The animals treated with *p*-synephrine also developed other effects such as respiratory distress and hypersalivation starting at a dose of 150 mg/kg, as well as piloerection and proptosis starting at a dose of 300 mg/kg. These effects, which are reversible, appeared 15 minutes after administration and persisted for two to four hours (Arbo *et al.* 2008).

3.4.2. Chronic toxicity

The animal toxicity studies are rather short: 28 days in mice (Arbo *et al.* 2009b), and varying between 15 days (Calapai *et al.* 1999) and 28 days (Hansen *et al.* 2012) in rats. These studies have many methodological shortcomings (for example, a single sex, an insufficient number of animals, lack of or insufficient histological examinations, type of isomer not always stated). The substances administered are highly disparate, ranging from *p*-synephrine alone (Arbo *et al.* 2009b) to *C. aurantium* extracts (Calapai *et al.* 1999; Hansen *et al.* 2012). In most cases, the amount of *p*-synephrine actually administered is not known precisely. These studies do not have a systemic aim and are only focused on the examination of certain cardiovascular parameters. Moreover, the main species used (the rat) is much less representative than the dog for studying this type of effect.

Calapai *et al.* (1999) administered hydroalcoholic extracts of *C. aurantium* fruits, containing up to 6% synephrine, to male Sprague-Dawley rats for 15 days at doses of 2.5; 5; 10 and 20 mg/kg/d. No effect on blood pressure was observed compared to the control group. However, ventricular arrhythmias and a prolongation of the QRS interval were reported starting at five days of treatment at the dose of 20 mg/kg/d (expressed in mg of 6% extract, or 1.2 mg/kg/d of synephrine).

In another study, female Sprague-Dawley rats received *C. aurantium* extracts titrated at 7.25% and 95% for 28 days, each administered at dilutions ensuring exposure to *p*-synephrine doses of 10 or 50 mg/kg/d. An increase in systolic pressure was observed with *p*-synephrine doses of 50 mg/kg/d, regardless of the extract, and an increase in diastolic pressure with the *p*-synephrine dose of 50 mg/kg/d was obtained from the 7.25% extract. An increase in heart rate was also observed with the *p*-synephrine dose of 10 mg/kg/d, regardless of the extract, but this was not confirmed at 50 mg/kg/d for the 95% extract. Furthermore, none of the extracts modified the length of the QT interval (Hansen *et al.* 2012).

Lastly, a 28-day toxicity study conducted in mice (CF1) found no significant change in biochemical and haematological parameters, except for a decrease in haematocrit in mice that had received doses of *C. aurantium* extracts (containing 7.5% *p*-synephrine) of 400 mg/kg/d (i.e. a *p*-synephrine dose of 30 mg/kg/d) and for a decrease in total proteins in mice that had also received a 99% pure *p*-synephrine dose of 30 mg/kg/d. However, these effects were not dose-dependent since the groups treated with the highest doses (up to 4000 mg/kg/d of extract [or 300 mg of *p*-synephrine/kg/d] and up to 300 mg/kg/d of 99% pure *p*-synephrine) did not exhibit statistically significant decreases. Cardiovascular parameters were not, however, explored in this study (Arbo *et al.* 2009b) .

In view of these various results, it is difficult to define a systemic or cardiovascular no observed adverse effect level (NOAEL) for *p*-synephrine. In addition, some studies very clearly show the potentiating role of caffeine (Haller *et al.* 2005; Hansen *et al.* 2012). Octopamine has not undergone any toxicity studies.

3.4.3. Genotoxicity

The available data are fragmentary and insufficient.

A mouse lymphoma assay was performed on L5178Y cells. Racemic *p*-synephrine is negative in this test up to the concentration of 2 mg/ml without metabolic activation, suggesting a lack of mutagenic potential under the study's operating conditions. However, this study is unable to confirm the absence of mutagenic potential of synephrine, since this was not tested in the presence of metabolic activation. Moreover, some biases in the study (mainly related to the dose spacing and the lack of replication of all doses) mean that this absence of a result should be treated with caution (McGregor *et al.* 1988).

No *in vivo* genotoxicity studies are available.

In view of all these factors, it is not possible to conclude as to the lack of genotoxicity of synephrine.

3.4.4. Reprotoxicity

A recent developmental toxicity study was conducted in Sprague-Dawley rats according to GLP. This exposed nine groups of 25 to 26 gravid females between the third and 20th day of gestation to *p*-synephrine doses of 10, 25, 50 or 100 mg/kg/d, from two extracts containing respectively 6% and 90% of synephrine (Hansen *et al.* 2011). In the group treated with the 90% extract at the dose of 100 mg of synephrine/kg/day, there was a decrease in the number of live foetuses per litter which was not found with treatment at the same dose of synephrine with the 6% extract. Conversely, in the group treated with the 6% extract at 100 mg/kg/day, but not in the group treated with the 90% extract at the same dose, two malformations were reported (an exencephaly and an unspecified mass in the thorax), but the authors do not believe them to be related to the treatment. Lastly, skeletal variations affecting the ribs were reported in all treatment groups, but also in the control group at the same frequency. The authors concluded as to a lack of teratogenicity for the tested extracts.

A uterotropic assay was conducted in immature female Wistar rats (aged 21 days) exposed by gavage for three days to 99% pure *p*-synephrine (50 mg/kg/d) or to a methanolic extract of C. *aurantium* containing 3% *p*-synephrine (25 and 50 mg/kg/d, expressed in mg of *p*-synephrine). No significant difference in relative uterine masses was observed compared to the control group, which therefore does not demonstrate any oestrogenic or anti-oestrogenic effect of *p*-synephrine (Arbo *et al.* 2009a).

3.5. Clinical data

Among the references analysed, it is necessary to distinguish cases of adverse effects (cases from the nutrivigilance scheme and cases from the literature) from clinical studies.

3.5.1.Cases of adverse effects

3.5.1.1. Cases from nutrivigilance

Since its nutrivigilance scheme was set up in 2009 and until 31 December 2013, ANSES recorded 40 reports of adverse effects possibly associated with the consumption of food supplements containing an ingredient obtained from *Citrus* spp. fruit that are sources of *p*-synephrine. Eight were received in 2010, six in 2011, 17 in 2012 and nine in 2013. Twenty-two of these reports were considered non-admissible, due to missing information (mainly concerning the dates on which the individuals started/stopped taking the food supplement) or lack of an adverse effect, for instance.
In order to identify the role of *p*-synephrine in the reports received, ANSES analysed the causality of the 18 cases declared admissible by applying the method defined in the ANSES Opinion of 11 May 2011 on the development of a method for determining causality in reports of adverse reactions in nutrivigilance. The causality ratings detailed below concern food supplements containing an ingredient obtained from *Citrus* spp. sources of *p*-synephrine, and do not concern *Citrus* spp. fruits or *p*-synephrine themselves. Moreover, some reports may involve several food supplements with different causality ratings (not detailed here).
Of the 18 cases examined, and for products containing a *Citrus* spp. source of *p*-synephrine:

- causality was considered very likely in one case (I4);
- causality was considered likely in five cases (I3);
- causality was considered possible in seven cases (I2);

- causality was considered doubtful in four cases (I1);
- causality was excluded in one case (I0).

The cases, types of effect and causalities established are detailed in Table 1.

Table 1: Analysis of the causality of adverse effects reported to ANSES involving food supplements containing an ingredient obtained from *Citrus* spp. sources of *p*-synephrine

Reference[1]	Type of effect	Serious effect[2]	Presence of caffeine	Remarks	Chronological (C) and semiological (S) scores	Causality
2012-012	Hyperphosphataemia	No	Yes		C3: timeframe consistent and progression suggestive S3: no other aetiology	very likely
2011-091	Cytolytic hepatitis and pericarditis	Yes	Yes	Presence of green tea	C4: timeframe consistent, progression suggestive and reintroduction positive S1: another possible aetiology	likely
2012-005	Hepatic cytolysis	Yes	Yes	Presence of green tea	C3: timeframe consistent and progression suggestive S1: another possible aetiology	likely
2012-231	Acute anxiety syndrome	No	Yes		C3: timeframe consistent and progression suggestive S1: another possible aetiology	likely
2013-133	Digestive disorders, agitation, insomnia, palpitations	No	Yes	Presence of green tea	C3: timeframe consistent and progression suggestive S1: another possible aetiology	likely
2013-184	Haematoma	No	No		C3: timeframe consistent and progression suggestive S1: another possible aetiology	likely
2010-026	Acute hepatitis	No	Yes	Presence of green tea	C2: timeframe not very consistent and progression suggestive S1: another possible aetiology	possible
2010-027	Myocardial infarction	Yes	Yes		C2: timeframe consistent and progression cannot be interpreted S1: another possible aetiology	possible
2011-037	Myocardial infarction	Yes	Yes		C2: time to onset imprecise and progression suggestive S2: another hypothetical aetiology	possible
2011-083	Bradycardia, convulsion and atrioventricular block	Yes	Yes		C2: timeframe consistent and progression inconclusive S1: another possible aetiology	possible
2012-059	Tachycardia, nausea, pallor of skin and connective tissue	No	Yes		C2: timeframe consistent and progression cannot be interpreted S1: another possible aetiology	possible
2012-124	Vascular purpura	Yes	Yes		C2: timeframe consistent and progression cannot be interpreted S2: another hypothetical aetiology	possible

[1] The numbers correspond to the references of the reports of adverse effects recorded in the nutrivigilance database.
[2] According to the definition of a serious adverse effect given in Article R1323-3 of the French Public Health Code.

Reference[1]	Type of effect	Serious effect[2]	Presence of caffeine	Remarks	Chronological (C) and semiological (S) scores	Causality
2013-174	Haematoma	No	No		C2: timeframe consistent and progression cannot be interpreted S1: another possible aetiology	possible
2010-016	Cytolytic hepatitis	No	Yes	Presence of green tea	C2: timeframe consistent and progression not suggestive S2: another hypothetical aetiology	doubtful
2010-029	Jaundice	No	Yes		C2: timeframe consistent and progression cannot be interpreted S0: other aetiology proven/very probable	doubtful
2011-047	Acute renal failure	Yes	Yes		C2: timeframe consistent and progression inconclusive S0: other aetiology proven/very probable (sarcoidosis)	doubtful
2012-156	Itching, abdominal aches and pains	Yes	No		C1: timeframe consistent and progression not suggestive S2: another hypothetical aetiology	doubtful
2010-025	Hepatitis	No	Yes		C0: timeframe inconsistent	excluded

The case in which causality was very likely (2012-012) concerns a 61-year-old woman without prior medical history, who consumed two slimming food supplements (one a "fat burner" containing an extract of *Citrus aurantium* L.) for at least four months. During a systematic examination in August 2011, blood tests revealed hyperphosphataemia at 1.78 mmol/L, combined with a vitamin D deficiency (8 ng/mL) in an asymptomatic context. The remainder of the examination showed normal renal, thyroid and parathyroid function. A subsequent examination conducted in October 2011 confirmed the hyperphosphataemia (1.81 mmol/L). Referred for a nephrology consultation on 20 December 2011, the subject immediately stopped taking the two food supplements. The next blood test, conducted on 4 January 2012, indicated that serum phosphate levels had returned to normal (1.39 mmol/L).
In view of these data, the time to onset was deemed to be consistent and the progression suggestive on cessation of treatment. In the absence of reintroduction, the chronological score was assessed at C3.
As the classic causes of hyperphosphataemia, i.e. kidney failure or massive cell lysis (such as rhabdomyolysis), have been ruled out, toxicity is the favoured aetiology. The semiological score was therefore assessed at S3.
The combination of these two scores (C3 and S3) gives an intrinsic causality score of I4, described as "very likely".

It should, however, be noted that as consumption of the two products began and ended simultaneously, this score corresponds to their association and not specifically to one or the other.

From a bibliography perspective, the literature does not report any similar cases, and as seen previously, neither clinical nor toxicological studies have examined phosphate levels. The lack of bibliographic data therefore means that it is impossible to confirm or disprove the role of *p*-synephrine in phosphorus metabolism.

While the majority of cases concern people who consumed these products for weight-loss purposes, two of them took these food supplements in a sport context (cases 2011-037 and 2012-059).

In general, the analysis of nutrivigilance cases reported to ANSES and the assessment of the causality of the adverse effect associated with *Citrus* spp. and *p*-synephrine are complicated by:

- the presence in all the products involved of other ingredients, especially caffeine (sometimes at very high doses), or green tea in the case of liver damage;
- the concomitant consumption of other products (food supplements, medicinal products, narcotics, etc.);

- the lack of information in some of the reports (mainly concerning the aetiologies investigated, the patient's medical history, the dosage used).

3.5.1.2. Cases from the literature

Isolated cases of severe adverse effects, mainly of a cardiovascular nature, have been reported in the literature. These are summarised in Annex 8. For these cases, it is rarely possible to unambiguously attribute the reported adverse effects to *Citrus aurantium* L. given the existence of other aetiologies, and sometimes co-morbidities, but mainly because of the complex associations.

Of all the 147 adverse events recorded by the FDA up to June 2004, relating to *Citrus aurantium* L. and its extracts, only one involved a product containing an ingredient derived from C. *aurantium* L. that was not in combination with ephedrine or caffeine. This case concerned a 74-year-old woman presenting with discomfort and oedema of the lower extremities while taking three Chinese herbal products in addition to her multiple chronic medical treatments (McGuffin 2006).

A study conducted by the FDA examined the consumption of food supplements containing *Citrus aurantium* in California. Of 70 people consuming such food supplements, five reported that they had experienced adverse effects; in three cases these effects were considered minor, the other two cases were classified respectively as moderate and severe. This study did not take synephrine levels into account, and the nature of the adverse effects was not reported (Klontz *et al.* 2006).

From 1998 to 2004, Health Canada collected 16 reports of adverse effects suspected of being associated with the presence of *Citrus aurantium* or synephrine. All of these cases were regarded as serious (and included two deaths). In seven cases, the suspect product contained caffeine, and in eight cases, caffeine and ephedrine (the two deaths implicated products with this combination) (Santé Canada 2004). From 2004 to 2006, Health Canada received a further 21 reports of adverse effects potentially associated with the presence of *Citrus aurantium* or synephrine, of which 15 cases concerned cardiovascular effects. Among these 15 cases, 10 were regarded as serious, including one myocardial infarction (Santé Canada 2007).

3.5.2. Clinical studies

The available clinical studies indicate contradictory results on cardiovascular function. The oral studies are detailed in Annex 7.

3.5.2.1. Single oral administration

A single dose of 450 mg of a standardised 6% extract of *p*-synephrine (i.e. 27 mg) in 18 healthy volunteers showed no effect on systolic or diastolic blood pressure or on the length of the QT interval (Min *et al.* 2005).

In a study in healthy adult subjects (n=10 per group), Stohs *et al.* (2011) assessed the effects associated with a single oral dose of 50 mg of *p*-synephrine alone or combined with two flavonoids, hesperidin and naringin. No significant variations in blood pressure or heart rate were observed up to 75 minutes after ingestion.

A study in healthy adult subjects (n=15) using a food supplement (54 mg of *p*-synephrine) showed an increase in systolic blood pressure between 1 and 5 hours after ingestion, and an increase in diastolic pressure between 4 and 5 hours after ingestion compared with the control group that received a placebo. The maximum difference for systolic pressure was 7.3 ± 4.6 mmHg, observed 3 hours after ingestion, while the maximum difference for diastolic pressure was 2.6 ± 3.8 mmHg, observed 5 hours after ingestion. Heart rate accelerated 2 to 5 hours after ingestion with a maximum deviation from the control group of 4.2 ± 4.5 beats per minute (bpm), observed 4 hours after ingestion (Bui *et al.* 2006).

Another study (n=10) compared the cardiovascular effects of two food supplements, one containing a *C. aurantium* extract corresponding to 46.9 mg of *p*-synephrine ("extract" group) and the other containing, among others, 5.5 mg of *p*-synephrine and 239.2 mg of caffeine ("mixture" group). Heart rate increased in both groups receiving the food supplements compared to the control group that received a placebo ("placebo" group). The greatest increase in heart rate was observed 6 hours after ingestion: an increase of 16.7 ± 12.4 bpm in the "mixture" group and 11.4 ± 10.8 bpm in the "extract" group. However, after ingestion, only the "mixture" group presented an increase in systolic and diastolic pressures, of 9.6 ± 6.2 mmHg and 9.1 ± 7.8 mmHg, respectively. Despite *p*-synephrine doses eight times higher than the "mixture" group, the "extract" group showed no increase in blood pressure compared to the "placebo" group. This may be

explained by the presence in the mixture of caffeine and other components (tyramine, theobromine, 2-dimethylaminoethanol, L-tyrosine, maté, green tea, etc.) (Haller *et al.* 2005).

In another study by the same team, seven men and three women ingested a food supplement containing, among others, 21 mg of synephrine and 304 mg of caffeine. Diastolic blood pressure 3 hours after ingestion was higher in this group (mean diastolic pressure of 71.7 ± 8.7 mmHg), than in the subjects who received a placebo (63.0 ± 4.9 mmHg). However, no significant difference was observed between the groups regarding systolic pressure and heart rate (Haller *et al.* 2008).

A single dose of a fortified coffee containing 360 mg of a *C. aurantium* extract containing 6% synephrine (or 21.6 mg of *p*-synephrine) and 450 mg of caffeine, consumed by 10 healthy volunteers (eight men and two women), significantly increased systolic blood pressure compared to consumption of a standard commercial coffee. The mean systolic pressure values in the "fortified coffee" group and in the "standard coffee" group were 118 ± 7 mmHg and 115 ± 8 mmHg, respectively. No effect was shown on heart rate or diastolic blood pressure averaged over the three hours following consumption of the coffee (Hoffman *et al.* 2006).

According to a study in 23 healthy subjects (14 women and 9 men), consumption of four capsules whose composition includes, among others, a *C. aurantium* extract containing 6% of synephrine, guarana and green tea extract (or 52 mg of *p*-synephrine and 704 mg of caffeine) did not result in any quantifiable effect on blood pressure, nor on heart rate, compared with a control group that received a placebo (Seifert *et al.* 2011).

3.5.2.2. Repeated oral administration

Studies after repeated administration were conducted in order to assess the efficacy - and not the safety - of the mixtures tested. Nevertheless, in addition to weight loss, blood pressure and heart rate were also often monitored. Also, studies mentioning adverse effects reported by volunteers are rare.

A placebo-controlled study conducted for 60 days (n=25 per group) compared the effects of an extract titrated in *p*-synephrine (98 mg/day) and a mixture consisting of a *C. aurantium* extract titrated in *p*-synephrine (98 mg/day), hesperidin (1152 mg/day) and naringin (200 mg/day). No significant differences in effects on blood pressure (systolic or diastolic) or on various biological parameters (hepatic, renal or haematopoietic) were found between the different groups. Regarding heart rate, no difference was noted between the group receiving the extract and the one receiving the placebo, although a slight difference was observed between the group receiving the mixture and the two other groups (mixture: 73.1 ± 9.7 bpm; extract: 71.3 ± 12.9 bpm; placebo: 65.5 ± 8.9 bpm). Although this difference is statistically significant, it was not deemed clinically significant by the authors, who concluded that daily consumption of 98 mg of *p*-synephrine for 60 days led to no adverse effects (Kaats *et al.* 2013).

In a placebo-controlled study reported at a conference, conducted on 30 normotensive subjects ingesting 80 mg of *p*-synephrine per day for six weeks, no effect on blood pressure or heart rate was observed. However, the abstract from the study (the only part available) provides no information on the variability of responses, the statistical test used, or the power of the test (Talbott *et al.* 2007).

Colker *et al.* (1999) administered to nine obese volunteers for six weeks a preparation providing 975 mg of a *C. aurantium* extract (containing 6% synephrine, or 58.5 mg/d of *p*-synephrine), 528 mg/d of caffeine and 900 mg of St John's wort. The authors state that they did not find any changes in blood pressure, heart rate, electrocardiogram, liver function (determination of transaminases and γ-GT), kidney function (determination of serum creatinine levels, uraemia, glycosuria and proteinuria) or blood count. However, no quantified values were reported for these parameters.

Similar results concerning blood pressure (systolic and diastolic) and heart rate were observed in an eight-week study in obese subjects (n=19 in the treated group, n=16 in the control group that received a placebo) ingesting six capsules per day, containing a *C. aurantium* extract (36 mg of *p*-synephrine per day) and caffeine provided by maté and guarana. Two adverse effects (nausea and urticaria) were however observed in the treated group (Zenk *et al.* 2005).

Similarly, Kalman *et al.* (2000) did not find any statistically significant differences in cardiac or haemodynamic parameters (systolic and diastolic pressures, heart rate, electrocardiogram), nor in liver (transaminases, γ-GT) and kidney parameters (serum creatinine levels, uraemia) between a group of 12 obese subjects who took capsules for eight weeks providing 10 mg/d of *p*-synephrine, 400 mg/d of caffeine, 40 mg/d ephedrine and 30 mg/d of salicin, and a control group of 13 obese subjects who received a placebo. Only the blood pressure and heart rate values were reported in this study.

A study in 19 obese subjects investigated the effects of administration of two capsules per day of a food supplement providing 5 mg/d of *p*-synephrine (provided by 85 mg of *C. aurantium*), 200 mg/d of caffeine (provided by 910 mg of guarana) and 20 mg/d of ephedrine (provided by 225 mg of *Ma Huang*, the Chinese name for *Ephedra*) for seven days, followed by a further seven days with administration of four capsules per day. No statistically significant difference was observed in blood pressure (systolic and diastolic), heart rate or the left ventricular ejection fraction between the treated group and the control group (which received a placebo). Furthermore, no electrocardiographic (ST wave, QRS complex) or echocardiographic differences were noted between the two groups. In contrast, the number of adverse effects reported in the treated group was higher than in the placebo group. These adverse effects were not considered by the authors to be serious (dry mouth, sleep disorders, etc.) (Kalman *et al.* 2002; Kalman 2004).

3.5.2.3. Comments on these studies

The available oral studies differ in their conclusions with regard to the reality of an effect of *p*-synephrine on the cardiovascular parameters, in particular the blood pressure profile. When a statistically significant difference is demonstrated, it is most often small, and without real physiological significance with regard to blood pressure (variations below 10 mmHg). As for heart rate, only one study showed an increase of more than 10 bpm. Although reassuring on some points, these results nevertheless suggest that a hypertensive effect of *p*-synephrine is still possible.

The cardiovascular nature of the adverse effects recorded and their occurrence when *p*-synephrine and caffeine taken together could be explained by the α-adrenergic properties of *p*-synephrine, without it being possible to establish a dose-response relationship. In addition, the wide variability in individual responses to caffeine has been clearly demonstrated (genetic polymorphism and non-genetic differences) (Brathwaite *et al.* 2011; Carrillo et Benitez 1996; Renda *et al.* 2012). This phenomenon, which was not taken into account in the clinical studies assessing the effects of caffeine, may explain the apparently contradictory results. However, the influence of this polymorphism on the response to other compounds such as *p*-synephrine is unknown.

Moreover, the clinical data available in the literature have the following limitations:

- several clinical studies were conducted with an observation time that was too short and/or on sample sizes that were too small and/or with a single administration;
- the chemical composition of *Citrus aurantium* extracts is often poorly defined and the profile of the extracts is almost never established. The exact *p*-synephrine concentration is not always clearly specified and may be associated with caffeine and/or other plant extracts which themselves are not characterised;
- no data are available for children or adolescents.

3.6. Vulnerable populations, at-risk situations and physical activity

3.6.1. Vulnerable populations and at-risk situations

In some people and in certain situations, there may be an increased risk of adverse effects associated with the use of products containing *Citrus* spp. or *p*-synephrine:

- people receiving chronic treatment for diseases such as diabetes, hypertension, rhythm disorders and other heart diseases, thyroid disease, depression (treatments with monoamine oxidase inhibitors) and glaucoma (Firenzuoli *et al.* 2005; Santé Canada 2007; Simmons et Schneir 2010);
- people who are overweight or obese, with cardiovascular risk factors, in particular high blood pressure;
- pregnant or breastfeeding women and children, for whom there are no relevant studies. In any case, weight-loss diets are not recommended for children and adolescents, even those suffering from overweight or obesity (HAS 2011);
- people suffering from eating disorders (anorexia) according to the case reported by Gray et Woolf (2005);

- people combining *p*-synephrine and caffeine (Haller *et al.* 2005; Hansen *et al.* 2012) or other products presented as having effects on weight, muscle mass or physical appearance, and claiming rapid results.

3.6.2.Physical activity

As mentioned above, the overweight or obese population is a target for sales of food supplements containing *p*-synephrine. Consumption of these products is often combined with physical activity, especially when this practice is advocated alongside nutritional monitoring as part of overall care for these patients. People who practice sports on a regular basis and who wish to reduce their body fat are also a target population, especially in sports where the performance level may be related to weight or body composition, such as sports with weight categories, disciplines with aesthetic components or sports involving movement of body weight (running, etc.).
Following safety recommendations and the ban on athletes using ephedrine, this compound is now regularly replaced by *p*-synephrine in many food supplements, in particular those claiming to help weight loss (Bent *et al.* 2004; Haaz *et al.* 2006).
Systolic pressure, and to a lesser degree diastolic pressure, increase naturally during physical activity. Due to its sympathomimetic properties, *p*-synephrine may impair the control of blood pressure and affect heart rate. Similar effects are expected following caffeine consumption. The possible consequences of consumption of food supplements providing *p*-synephrine (and sometimes caffeine) during and after exercise have been the subject of experimental studies in animals and humans.

In a study conducted in female Sprague-Dawley rats, *C. aurantium* extracts titrated at 7.25% and 95% of *p*-synephrine were administered for 28 days each at dilutions ensuring exposure to *p*-synephrine doses of 10 or 50 mg/kg/d (Hansen *et al.* 2013). Each rat was subjected to 30 minutes of physical activity, three days a week. The results show a statistically significant increase in systolic and diastolic pressure, observed up to 8 hours after administration of *p*-synephrine at the doses of 10 and 50 mg/kg/d, regardless of the extract. Concerning systolic pressure, a maximum increase of 7.5 mmHg was observed 4 hours after administration of a concentrated extract providing 50 mg/kg/d of *p*-synephrine (this increase was 4.7 mmHg with a concentrated extract providing 10 mg/kg/d of *p*-synephrine). Concerning diastolic pressure, a maximum increase of 6 mmHg was observed 8 hours after administration of a diluted extract providing 50 mg/kg/d of *p*-synephrine (this increase was 3.7 mmHg with a diluted extract providing 10 mg/kg/d of *p*-synephrine). On the other hand, heart rate was not affected by the consumption of these extracts.

In a previous study, conducted in rats of the same strain that received the same extracts but without any physical activity, the same team only observed an increase in systolic pressure in the groups exposed to extracts providing 50 mg/kg/d of *p*-synephrine, with a maximum increase of 6.4 mmHg observed 4 hours after administration of the substance (Hansen *et al.* 2012). In 2013 the authors concluded that physical exercise potentiates the effect of *p*-synephrine on the α and β adrenergic receptors.

The scope of the results from this experimental study should however be qualified with regard to the data obtained in humans (detailed in Annex 7).

Accordingly, a study was conducted in seven men and three women, all in good health, not overweight and without any cardiovascular risk factor, who ingested a food supplement containing 21 mg of *p*-synephrine and 304 mg of caffeine, followed by either a resting phase or exhaustive exercise on an exercise bike (Haller *et al.* 2008). This study's results confirm the hypertensive effects of the food supplement as of the resting phase. During the exercise, systolic hypertension developed in parallel with or without the food supplement; the result was a peak systolic pressure that was higher with the food supplement than with the placebo. In addition, the vasodilation regularly observed during recovery from exercise, which is expressed as a decrease in diastolic pressure, was seen in the placebo consumption situation but not with the food supplement. This resulted in the vasopressor effect of the *p*-synephrine/caffeine combination being maintained during recovery from the exercise.

This observation was also reported in another study in 17 athletes, receiving either a food supplement mainly composed of 20 mg of synephrine, 200 mg of caffeine, 120 mg of phenylethylamine, 4 mg of yohimbine and 100 µg of a mixture of diiodothyronines (n=10), or a placebo (n=7) 30 minutes before being subjected to 40 minutes of physical exercise on an exercise bike. No effect on heart rate related to consumption of the food supplement was shown at the end of the test. However, taking the food supplement seemed to have decreased the post-exercise hypotension deemed by the authors to be a benefit of physical exercise (Magalhães *et al.* 2013).

This decrease in the hypotensive response during the recovery phase, observed after a single dose of synephrine associated with other sympathomimetics, should be verified after repeated doses of *p*-synephrine alone.

In conclusion, in rats, *p*-synephrine potentiates the hypertensor effect of physical exercise: in humans an additive effect of exercise and *p*-synephrine on systolic pressure is noted, while during recovery, there is lesser diastolic hypotension, which could indicate impaired vasomotor control of sympathomimetic origin with potential cardiovascular consequences.

3.7. Maximum permissible dose

Since a cardiovascular NOAEL cannot be defined for *p*-synephrine, either from animal or clinical studies, and especially because in most cases *Citrus* spp. extracts are used, a maximum dose cannot be defined for *p*-synephrine from the literature data analysed.
Calculating a maximum permissible dose is difficult when it relates to food supplements whose composition is complex and varies widely. It is nevertheless important to take the different associated substances into account. Indeed, while the studies often highlight the lack of cardiovascular effects for low doses of *p*-synephrine alone, this type of effect may become apparent with the *Citrus aurantium* extract and especially in the presence of other compounds such as caffeine (isolated or provided by ingredients obtained from coffee, maté and guarana, in particular).

3.8. National and international recommendations

On 12 April 2012, the French National Agency for Medicines and Health Products Safety (ANSM) made a prohibition decision concerning the use of three plants and 26 active substances in magistral or officinal weight-loss formulae prepared in pharmacies (published 10 June 2012 in the Official Journal). The ANSM stated that "these preparations have not proven their efficacy and may expose the patient to health risks". Synephrine and the green fruit of *Citrus aurantium* L. ssp. *aurantium* (syn. *Citrus aurantium* L. var. amara) are among the substances and plants prohibited in these preparations.

Although ephedrine and certain substances with a similar chemical structure or biological effects are regarded as doping substances and are prohibited in competitive sport, synephrine is not prohibited, although it is nevertheless included in the World Anti Doping Agency (WADA) 2013 surveillance programme.

The European Food Safety Authority considers that exposure to conventional foods does not present a risk, but that for preparations containing *Citrus aurantium*, additional safety data are required for doses higher than 20 mg/d of *p*-synephrine. It also stresses the weakness of the genotoxicity assessment and the lack of long-term studies (EFSA Scientific cooperation 2009).

The Australian authorities have set the maximum permissible dose of *p*-synephrine at 30 mg per day, due to its potential cardiotoxicity (NDPSC 2003).

In its 2010 opinion, Health Canada set the maximum permissible dose of the combination of synephrine and octopamine at 30 mg per day, due to the potential risk of cardiovascular toxicity. In addition, it prohibits sources of caffeine (coffee, tea, cola, maté, guarana, etc.) in products containing synephrine due to the lack of sufficient clinical data on the safety of this combination in humans. Lastly, considering that the data are insufficient to determine whether the long-term consumption of products containing synephrine is without risk, the advice of a medical practitioner is required if they are used for longer than eight weeks (Santé Canada 2010).

In the United States, the National Center for Complementary and Alternative Medicine (NCCAM) of the National Institute of Health (NIH) recommends that people with heart disorders or high blood pressure, as well as people using products (medication, caffeine, etc.) likely to accelerate heart rate, avoid taking food supplements containing *Citrus aurantium* (NCCAM-NIH 2012).

The German Federal Institute for Risk Assessment (BfR) adopted an approach based on a study of consumption of foodstuffs that may contain *p*-synephrine: oranges, mandarins, lemons and their juice. The BfR also took jams into account but did not distinguish between the fruits used. For the maximalist approach to *p*-synephrine levels in these foods, the median consumption of *p*-synephrine reached 6.7 mg/d and the 95th percentile of consumption reached 25.7 mg/d. The BfR considers that the quantities of constituents of *Citrus aurantium* likely to have effects on health, especially *p*-synephrine, should not exceed the median value of dietary intake observed, and therefore believes that the quantities of *p*-synephrine provided by the

food supplements should not exceed 6.7 mg/d (BfR 2012). Nevertheless, the method used may have led to a slight overestimation of the amounts consumed.

3.9. French consumption data

ANSES used the results from the Second French Individual Survey on Food Consumption (INCA 2) (Anses 2009) to estimate synephrine intakes in the French population and compare them with the German data from the BfR (BfR 2012). This work is detailed in Annex 9.

To estimate the total daily intakes of synephrine, the minimum and maximum synephrine concentrations in each type of citrus fruit (fresh fruit, juice and jams) were applied to the individual consumptions from the INCA 2 study, which enabled the minimum and maximum individual synephrine intakes to be defined, and the mean and the value of the 95th percentile to be calculated.

For the entire French population, and considering the maximum levels in citrus fruits, total synephrine intakes are 4.3 mg/day on average and 17.7 mg/day at the 95th percentile.

If only those people who consume citrus fruits are included, total synephrine intakes are 6.2 mg/day on average and 20.0 mg/day at the 95th percentile for the maximum levels.

3.10. Conclusions of the Expert Committee on Human Nutrition

It seems important to differentiate between intakes from food supplements and normal dietary intakes of *p*-synephrine from consumption of citrus fruit juice, which can vary from a few milligrams to several tens of milligrams per glass. Regarding normal dietary intakes, the general population does not seem to be at risk from the presence of *p*-synephrine in a balanced diet, which may contain up to 20 mg/d of *p*-synephrine. The food supplements containing extracts of *Citrus* spp., whose cardiovascular effects were reported to the nutrivigilance scheme, provide between 1 and 72 mg of *p*-synephrine per day at the doses recommended by the manufacturer, and all contain caffeine. In this context, the Expert Committee (CES) on Human Nutrition:

- considers that it is not possible to establish a maximum permissible dose of *p*-synephrine in food supplements, due to the lack of exploitable data from the literature. Nevertheless, the dose of 20 mg/day, corresponding to the 95th percentile of French consumption, may be regarded as a reference intake for *p*-synephrine that must not be exceeded for food supplements; this reference does not constitute a safety limit in the true sense of the term, in the absence of any certainty as to the safety of this dose for the French general population:
- recommends avoiding combining *p*-synephrine with caffeine or preparations containing it;
- draws attention to the risks of combining *p*-synephrine with any substance having cardiovascular effects similar to those of caffeine;
- warns against the use of food supplements containing *p*-synephrine or an ingredient obtained from *Citrus* spp. in populations at higher risk from adverse effects, such as people receiving chronic treatment, in particular for hypertension, heart disease or depression;
- strongly advises against the consumption of *p*-synephrine by pregnant or breastfeeding women, children and adolescents;
- notes that the studies involving combinations of ingredients including *p*-synephrine report a greater increase in systolic pressure during exercise and a lesser post-exercise decrease in diastolic pressure. These data suggest that *p*-synephrine may modify blood pressure tolerance to exercise and therefore increase acute cardiovascular risk. Only further data would enable a long-term risk to be ruled out. Moreover, additional studies on the short- and long-term cardiovascular risk of *p*-synephrine alone are needed;
- considers that this change in blood pressure tolerance to exercise may be compounded by the presence of cardiovascular risk factors. This is particularly the case in overweight or obese subjects seeking to lose weight.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety adopts the conclusions of the CES on Human Nutrition.

ANSES therefore:

- considers that the dose of 20 mg/day, corresponding to the 95th percentile of French consumption, can be regarded as a reference intake for *p*-synephrine that must not be exceeded for food supplements;
- notes that many food supplements on the market lead to a daily intake higher than this reference value; such food supplements are therefore unsuitable for sale to consumers;
- recommends avoiding combining *p*-synephrine with caffeine or preparations containing it, and draws attention to the risks of combining *p*-synephrine with any substance having cardiovascular effects similar to those of caffeine. Similarly, food supplements with such synephrine/caffeine combinations are also unsuitable for sale to consumers;
- strongly advises against the consumption of *p*-synephrine by populations at higher risk from adverse effects (people receiving chronic treatment, in particular for hypertension, heart disease or depression), by pregnant or breastfeeding women, children and adolescents. The consumer must be explicitly made aware of such information;
- notes that the use of mixtures containing *p*-synephrine during physical activity may modify blood pressure tolerance to exercise and therefore increase acute cardiovascular risk. This change may be compounded by the presence of cardiovascular risk factors (particularly in overweight or obese subjects seeking to lose weight). Further data are needed on 1/ the long-term risk of the use of mixtures containing *p*-synephrine during physical activity; 2/ the short- and long-term cardiovascular risk of *p*-synephrine alone.
  It should also be noted that while regular practice of long-duration (stamina-building) physical exercise helps correct the resting blood pressure profile in hypertensive subjects, the benefit of this practice could be impaired by the consumption of food supplements containing *p*-synephrine.

Given the context in which food supplements containing *p*-synephrine are used, ANSES reiterates the main recommendations it issued in 2010 following its assessment of the risks related to dietary weight-loss practices (Anses 2010). Weight-loss diets pose a risk to health and may have harmful effects on the bones, kidneys, heart, as well as on behaviour and psychological well-being. In addition, the metabolic changes induced by these diets and their repetition are often behind the vicious circle of weight regain. Lastly, apart from certain situations where weight loss is medically justified (such as obesity, overweight or significant weight gain), which are subject to specialised care, weight-loss diets are not recommended due to the short-, medium- and long-term risks involved.

Marc Mortureux

KEYWORDS

Synephrine, *Citrus*, bitter orange, bigarade, food supplement, slimming, weight-loss diet, physical activity, caffeine, interaction, cardiovascular, nutrivigilance, adverse effect.

REFERENCES

Allison DB, Cutter G, Poehlman ET, Moore Research Director, Barnes S (2005) Exactly which synephrine alkaloids does *Citrus aurantium* (bitter orange) contain? *International Journal of Obesity* **29**(4), 443-446.

ANSES (2009) Individual and National Study of Food Consumption 2 (INCA 2) (2006-2007).

ANSES (2010) Assessment of the risks related to dietary weight-loss practices - Collective expert appraisal report.

Arbo MD, Franco MT, Larentis ER, Garcia SC, Sebben VC, Leal MB, Dallegrave E, Limberger RP (2009a) Screening for in vivo (anti)estrogenic activity of ephedrine and *p*-synephrine and their natural sources Ephedra sinica Stapf. (Ephedraceae) and *Citrus aurantium* L. (Rutaceae) in rats. *Archives of Toxicology* **83**(1), 95-99.

Arbo MD, Larentis ER*, et al.* (2008) Concentrations of *p*-synephrine in fruits and leaves of *Citrus* species (Rutaceae) and the acute toxicity testing of *Citrus aurantium* extract and *p*-synephrine. *Food and Chemical Toxicology* **46**(8), 2770-2775.

Arbo MD, Schmitt GC*, et al.* (2009b) Subchronic toxicity of *Citrus aurantium* L. (Rutaceae) extract and *p*-synephrine in mice. *Regul Toxicol Pharmacol* **54**(2), 114-7. [In eng]

Avula B, Upparapalli SK, Khan IA (2007) Simultaneous analysis of adrenergic amines and flavonoids in *Citrus* peel jams and fruit juices by liquid chromatography: Part 2. *Journal of AOAC International* **90**(3), 633-640.

Avula B, Upparapalli SK, Navarrete A, Khan IA (2005) Simultaneous quantification of adrenergic amines and flavonoids in C. *aurantium*, various *Citrus* species, and dietary supplements by liquid chromatography. *Journal of AOAC International* **88**(6), 1593-1606.

Bartley GE, Breksa AP, Ishida BK (2010) PCR amplification and cloning of tyrosine decarboxylase involved in synephrine biosynthesis in *Citrus*. *New Biotechnology* **27**(4), 308-316.

Bent S, Padula A, Neuhaus J (2004) Safety and efficacy of *Citrus aurantium* for weight loss. *American Journal of Cardiology* **94**(10), 1359-1361.

BfR (2012) Health assessment of sports and weight loss products containing synephrine and caffeine Opinion No. 004/2012, 16 November 2012.

Bouchard NC, Howland MA, Greller HA, Hoffman RS, Nelson LS (2005) Ischemic stroke associated with use of an ephedra-free dietary supplement containing synephrine. *Mayo Clinic Proceedings* **80**(4), 541-545.

Brathwaite J, Da Costa L, El-Sohemy A (2011) Catechol-O-Methyltransferase Genotype Is Associated with Self-Reported Increased Heart Rate Following Caffeine Consumption *Journal of Caffeine Research* **1**(2), 123-130.

Bressler R (2006) Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs. *Geriatrics* **61**(11), 12-18. [In eng]

Bui LT, Nguyen DT, Ambrose PJ (2006) Blood pressure and heart rate effects following a single dose of bitter orange. *Annals of Pharmacotherapy* **40**(1), 53-57.

Burke J, Seda G, Allen D, Knee TS (2007) A case of severe exercise-induced rhabdomyolysis associated with a weight-loss dietary supplement. *Military Medicine* **172**(6), 656-658.

Calapai G, Firenzuoli F, Saitta A, Squadrito F, R. Arlotta M, Costantino G, Inferrera G (1999) Antiobesity and cardiovascular toxic effects of *Citrus aurantium* extracts in the rat: A preliminary report. *Fitoterapia* **70**(6), 586-592.

Carrillo JA, Benitez J (1996) CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine. *British Journal of Clinical Pharmacology* **41**(6), 605-608.

Chuang CC, Wen WC, Sheu SJ (2007) Origin identification on the commercial samples of Aurantii Fructus. *Journal of Separation Science* **30**(9), 1235-1241.

Chung H, Kwon SW, Kim TH, Yoon JH, Ma DW, Park YM, Hong BK (2013) Synephrine-containing dietary supplement precipitating apical ballooning syndrome in a young female. *Korean Journal of Internal Medicine* **28**(3), 356-360.

Clement BA, Goff CM, Forbes TDA (1998) Toxic amines and alkaloids from Acacia rigidula. *Phytochemistry* **49**(5), 1377-1380.

Colker CM, Kalman DS, Torina GC, Perlis T, Street C (1999) Effects of *Citrus aurantium* extract, caffeine, and St. John's Wort on body fat loss, lipid levels, and mood states in overweight healthy adults. *Current Therapeutic Research - Clinical and Experimental* **60**(3), 145-153.

Crowley JR, Couch MW, Williams CM (1982) Identification and quantitative determination of m-hydroxyphenylglycol in mammalian urine. *Biomedical Mass Spectrometry* **9**(4), 146-152.

Di Marco MP, Edwards DJ, Wainer IW, Ducharme MP (2002) The effect of grapefruit juice and Seville orange juice on the pharmacokinetics of dextromethorphan: The role of gut CYP3A and *P*-glycoprotein. *Life Sciences* **71**(10), 1149-1160.

Dragull K, Breksa Iii AP, Cain B (2008) Synephrine content of juice from Satsuma mandarins (*Citrus* unshiu Marcovitch). *Journal of Agricultural and Food Chemistry* **56**(19), 8874-8878.

Dugo P, Mondello L (Eds) (2010) '*Citrus* Oils, composition, advanced analytical techniques, contaminants and biological activity.' (New-York)

Dugo P, Mondello L, Cogliandro E, Verzera A, Dugo G (1996) On the genuineness of citrus essential oils. 51. Oxygen heterocyclic compounds of bitter orange oil (*Citrus aurantium* L.). *Journal of Agricultural and Food Chemistry* **44**(2), 544-549.

Edwards DJ, Fitzsimmons ME, Schuetz EG, Yasuda K, Ducharme MP, Warbasse LH, Woster PM, Schuetz JD, Watkins P (1999) 6',7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: Effects on cyclosporine disposition, enterocyte CYP3A4, and *P*-glycoprotein. *Clinical Pharmacology and Therapeutics* **65**(3), 237-244.

EFSA Scientific cooperation E (2009) Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies.

Firenzuoli F, Gori L, Galapai C (2005) Adverse reaction to an adrenergic herbal extract (*Citrus aurantium*). *Phytomedicine* **12**(3), 247-248.

Gange CA, Madias C, Felix-Getzik EM, Weintraub AR, Estes Iii NAM (2006) Variant angina associated with bitter orange in a dietary supplement. *Mayo Clinic Proceedings* **81**(4), 545-548.

Gray S, Woolf AD (2005) *Citrus aurantium* used for weight loss by an adolescent with anorexia nervosa. *Journal of Adolescent Health* **37**(5), 415-416.

Gurley BJ, Gardner SF, Hubbard MA, Williams DK, Gentry WB, Carrier J, Khan IA, Edwards DJ, Shah A (2004) In vivo assessment of botanical supplementation on human cytochrome P450 phenotypes: *Citrus*

aurantium, Echinacea purpurea, milk thistle, and saw palmetto. *Clinical Pharmacology and Therapeutics* **76**(5), 428-440.

Haaz S, Fontaine KR, Cutter G, Limdi N, Perumean-Chaney S, Allison DB (2006) *Citrus aurantium* and synephrine alkaloids in the treatment of overweight and obesity: An update. *Obesity Reviews* **7**(1), 79-88.

Haller CA, Benowitz NL, Jacob Iii P (2005) Hemodynamic effects of ephedra-free weight-loss supplements in humans. *American Journal of Medicine* **118**(9), 998-1003.

Haller CA, Duan M, Jacob Iii P, Benowitz N (2008) Human pharmacology of a performance-enhancing dietary supplement under resting and exercise conditions. *British Journal of Clinical Pharmacology* **65**(6), 833-840.

Hansen DK, George NI, White GE, Abdel-Rahman A, Pellicore LS, Fabricant D (2013) Cardiovascular toxicity of *Citrus aurantium* in exercised rats. *Cardiovascular Toxicology* **13**(3), 208-219.

Hansen DK, George NI, White GE, Pellicore LS, Abdel-Rahman A, Fabricant D (2012) Physiological effects following administration of *Citrus aurantium* for 28 days in rats. *Toxicology and Applied Pharmacology* **261**(3), 236-247.

Hansen DK, Juliar BE, White GE, Pellicore LS (2011) Developmental toxicity of *Citrus aurantium* in rats. *Birth Defects Research Part B - Developmental and Reproductive Toxicology* **92**(3), 216-223.

HAS (2011) Recommandations de bonnes pratiques - Surpoids et obésité de l'enfant et de l'adolescent (Actualisation des recommandations 2003)

He D, Shan Y, Wu Y, Liu G, Chen B, Yao S (2011) Simultaneous determination of flavanones, hydroxycinnamic acids and alkaloids in citrus fruits by HPLC-DAD-ESI/MS. *Food Chemistry* **127**(2), 880-885.

Hibino T, Yuzurihara M, Kase Y, Takeda A (2009) Synephrine, a component of Evodiae fructus, constricts isolated rat aorta via adrenergic and serotonergic receptors. *Journal of Pharmacological Sciences* **111**(1), 73-81.

Hoffman J, Ratamess N, Jennings P, Mangine G, Faigenbaum A (2006) Thermogenic Effect from Nutritionally Enriched Coffee Consumption. *Journal of the International Society of Sports Nutrition* **3**(1), 35-41.

Holmes Jr RO, Tavee J (2008) Vasospasm and stroke attributable to ephedra-free xenadrine: Case report. *Military Medicine* **173**(7), 708-710.

Hou YC, Hsiu SL, Tsao CW, Wang YH, Chao PDL (2000) Acute intoxication of cyclosporin caused by coadministration of decoctions of the fruits of *Citrus aurantium* and the pericarps of *Citrus* grandis. *Planta Medica* **66**(7), 653-655.

Ibrahim KE, Midgley JM, Crowley JR, Williams CM (1983) The mammalian metabolism of R-(-)-m-synephrine. *Journal of Pharmacy and Pharmacology* **35**(3), 144-147.

Iijima OT, Minematsu S, Maemura S, Waterson LA, Gopinath C (1995) A single oral dose toxicity study and a 13-week repeated dose study with a 4-week recovery period of TSUMURA Hochn-ekki-to (TJ-41) in rats. *Japanese Pharmacology and Therapeutics* **23**(SUPPL. 7), 209-224.

Jordan R, Midgley JM, Thonoor CM, Williams CM (1987) β-Adrenergic activities of octopamine and synephrine stereoisomers on guinea-pig atria and trachea. *Journal of Pharmacy and Pharmacology* **39**(9), 752-754.

Kaats G, Miller H, Preuss H, Stohs S (2013) A 60 day double-blind, placebo-controlled safety study involving *Citrus aurantium* (bitter orange) extract. *Food and Chemical Toxicology* **55**, 358-362.

Kalman D, Incledon T, Gaunaurd I, Schwartz H, Krieger D (2002) An acute clinical trial evaluating the cardiovascular effects of an herbal ephedra-caffeine weight loss product in healthy overweight adults. *International Journal of Obesity* **26**(10), 1363-1366.

Kalman DS (2004) An acute clinical trial evaluating the cardiovascular effects of an herbal ephedra-caffeine weight loss product in healthy overweight adults [2]. *International Journal of Obesity* **28**(10), 1355-1356.

Kalman DS, Colker CM, Shi Q, Swain MA (2000) Effects of a weight-loss aid in healthy overweight adults: Double-blind, placebo-controlled clinical trial. *Current Therapeutic Research - Clinical and Experimental* **61**(4), 199-205.

Klontz KC, Timbo BB, Street D (2006) Consumption of dietary supplements containing *Citrus aurantium* (Bitter orange) - 2004 California Behavioral Risk Factor Surveillance Survey (BRFSS). *Annals of Pharmacotherapy* **40**(10), 1747-1751.

Kusu F, Li XD, Takamura K (1992) Determination of synephrine and N-methyltyramine in Zhishi and Zhike (immature citrus fruits) by high-performance liquid chromatography with electrochemical detection. *Chemical and Pharmaceutical Bulletin* **40**(12), 3284-3286.

Kusu F, Matsumoto K, Arai K, Takamura K (1996) Determination of synephrine enantiomers in food and conjugated synephrine in urine by high-performance liquid chromatography with electrochemical detection. *Analytical Biochemistry* **235**(2), 191-194.

Lagarto Parra A, Silva Yhebra R, Guerra Sardiñas I, Iglesias Buela L (2001) Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine* **8**(5), 395-400.

Lim GE, Li T, Buttar HS (2003) Interactions of grapefruit juice and cardiovascular medications: A potential risk of toxicity. *Experimental and Clinical Cardiology* **8**(2), 99-107.

Ma G, Bavadekar SA, Schaneberg BT, Khan IA, Feller Research Director (2010) Effects of synephrine and β-phenethylamine on human α-adrenoceptor subtypes. *Planta Medica* **76**(10), 981-986.

Magalhães LM, de Oliveira CVC, Gonçalves MCR, de Souza AA, Silva AS (2013) Single dose of dietary supplement Nutrex Lipo-6 Black® limits the post exercise hypotension induced by aerobic exercise in young adults. *Journal of Pharmacy and Nutrition Sciences* **3**(2), 127-133.

Malhotra S, Bailey DG, Paine MF, Watkins PB (2001) Seville orange juice-felodipine interaction: Comparison with dilute grapefruit juice and involvement of furocoumarins. *Clinical Pharmacology and Therapeutics* **69**(1), 14-23.

Mattoli L, Cangi F, Maidecchi A, Ghiara C, Tubaro M, Traldi P (2005) A rapid liquid chromatography electrospray ionization mass spectrometry n method for evaluation of synephrine in *Citrus aurantium* L. samples. *Journal of Agricultural and Food Chemistry* **53**(26), 9860-9866.

McGregor DB, Riach CG, Brown A, Edwards I, Reynolds D, West K, Willington S (1988) Reactivity of catecholamines and related substances in the mouse lymphoma L5178Y cell assay for mutagens. *Environmental and Molecular Mutagenesis* **11**(4), 523-544.

McGuffin M (2006) Media Spins Numbers on Bitter Orange AERs Based on Erroneous Information from FDA Review Finds All But One "Report" Associated with Ephedrine or Caffeine. *Herbalgram* **69**, 52-55.

Min B, Cios D, Kluger J, White CM (2005) Absence of QTc-interval-prolonging or hemodynamic effects of a single dose of bitter-orange extract in healthy subjects. *Pharmacotherapy* **25**(12 I), 1719-1724.

Moaddeb J, Tofade TS, Bevins MB (2011) Hypertensive urgency associated with xenadrine EFX use. *Journal of Pharmacy Practice* **24**(4), 400-403.

Mosqueda-Garcia R, Tseng CJ, Biaggioni I, Robertson RM, Robertson D (1990) Effects of caffeine on baroreflex activity in humans. *Clinical Pharmacology and Therapeutics* **48**(5), 568-574.

Nabekura T, Yamaki T, Kitagawa S (2008) Effects of chemopreventive citrus phytochemicals on human *P*-glycoprotein and multidrug resistance protein 1. *European Journal of Pharmacology* **600**(1-3), 45-49.

Nasir JM, Durning SJ, Ferguson M, Barold HS, Haigney MC (2004) Exercise-induced syncope associated with QT prolongation and ephedra-free Xenadrine. *Mayo Clinic Proceedings* **79**(8), 1059-1062.

NCCAM-NIH (2012) Herbs at a glance - Bitter Orange. Available online from: http://nccam.nih.gov/health/bitterorange [consulted on 17 October 2013].

NDPSC (2003) (National Drugs and Poisons Schedule Committee) Record of the Reasons, 37th meeting, February 25-26 2003. Available online from: http://www.tga.gov.au/pdf/archive/ndpsc-record-37.pdf [consulted on 15 January 2013].

Nykamp DL, Fackih MN, Compton AL (2004) Possible Association of Acute Lateral-Wall Myocardial Infarction and Bitter Orange Supplement. *Annals of Pharmacotherapy* **38**(5), 812-816.

Pellati F, Benvenuti S (2007) Chromatographic and electrophoretic methods for the analysis of phenetylamine alkaloids in *Citrus aurantium*. *Journal of Chromatography A* **1161**(1-2), 71-88.

Pellati F, Benvenuti S, Melegari M (2004) High-performance liquid chromatography methods for the analysis of adrenergic amines and flavanones in *Citrus aurantium* L. var. amara. *Phytochemical Analysis* **15**(4), 220-225.

Pellati F, Benvenuti S, Melegari M (2005) Enantioselective LC analysis of synephrine in natural products on a protein-based chiral stationary phase. *Journal of Pharmaceutical and Biomedical Analysis* **37**(5), 839-849.

Pellati F, Benvenuti S, Melegari M, Firenzuoli F (2002) Determination of adrenergic agonists from extracts and herbal products of *Citrus aurantium* L. var. amara by LC. *Journal of Pharmaceutical and Biomedical Analysis* **29**(6), 1113-1119.

Pellati F, Cannazza G, Benvenuti S (2010) Study on the racemization of synephrine by off-column chiral high-performance liquid chromatography. *Journal of Chromatography A* **1217**(21), 3503-3510.

Penzak SR, Acosta EP, Turner M, Edwards DJ, Hon YY, Desai HD, Jann MW (2002) Effect of Seville orange juice and grapefruit juice on indinavir pharmacokinetics. *Journal of Clinical Pharmacology* **42**(10), 1165-1170.

Renda G, Zimarino M, Antonucci I, Tatasciore A, Ruggieri B, Bucciarelli T, Prontera T, Stuppia L, De Caterina R (2012) Genetic determinants of blood pressure responses to caffeine drinking. *American Journal of Clinical Nutrition* **95**(1), 241-248.

Rossato LG, Costa VM, De Pinho PG, Carvalho F, De Lourdes Bastos M, Remião F (2011a) Structural isomerization of synephrine influences its uptake and ensuing glutathione depletion in rat-isolated cardiomyocytes. *Archives of Toxicology* **85**(8), 929-939.

Rossato LG, Costa VM, Limberger RP, Bastos MDL, Remião F (2011b) Synephrine: From trace concentrations to massive consumption in weight-loss. *Food and Chemical Toxicology* **49**(1), 8-16.

Saito M, Hirata-Koizumi M, Matsumoto M, Urano T, Hasegawa R (2005) Undesirable effects of citrus juice on the pharmacokinetics of drugs: Focus on recent studies. *Drug Safety* **28**(8), 677-694.

Health Canada (2004) Products containing bitter orange or synephrine: suspected cardiovascular adverse reactions. *Canadian Adverse Reaction Newsletter* **14**(4), 3-4.

Health Canada (2007) Bitter orange or synephrine: update on cardiovascular adverse reactions. *Canadian Adverse Reaction Newsletter* **17**(2), 2-3.

Health Canada (2010) Notice - Guidelines for the use of synephrine in natural health products.

Scholz EP, Zitron E*, et al.* (2005) Inhibition of cardiac HERG channels by grapefruit flavonoid naringenin: Implications for the influence of dietary compounds on cardiac repolarisation. *Naunyn-Schmiedeberg's Archives of Pharmacology* **371**(6), 516-525.

Seifert JG, Nelson A, Devonish J, Burke ER, Stohs SJ (2011) Effect of acute administration of an herbal preparation on blood pressure and heart rate in humans. *International Journal of Medical Sciences* **8**(3), 192-197.

Simmons T, Schneir AB Hypertensive Crisis from a MAOI/Supplement Interaction Leading to Myocardial Infarction and Acute heart Failure. In 'North American Congress of Clinical Toxicology', 2010, pp. 604-667

Smedema JP, Müller GJ (2008) Coronary spasm and thrombosis in a bodybuilder using a nutritional supplement containing synephrine, octopamine, tyramine and caffeine. *South African Medical Journal* **98**(5), 372-373.

Stephensen TA, Sarlay Jr R (2009) Ventricular fibrillation associated with use of synephrine containing dietary supplement. *Military Medicine* **174**(12), 1313-1319.

Stohs SJ, Preuss HG, Keith SC, Keith PL, Miller H, Kaats GR (2011) Effects of *p*-synephrine alone and in combination with selected bioflavo-noids on resting metabolism, blood pressure, heart rate and self-reported mood changes. *International Journal of Medical Sciences* **8**(4), 295-301.

Sultan S, Spector J, Mitchell RM (2006) Ischemic colitis associated with use of a bitter orange - Containing dietary weight-loss supplement. *Mayo Clinic Proceedings* **81**(12), 1630-1631.

Suzuki O, Matsumoto T, Oya M, Katsumata Y (1979) Oxidation of synephrine by type A and type B monoamine oxidase. *Experientia* **35**(10), 1283-1284.

Talbott S, Christopulos AM, Richards E *Citrus aurantium* extract has no effect on blood pressure or heart rate in healthy adults. In 'Experimental Biology', 2007, Washington, D.C,

Thomas JE, Munir JA, McIntyre PZ, Ferguson MA (2009) STEMI in a 24-year-old man after use of a synephrine-containing dietary supplement: A case report and review of the literature. *Texas Heart Institute Journal* **36**(6), 586-590.

Uckoo RM, Jayaprakasha GK, Nelson SD, Patil BS (2011) Rapid simultaneous determination of amines and organic acids in citrus using high-performance liquid chromatography. *Talanta* **83**(3), 948-954.

Vidal (Ed.) (2012) 'Vidal 2012: le dictionnaire (88e edn).' (Paris)

Vieira SM, Theodoro KH, Glória MBA (2007) Profile and levels of bioactive amines in orange juice and orange soft drink. *Food Chemistry* **100**(3), 895-903.

Wheaton TA, Stewart I (1965) Quantitative analysis of phenolic amines using ion-exchange chromatography. *Analytical Biochemistry* **12**(3), 585-592.

Wheaton TA, Stewart I (1969) Biosynthesis of synephrine in citrus. *Phytochemistry* **8**(1), 85-92.

Wheaton TA, Stewart I (1970) The distribution of tyramine, N-methyltyramine, hordenine, octopamine, and synephrine in higher plants. *Lloydia* **33**(2), 244-254.

Zenk JL, Leikam SA, Kassen LJ, Kuskowski MA (2005) Effect of lean system 7 on metabolic rate and body composition. *Nutrition* **21**(2), 179-185.

ANNEXES

Annex 1: Rapporteurs, members of the Nutrivigilance Working Group and the CES on Human Nutrition

Annex 2: *P*-synephrine levels in fresh fruit

Annex 3: *P*-synephrine levels in whole dried fruit

Annex 4: *P*-synephrine levels in fruit juice

Annex 5: *P*-synephrine levels in dried extracts

Annex 6: *P*-synephrine levels in food supplements

Annex 7: Clinical studies via the oral route

Annex 8: Case studies of adverse events relating to food supplements containing synephrine or a concentrated extract of *Citrus* spp. containing synephrine

Annex 9: ANSES internal scientific and technical support memorandum on the "Estimate of synephrine intakes in the adult French population"

ANNEX 1: INITIAL RAPPORTEURS, MEMBERS OF THE NUTRIVIGILANCE WORKING GROUP AND THE CES ON HUMAN NUTRITION

FOREWORD: Outside experts, whether Expert Committee and WG members or designated rapporteurs, are all appointed in their personal capacity, *intuitu personae*, and do not represent their parent organisation.

RAPPORTEURS

Mr Jacques BELEGAUD – Honorary UP – Picardie-Amiens University, Pharmaceutical Sciences Training & Research Unit (UFR)

Mr Xavier BIGARD – UP – Scientific adviser to the Chair of the French Anti Doping Agency, Paris

Ms Sabrina BOUTEFNOUCHET – UL – Paris-Descartes University, Pharmacy UFR

Mr Patrick FRIOCOURT – HP – Blois Hospital Centre

Mr Thierry HENNEBELLE – UL – Lille II University, Pharmacy UFR

NUTRIVIGILANCE WORKING GROUP (MANDATE FROM 2012 TO 2015)

Chairperson

Mr Alexandre MACIUK – UL – Paris-Sud University, Pharmacy UFR

Members

Ms Catherine ATLAN – UL-HP – Aix-Marseille II University, Medicine UFR

Mr Alain BOISSONNAS – Retired, UP-HP – Paul Brousse University Hospital, Department of Infectious and Tropical Medicine, Internal Medicine and Addiction Medicine

Ms Sabrina BOUTEFNOUCHET – UL – Paris-Descartes University, Pharmacy UFR

Mr Pierre CHAMPY – UP – Paris-Sud University, Pharmacy UFR

Ms Fatiha EL BABILI – Researcher – Paul Sabatier Toulouse III University

Mr Fabien GUIBAL – HP – Paris Public Hospital System (AP-HP) Saint Louis Hospital, Paris

Mr Thierry HENNEBELLE – UL – Lille II University, Pharmacy UFR

Ms Christine LAFFORGUE – UL – Paris-Sud University, Pharmacy UFR

Ms Anne-Marie MARIOTTE – Honorary UP – Grenoble University, Pharmacy UFR

Mr Jean-Marie RENAUDIN – HP – Emile Durkheim Hospital Centre, Epinal

Mr Mohamed Faouzi SAFRAOU – HP – Former Practitioner at the European Georges Pompidou Hospital, Paris

Mr Alain SERVIN – Retired, formerly Inserm Research Director – Paris-Sud University, Pharmacy UFR, CNRS joint research unit (UMR) 8076

Mr Bernard WENIGER – Retired, UL – Strasbourg University, Pharmacy UFR

EXPERT COMMITTEE ON HUMAN NUTRITION (MANDATE FROM 2012 TO 2015)

Chairperson

Mr François MARIOTTI – MC – AgroParisTech, Biology and Human Nutrition UFR

Members

Ms Latifa ABDENNEBI-NAJAR – Research Director – Institut Polytechnique la Salle de Beauvais, UP 2012.10.101 EGEAL Team

Mr Jacques BELEGAUD – Honorary University Professor – Picardie-Amiens University, Pharmaceutical Sciences Training & Research Unit (UFR)

Ms Catherine BENNETAU-PELISSERO – UP – Bordeaux Sciences Agro, U862 Inserm

Ms Marie BODINIER – Research Officer – INRA Nantes, UR1268 Biopolymers Interactions Assemblies (BIA), allergy team

Mr Marc BONNEFOY – UP-HP – Claude-Bernard Lyon 1 University, Lyon Sud Medicine UFR, Hospices Civils de Lyon, Inserm 1060

Ms Marie-Christine BOUTRON-RUAULT – Research Director – CESP Inserm U1018 team 9 "Nutrition, hormones and women's health"

Mr Jean-Louis BRESSON – UP-HP – AP-HP Necker Hospital for Sick Children, Centre for Clinical Investigation, CIC 0901

Mr Olivier BRUYERE – UP – Liège University, Department for Public Health, Epidemiology and Health Economics, Unit for Methodological Support in Epidemiology and Biostatistics

Ms Sybil CHARRIERE – UL-HP – Claude Bernard Lyon I University, Lyon Est Medicine UFR, Hospices Civils de Lyon

Mr Gérard CROS – UP – Montpellier 1 University, Pharmacy UFR, Unit: Max Mousseron Biomolecular Institute, CNRS-UMR 5247

Mr Anthony FARDET – Research Officer – INRA Clermont-Ferrand/Theix, Human Nutrition Unit, UMR 1019 INRA/University of Auvergne

Ms Anne GALINIER – UL-HP – Paul Sabatier University, Toulouse University Hospital

Mr Jean-François HUNEAU – UP – AgroParisTech, Biology and Human Nutrition UFR

Mr Alexandre MACIUK – UL – Paris-Sud University, Pharmacy UFR

Mr André MAZUR – Research Director – INRA Clermont-Ferrand/Theix, Human Nutrition Unit, UMR 1019 INRA/University of Auvergne

Mr Gilles MITHIEUX – DR1 – CNRS, Inserm unit 855 "nutrition and brain", Lyon

Ms Béatrice MORIO-LIONDORE – Research Director – INRA Lyon, Laboratory for Cardiovascular, Metabolism, Diabetology and Nutrition Research

Mr Claude MOULIS – Emeritus UP – Paul-Sabatier University, Toulouse, Pharmaceutical Sciences UFR, UMR152

Ms Annie QUIGNARD-BOULANGE – Emeritus Research Director – Inserm, UMR 914 INRA/AgroParisTech

Mr Alain SERVIN – Retired, formerly Inserm Research Director – Paris-Sud University, Pharmacy UFR, CNRS joint research unit (UMR) 8076

Ms Ariane SULTAN – UL-HP – Montpellier University Hospital, Lapeyronie Hospital, Nutrition-Diabetes team

Mr Stéphane WALRAND – Research Director – INRA Clermont-Ferrand/Theix, Human Nutrition Unit, UMR 1019 INRA/University of Auvergne

ANSES COORDINATION

Ms Irène MARGARITIS – UP – Head of the Nutritional Risk Assessment Unit - ANSES

Mr Aymeric DOPTER – Pharm D – Nutrivigilance Unit Coordinator – ANSES

Ms Gwenn VO VAN-REGNAULT – Pharm D – Nutrivigilance Project Manager – ANSES

ANNEX 2: *P*-SYNEPHRINE LEVELS IN FRESH FRUIT

Species	Harvest areas	Levels of *p*-synephrine (mg/g)	References
Whole fresh fruit			
Citrus aurantium L.	Brazil (immature)	0.41-0.48	Arbo *et al.* (2008)
C. sinensis L. Osbeck	Brazil (immature)	0.62-0.99	Arbo *et al.* (2008)
C. sinensis L. Osbeck	United States (Florida)	0.019	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
C. deliciosa	Brazil (immature)	0.77-1.97	Arbo *et al.* (2008)
C. reticulata Blanco Tangerine	United States (Florida)	0.125	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
C. reticulata Blanco Cleopatra mandarin	United States (Florida)	0.280	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
C. limon	Brazil (immature)	0.37-0.45	Arbo *et al.* (2008)
C. limonia	Brazil (immature)	0.12-0.51	Arbo *et al.* (2008)
C. paradisi Mac Grapefruit	United States (Florida)	ND	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
Fresh hybrid fruit pulp (mature)			
C. aurantium L.	China	0.013-0.035	Mattoli *et al.* (2005)
Citrus hybrids	China	0.008-0.054	He *et al.* (2011)
Fresh hybrid fruit peel (mature)			
Citrus hybrids	China	0.010-1.02	He *et al.* (2011)
Leaves			
Citrus reticulata Blanco Tangerine	United States (Florida)	2.031	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
Citrus reticulata Blanco Cleopatra mandarin	United States (Florida)	2.215	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
Citrus sinensis Obeck	United States (Florida)	0.321	Wheaton et Stewart (1969); Wheaton et Stewart (1970)
Citrus paradisi Mac. Grapefruit	United States (Florida)	ND	Wheaton et Stewart (1969); Wheaton et Stewart (1970)

ND = not detected

ANNEX 3: *P*-SYNEPHRINE LEVELS IN DRIED FRUIT

Species	Harvest areas	Levels of *p*-synephrine (mg/g)	References
Citrus aurantium L.	China (immature) China (nearly mature) Japan (immature) fruit Japan (mature) peel Italy (mature) fruit	1.3-3.1 1.1-2.3 2.52 0.75 1-3.5	Kusu *et al.* (1992) Kusu *et al.* (1992) Pellati et Benvenuti (2007) Pellati et Benvenuti (2007) Pellati *et al.* (2002); Pellati *et al.* (2004); Pellati *et al.* (2005)
C. unshiu Markovich	Japan (mature) peel	3.07	Pellati et Benvenuti (2007)
C. reticulata Blanco	Japan (immature) peel Japan (mature) peel	6.23 2.38	Pellati and Benvenuti (2007)
Citrus aurantium Other *Citrus* spp. *C. maxima, C. paradisi, C. medica, C. limon, C. grandis, C. aurantifolia, C. meyeri, Poncirus trifoliatus*	Different origins (mature) fruit	0.38-4.07 0.11 - 2 ND	Avula *et al.* (2005)

ND = not detected

ANNEX 4: *P*-SYNEPHRINE LEVELS IN FRUIT JUICES AND JAMS

Fruits		Levels of *p*-synephrine (mg/L)	Other	References
Orange juice				
Orange	Hamlin Navel Pineapple Valencia	22 15 27 19		Wheaton et Stewart (1965)
C. sinensis Tan.	Marrs sweet orange	85	Tyramine: 4.82 mg/L	Uckoo *et al.* (2011)
C. sinensis	Valencia	23.3 *		Kusu *et al.* (1996)
Citrus aurantium L. var. *sinensis*	Tarocco Naveline Navel 'White' juice 'Red' juice	26-35 * 12-15 * 13.2 * 13-16 * 30-32 *		Mattoli *et al.* (2005)
Orange	Brand A Brand B Brand C Brand D Brand E Brand F Brand G	16.3 21.8 15.8 14.7 17.9 15.5 10.1	Other amines detected including putrecine (22.6 - 43.7 mg/L)	Vieira *et al.* (2007)
Orange	Different species including *C. aurantium*	3.65 - 60.66		Avula *et al.* (2007)
Lemon juice				
Lemon	Bearss Meyer	2	Octopamine: 4 mg/L Tyramine: 25 mg/L	Wheaton et Stewart (1965)
C. limon Tan.	Meyer lemon	2.75	Octopamine: 16.29 mg/L Tyramine: 9.22 mg/L	Uckoo *et al.* (2011)
Lemon		ND		Avula *et al.* (2007)
Mandarin, clementine or tangerine juice				
C. reshni hort. Ex Tanaka *C. reticulata ?*	Cleopatra mandarin	280	*N*-MeTyramine: 58 mg/L	Wheaton et Stewart (1965)
C. unshiu Markovich	Satsuma mandarin	54.5-160		Dragull *et al.* (2008)

Fruits		Levels of *p*-synephrine (mg/L)	Other	References
C. unshiu mikan		35.5		Kusu *et al.* (1996)
C. clementina Tan.	Clementine	114.61	Tyramine: 17 mg/L	Uckoo *et al.* (2011)
C. reticulata Tan.	Nova tangerine	78.28		Uckoo *et al.* (2011)
Tangerine	Dancy	125	Octopamine: 1mg/L Tyramine: 1 mg/L	Wheaton et Stewart (1965)
Grapefruit juice				
Grapefruit	Duncan March Ruby red	ND		Wheaton et Stewart (1965)
C. paradisi Macf.	Rio Red grapefruit	ND		Uckoo *et al.* (2011)
C. grandis Tan.	Red-fleshed pummelo	ND		Uckoo *et al.* (2011)
Grapefruit		ND		Avula *et al.* (2007)
Jams (in mg/kg)				
Citrus		121		Kusu *et al.* (1996)
C. aurantium		0.9-51		Avula *et al.* (2007)
Grapefruit		ND		Avula *et al.* (2007)

ND = not detected

*: approximate values based on measurements in µg/g or mg/100g

ANNEX 5: *P*-SYNEPHRINE LEVELS IN DRIED EXTRACTS

	Supplier laboratory	Claimed titre	Levels of *p*-synephrine (%)	Remarks	References
Dry extract *Citrus aurantium* L. var. *amara*	Local market Italy		3.003-3.079	Octopamine 0.023-0.028% Tyramine 0.055-0.056%	Pellati *et al.* (2002); Pellati *et al.* (2004)
Hydroalcoholic extract *Citrus aurantium* L. var. *amara*	Polichimica, Bologna, Italy	Titrated 6% Titrated 4%	4.112-2.698	Partial racemisation observed	Pellati *et al.* (2005)
Hydroalcoholic extract *Citrus aurantium* L. var. *amara*	Polichimica, Bologna, Italy	Titrated 6% Titrated 4%	6.571-4.228	Presence of tyramine, octopamine, hordenine, *N*-methyltyramine	Pellati et Benvenuti (2007)

ANNEX 6: *P*-SYNEPHRINE LEVELS IN FOOD SUPPLEMENTS

Source region	Number of products analysed	Levels of *p*-synephrine (mg/g)	Remarks	References
Local market Italy	3	2.3-10.5	Partial racemisation observed	Pellati *et al.* (2002); Pellati *et al.* (2004)
Various sources	8	0.073-18.62	Presence of tyramine, octopamine, *N*-methyltyramine	Avula *et al.* (2005)
Local market Italy	5	0.49-22.39	Partial racemisation observed	Pellati *et al.* (2005)
Pharmacy, Italy	5	0.65-27.41	Presence of tyramine, octopamine, hordenine, *N*-methyltyramine	Pellati et Benvenuti (2007)

ANNEX 7: CLINICAL STUDIES VIA THE ORAL ROUTE

Products	Subjects treated	Comments on the subjects	Comments on the study	Duration	Dose of *p*-synephrine (mg/d)	Dose of caffeine (mg/d)	Adverse effects (parameters studied)	References
SINGLE DOSE								
Extract of *Citrus aurantium*	18	No cardiac history	Randomised, double-blind, placebo-controlled crossover study in two stages: extract of C. *aurantium* / WO 1 week / placebo No intake of caffeine, FS or stimulant 12 h before and during study phases	8h	27	-	NO (blood pressure, QT)	Min *et al.* (2005)
Extract of *Citrus aurantium*	10 (per group)	No caffeine or physical exercise for 8 to 10h	Randomised, double-blind, placebo-controlled study Control group: placebo (n=10) Treated groups: 4 groups (n=10) : synephrine +/- hesperidin and naringin	75 min	50	-	NO (blood pressure, heart rate)	Stohs *et al.* (2011)
Extract of *Citrus aurantium*	15	22-29 years, no history of heart disease or hypertension, non-smokers, non-obese, no use of FS or OTC containing *Citrus aurantium* 1 month before study	Randomised, double-blind, placebo-controlled crossover study in two stages: extract of C. *aurantium* / WO 1 week / placebo No caffeine intake on day of study	6h	54	-	Increase in blood pressure and heart rate	Bui *et al.* (2006)

Products	Subjects treated	Comments on the subjects	Comments on the study	Duration	Dose of *p*-synephrine (mg/d)	Dose of caffeine (mg/d)	Adverse effects (parameters studied)	References
Extract of *Citrus aurantium*	10	Non-smoking subjects 19 to 42 years, non obese, no cardiac history, no hypertension	Randomised, double-blind, placebo-controlled crossover study in three stages: extract of C. *aurantium* / WO 1 week / mixture / WO 1 week / placebo No intake of caffeine, FS or OTC 24h before the study	6h	46.9	-	Increase in heart rate	Haller *et al.* (2005)
Mixture	10			6h	5.5	239.2	Increase in heart rate and blood pressure	
Mixture	10	20-31 years, practising sport at least 3 times a week, non-obese, non-smokers	Randomised, double-blind, placebo-controlled crossover study in three stages: Mixture + rest / WO 1 week / Mixture + 30 min exercise 1h after consumption / WO 1 week / placebo + 30 min exercise 1h after consumption No intake of caffeine, FS or OTC 24h before the study	2h	21	303	Increase in diastolic blood pressure (with or without exercise)	Haller *et al.* (2008)
Mixture (+ Yohimbine and diiodothyronine)	10	19-32 years, engaging in regular physical activity	Randomised, double-blind, placebo-controlled study Control group: placebo (n=7) Treated groups: mixture (n=10)	2h	20	200	NO (variability in heart rate, blood pressure) But decrease in post-exercise hypotension	Magalhães *et al.* (2013)

Products	Subjects treated	Comments on the subjects	Comments on the study	Duration	Dose of *p*-synephrine (mg/d)	Dose of caffeine (mg/d)	Adverse effects (parameters studied)	References
Mixture (+ *Garcinia cambogia* + chromium polynicotinate)	10	No cardiac history, non-smokers, no FS for 6 weeks	Randomised, double-blind, placebo-controlled crossover study in two stages: mixture / WO 4 days / placebo (=coffee)	3h	21.6	450	Increase in systolic blood pressure ——————— NO (heart rate and diastolic pressure)	Hoffman *et al.* (2006)
Mixture	23	Average age = 24.5 years Average BMI = 26.6	Randomised, double-blind, placebo-controlled crossover study in two stages: mixture / WO 1 week / placebo	24h	52	704	NO (blood pressure, heart rate)	Seifert *et al.* (2011)
REPEATED DOSES								
Extract of *Citrus aurantium* (whether or not associated with naringin and hesperidin)	25 (per group)	27-76 years, mean BMI = 30.8 Male/female ratio 8:2	Randomised, double-blind, placebo-controlled study Group A: standardised *p*-synephrine extract (98mg) without naringin or hesperidin (n=25) Group B: extract of *C. aurantium* containing *p*-synephrine (98mg), naringin (200mg) and hesperidin (1152 mg) (n=25) Group C: placebo (n=25) 8 subjects abandoned for undetermined reason, breakdown among 3 groups regarded as homogeneous but not detailed	60 days	98 mg	-	Small but statistically significant differences for heart rate NO (for blood pressure and renal, hepatic and haematopoietic factors. No adverse effect reported)	Kaats *et al.* (2013)

Products	Subjects treated	Comments on the subjects	Comments on the study	Duration	Dose of *p*-synephrine (mg/d)	Dose of caffeine (mg/d)	Adverse effects (parameters studied)	References
Extract of *Citrus aurantium*	15	Normotensive	Placebo-controlled study	6 weeks	80	-	NO (blood pressure, heart rate)	Unpublished Talbott *et al.* (2007)
Mixture (+ St John's wort)	9	Obese subjects, no cardiac history	Randomised, double-blind, placebo-controlled study Group A: mixture (n=9) Group B: placebo (n=7) Group C: nothing (n=4) For all the groups: sport 3 days/week + diet controlled at 1800 kcal/d	6 weeks	58.5	528	NO (blood pressure, heart rate, electrocardiogram)	Colker *et al.* (1999)
Mixture	19	Obese subjects, no "non-stabilised" heart disease or uncontrolled hypertension	Randomised, double-blind, placebo-controlled crossover study Group 1: mixture (n=19) Group 2: placebo (rice) (n=16) Controlled diet, 3 sport sessions/week, no caffeine intake	8 weeks	36	? (guarana, maté)	NO (blood pressure, heart rate)	Zenk *et al.* (2005)
Mixture (+ ephedrine)	12	Obese subjects, no history of heart disease or hypertension	Randomised, double-blind, placebo-controlled study: treated group (n=12) placebo group (maltodextrin) (n=13)	8 weeks	10	400	NO (liver tests, blood pressure, heart rate)	Kalman *et al.* (2000)

Products	Subjects treated	Comments on the subjects	Comments on the study	Duration	Dose of *p*-synephrine (mg/d)	Dose of caffeine (mg/d)	Adverse effects (parameters studied)	References
Mixture (+ ephedrine)	19.	Obese subjects, no history of heart disease, no hypertension	Randomised, double-blind, placebo-controlled single-centre study: Treated group (n=19): for 7 days: 2 capsules/day the next 7 days: 4 capsules/d Placebo group (n=8) controlled coffee consumption, no other FS	14 days	10.	400.	NO (pressure, heart rate) But: more (non-serious) adverse effects in the treated subjects (dry mouth, sleep disorders, etc.)	Kalman *et al.* (2002); Kalman (2004)

WO: washout (period without treatment to eliminate the product)
FS: food supplement
OTC: over the counter (non-prescription medicinal product)

ANNEX 8: CASE STUDIES OF ADVERSE EVENTS RELATING TO FOOD SUPPLEMENTS CONTAINING SYNEPHRINE OR A CONCENTRATED EXTRACT OF *CITRUS* SPP. CONTAINING SYNEPHRINE

Adverse effects reported	Existence of prior medical history or risk factors/*context*	Presence of other ingredients/caffeine	Remarks on progression	References
Takotsubo syndrome (acute cardiomyopathy)	Subject 21 years old	? / Yes	Hospitalised for 10 days. No sequelae. No new event after use of the product ceased	Chung *et al.* (2013)
Angina pectoris while taking Cortislim®	Obese former smoker, taking fibrates and aspirin/*no particular exercise*	Yes / Yes (green tea extract)	Resolved when use ceased, but symptoms returned after several months without reintroduction	Gange *et al.* (2006)
Myocardial infarction while taking Edita's Skinny Pill®	Smoker (one and a half packets/day), sedentary, 55 years old, heart murmur/*no particular physical activity*	Yes / Yes (guarana, green tea) + heavy coffee consumption	Symptomatic treatment (thrombolysis)	Nykamp *et al.* (2004)
Myocardial infarction while taking Nutrex Lipo-6x®	No personal or family history. Young subject / *usually taken before sport; the event occurred after 2 hours of sport*	Yes (yohimbine, guggulsterones, phenylephrine, tyramine, hordenine) / Yes + concomitant consumption of a caffeine-rich beverage	Symptomatic treatment	Thomas *et al.* (2009)
Syncope while taking Xenadrine EFX®	No history, young, athletic subject / *the event occurred while practising sport on an empty stomach*	Yes / Yes (green tea, maté)	No new event after use of the product ceased	Nasir *et al.* (2004)
Ventricular fibrillation while taking Hi-Tech Lipodrene®	No history (5 packets of cigarettes/year), subject young and likely to be athletic (military) / *the event occurred while doing physical exercise (press-ups)*	Yes (depending on the sources: presence of ephedrine and/or *Acacia rigidula,* known to contain many phenylethylamine-type compounds other than synephrine (Clement *et al.* 1998) / Yes	Symptomatic treatment	Stephensen et Sarlay Jr (2009)
Tachyarrhythmia while taking extract of C. *aurantium* containing 6% synephrine	Little information, patient taking thyroxine/*no particular physical exercise*	No / No	Reappeared on reintroduction	Firenzuoli *et al.* (2005)
Hypertension while taking Xenadrine EFX®	34 years old, smoker (10 cigarettes/day), prior history of pre-eclampsia	Yes / Yes (green tea, maté)	Symptomatic treatment	Moaddeb *et al.* (2011)

Adverse effects reported	**Existence of prior medical history or risk factors/*context***	**Presence of other ingredients/caffeine**	**Remarks on progression**	**References**
Ischemic stroke while taking Stacker 2®	Subject aged 38 years with no particular prior history/*no particular physical exercise*	Yes / Yes	Seemed to resolve when use of the product ceased	Bouchard *et al.* (2005)
Vasospasm and stroke while taking Xenadrine EFX®	Subject aged 36 years with no particular prior history	Yes / Yes (green tea, maté)	Symptomatic treatment	Holmes Jr et Tavee (2008)
Myocardial infarction	Subject aged 39 years, no prior history, body-building competition (reduced liquid intake and high-carbohydrate diet)	Yes (octopamine, tyramine, St John's wort) / Yes	Symptomatic treatment	Smedema et Müller (2008)
Ischemic colitis while taking NaturalMax Skinny Fast®	No prior history	Yes / Yes	No new event after use of the product ceased	Sultan *et al.* (2006)
Rhabdomyolysis	Subject aged 22 years with no prior history	Yes (yohimbine) / Yes	Symptomatic treatment Permanent sensory and motor deficit in extremities	Burke *et al.* (2007)

ANNEX 9: ANSES INTERNAL SCIENTIFIC AND TECHNICAL SUPPORT MEMORANDUM ON THE "ESTIMATE OF SYNEPHRINE INTAKES IN THE ADULT FRENCH POPULATION"

Maisons-Alfort, 22 January 2014

MEMORANDUM:
Internal scientific and technical support of the French Agency for Food, Environmental and Occupational Health & Safety

on the "Estimate of synephrine intakes in the adult French population"

On 6 January 2014, ANSES's Food Consumption Observatory Unit (UOCA) received an internal request for scientific and technical support from the Nutrivigilance Unit in ANSES's Risk Assessment Department (DER), regarding the estimate of synephrine intakes in the adult French population.

1. BACKGROUND AND PURPOSE OF THE REQUEST

ANSES's Nutrivigilance Unit is currently preparing an opinion on the risks associated with the presence in food supplements of *p*-synephrine or ingredients obtained from *Citrus* spp. fruits containing this substance (Request No. 2012-SA-0200).

This opinion refers to a report[3] by the German Federal Institute for Risk Assessment (BfR), which assessed consumption levels in the German population of citrus fruit that may contain synephrine as well as the minimum and maximum synephrine intakes that may be associated with each type of citrus fruit consumed.

In order to make a comparison between these results and those relating to the French population, the Nutrivigilance Unit called on the UOCA to provide data on intakes in the French population based on the INCA 2 study. The information requested was the mean and the value of the 95th percentile, for consumption of citrus fruit that may contain synephrine, as well as a simulation of minimum and maximum synephrine intakes in the adult French population.

2. ORGANISATION OF THE WORK

The internal scientific and technical support study was carried out by the UOCA of ANSES's DER according to the methodology presented below.

■ Source and quality of consumption data used: INCA 2 study

The consumption data used came from the INCA 2 study conducted in three phases in 2006-07 among 4079 individuals aged from 3 to 79 years old (1455 children from 3-17 years old and 2624 adults from 18-79 years old).
Participants were selected according to a three-stage design, stratified by the size of the urban area and the region. The sampling frames used were the 1999 population census and new housing built between 1999 and 2004.
Data on consumption by the individuals in this sample were collected using a 7-day food consumption diary in which they noted the types of foods and quantities consumed, estimated using a photograph manual, standard units or household measures. Each line in the diary corresponded to a food (or beverage) consumed. In the case of industrial products, the trade name and brand name of the consumed food had to be provided. Each collected line of food was codified using a nomenclature specially developed for the INCA 2 study and containing 1280 items.

[3] Health assessment of sports and weight loss products containing synephrine and caffeine. BfR Opinion No. 004/2012, of 16 November 2012

A weighting was applied to each individual in the two samples (children 3-17 years old and adults 18-79 years old) to ensure their representativeness at national level (French mainland, i.e. excluding Corsica).
As part of this scientific and technical support study, under-reporting individuals were not excluded for the estimation of consumption levels, and only adults were considered.

Foods taken into account for estimating consumption levels

To allow easy comparison between the German and French consumption levels, the choice of INCA 2 foods taken into account for this scientific and technical support study was based on the list of foods monitored in the BfR report, namely:
- oranges;
- orange juice;
- mandarins/clementines;
- mandarin juice;
- lemons;
- lemon juice;
- jam/marmalade.

The BfR report states that as the consumption data used do not distinguish between citrus jams and jams made with other fruit, these are therefore grouped together in a single category: jam/marmalade.

Identification of product categories monitored from INCA 2 data

The above-mentioned citrus fruits are consumed not only as foods but also frequently as recipe ingredients (fruit salads, juice mixtures, etc.). Therefore, to avoid underestimating consumption, it seemed preferable to also take into account consumption of these citrus fruits via recipes that may contain them. When it was not possible to determine from the wording in the food diaries whether or not the studied citrus fruits were actually contained in the recipes considered, all the lines referring to them were included. Proportions of amounts of citrus fruit were then assigned to each recipe, defined on the basis of a comparison with similar recipes, industrial or home-made. Only recipes containing a significant proportion of citrus fruit (accounting for more than 5% of their weight) were taken into account for the study and are presented in the tables below.

- Oranges

Consumers of oranges were identified from consumption lines in the food diaries referring to the item from the nomenclature: "fresh orange" but also to those of the following recipes:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
13134	fresh fruit salad	13034	fresh orange	16
13999	unspecified fruit	13034	fresh orange	15
24680	soft orange-filled sponge cake such as Chamonix	13034	fresh orange	11.8
1017	sangria	13034	fresh orange	5.8

- Orange juice

Consumers of orange juice were identified from consumption lines in the food diaries referring to the following items from the nomenclature: "pasteurised orange juice made from concentrate", "home-squeezed orange juice", "pure pasteurised orange juice", "pasteurised orange nectar". All the lines corresponding to the following recipes were also included:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
2069	pasteurised fruit juice cocktail made from concentrate	2012	pasteurised orange juice made from concentrate	65
2004	unspecified fruit juice	2013	home-squeezed orange juice/pasteurised orange juice made from concentrate/pure pasteurised orange juice	62
18304	still fruit drink with 10 to 50% juice, reduced sugar content, such as Minute Maid Fresh Mix	2012	pasteurised orange juice made from concentrate	32
42	cocktail such as planter's punch	2012	pasteurised orange juice made from concentrate	22
1017	sangria	2012	pasteurised orange juice made from concentrate	16.8
18342	still orange drink with 10 to 50% juice, sweetened, such as Oasis Orange	2012	pasteurised orange juice made from concentrate	13
18343	fruit juice and milk drink such as Danao	2012	pasteurised orange juice made from concentrate	13
18301	sparkling orange juice drink with pulp, around 14% fruit, sweetened, such as Orangina	2012	pasteurised orange juice made from concentrate	11.5
18019	sparkling fruit drink with 10 to 50% juice, sweetened, such as Fanta Orange	2012	pasteurised orange juice made from concentrate	10
18330	still exotic fruit drink, 10 to 50% sweetened juice, such as Banga Exotique or Oasis Cocktail Tropical	2012	pasteurised orange juice made from concentrate	6
18340	sparkling fruit drink with sweeteners, such as Fanta Light Orange or Orangina Light Tentation	2012	pasteurised orange juice made from concentrate	5.7
2008	non-alcoholic cocktail with fruit juice and syrup	2013	home-squeezed orange juice	30
2035	cocktail of pure pasteurised fruit juices	2070	pure pasteurised orange juice	28
2002	cocktail of pure pasteurised multivitamin fruit juices	2070	pure pasteurised orange juice	20
43	alcoholic cocktail	2070	pure pasteurised orange juice	8

- Mandarins/clementines

Consumers of mandarins and/or clementines were identified from consumption lines in the food diaries referring to the item from the nomenclature: "Fresh clementine or mandarin" as well as the recipe:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
13999	unspecified fruit	13024	fresh clementine or mandarin	9

- Mandarin juice

Consumers of mandarin juice were identified from consumption lines in the food diaries referring to the item from the nomenclature: "Pure pasteurised mandarin or clementine juice" and also that of the recipe:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
2002	cocktail of pure pasteurised multivitamin fruit juices	2034	pure pasteurised mandarin or clementine juice	6

- Lemons

Consumers of lemons were identified from consumption lines in the food diaries referring to the items from the nomenclature: "Fresh lemon" and "Lime pulp". The lines corresponding to the following recipes were also included:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
31025	all types of sorbet	13009	fresh lemon	6
1017	sangria	13009	fresh lemon	5.8
25488	smoked salmon and butter baguette sandwich	13009	fresh lemon	5.2

- Lemon juice

Consumers of lemon juice were identified from consumption lines in the food diaries referring to the items from the nomenclature: "Home-squeezed lemon juice" and "Pure pasteurised lime juice" as well as to the items from the following recipes:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
25537	salmon carpaccio	2007	home-squeezed lemon juice	19.7
25538	beef carpaccio	2007	home-squeezed lemon juice	14
25621	houmous	2007	home-squeezed lemon juice	8

- Jams

Consumers of jams were identified from consumption lines in the food diaries referring to the items from the nomenclature: "orange marmalade" and "any type of jam or marmalade", when the wording of the consumption diary specified a jam containing citrus fruits, orange, mandarin, clementine or lemon. The lines in the food diaries containing the following item were also included:

CODAL for the recipe	RECIPES	CODAL for the ingredient	INGREDIENTS	% of the Ingredient in the Recipe (in weight)
24686	soft orange-filled, chocolate-covered sponge cake such as Pim's	31039	orange marmalade	37

Insofar as it was possible to precisely identify the citrus jams consumed in the INCA 2 study, only these foods were taken into account for this scientific and technical support study, in contrast to the BfR report which did not distinguish the fruits used in the jams monitored, and therefore overestimated synephrine intake via this food.

Synephrine concentration data

The ranges of synephrine concentrations in citrus fruits used to estimate intake were established according to the literature data listed in the annexes of the ANSES opinion[4].

Table 1: Minimum and maximum synephrine concentrations (in mg/g) in citrus fruits, used to simulate intakes in the adult French population

Synephrine concentrations in mg/g	Species	Minimum (in mg/g)	Maximum (in mg/g)	References
Oranges	*C. sinensis*	0.013	0.035	Matolli et al. (2005)
Orange juice	Orange / *C. sinensis* Tan.	0.00365	0.085	Avula et al. (2007) / Uckoo et al. (2011)
Mandarins/Clementines	*Citrus* hybrids	0.008	0.054	He et al. (2011)
Mandarin juice	*C. unshiu mikan* / *C. reshni hort. Ex Tanaka C. reticulata?*	0.0355	0.28	Kusu et al. (1996) / Wheaton and Stewart (1965)
Lemons	*C. limon*	0.37	0.45	Arbo et al. (2008)
Lemon juice	Citron / *C. limon* Tan.	0.002	0.00275	Wheaton and Stewart (1965) / Uckoo et al. (2011)
Jams	*C. aurantium / Citrus*	0.0009	0.121	Avula et al. (2007) / Kusu et al. (1996)

For fruits, the data come from the table in Annex 2: *P*-synephrine levels in fresh fruit; more specifically the part "Fresh hybrid fruit pulp (mature)" for oranges and mandarins/clementines, and the part "Whole fresh fruit" for lemons (only data available for this citrus fruit). For the concentrations in fruit juices and jams, the minimum and maximum synephrine levels per fruit juice were taken from Annex 4.

[4] ANSES Opinion on the risks associated with the presence in food supplements of *p*-synephrine or ingredients obtained from *Citrus* spp. fruits containing this substance (unpublished, currently being finalised). Request No 2012-SA-0200

The synephrine levels used to simulate intakes provided in this scientific and technical support document are given in Table 1.

■ Estimated minimum and maximum synephrine intakes

The BfR report presents synephrine intakes in the German population associated with the consumption of the various citrus fruits studied, and proposes a range of values, according to whether all the citrus fruits consumed have the same minimum synephrine concentration or the same maximum concentration. The concentrations by type of citrus fruit were chosen from a list of the different concentrations available in the literature[5].
To estimate these ranges of intakes, the consumption estimates (mean and 95th percentile) were multiplied by the minimum and maximum concentrations chosen.
A range for total exposure to synephrine is also shown, for average and high consumption of citrus fruits. However, the method used to calculate this range is not specified.

To be able to provide comparable data to those in the BfR report, the minimum and maximum synephrine intakes in the adult French population by type of citrus fruit were simulated in the same way in this support study: the mean and 95th percentile of individual daily consumption for each type of citrus fruit were multiplied by the minimum and maximum concentrations shown in Table 1.

In the absence of specific details on the calculation of total synephrine exposure in the BfR report, the method that seems most appropriate for obtaining this information was used in this support study: the minimum and maximum synephrine concentrations for each type of citrus fruit were applied to the individual consumptions from the INCA 2 study, in order to define the maximum and minimum individual synephrine intakes and to calculate the mean and the value for the 95th percentile.

3. ANALYSIS AND CONCLUSIONS

The results presented below show, for each type of citrus fruit, the mean consumption and the value of the 95th percentile, for the entire adult population and then only for adult consumers of each type of citrus fruit. The ranges of total synephrine intakes for average or high consumption of citrus fruits are also shown. To ensure that the results were as representative as possible, they were estimated taking into account the constraints related to the complex sampling in the INCA 2 study and the individual weighting.

■ In the entire adult population

Table 2 shows the levels of consumption of the citrus fruits studied as well as a range of estimated synephrine intakes for each type of citrus fruit in question, in the entire adult French population.

Table 2: Consumption levels (g/day) and minimum and maximum synephrine intakes (mg/day) for each type of citrus fruit consumed in the entire adult French population

[5] Health assessment of sports and weight loss products containing synephrine and caffeine. BfR Opinion No. 004/2012, of 16 November 2012

In the entire adult population		Total consumption (in g/day)	Synephrine intakes with minimum levels (in mg/day)	Synephrine intakes with maximum levels (in mg/day)
Oranges	N	2624		
	Mean	7.6	0.1	0.3
	P95	43.9	0.6	1.5
Orange juice	N	2624		
	Mean	40.3	0.1	3.4
	P95	185.2	0.7	15.7
Mandarins, Clementines	N	2624		
	Mean	7.1	0.1	0.4
	P95	47.1	0.4	2.5
Mandarin juice	N	2624		
	Mean	0.5	0.02	0.1
	P95	0	0	0
Lemons	N	2624		
	Mean	0.2	0.1	0.1
	P95	1.1	0.4	0.5
Lemon juice	N	2624		
	Mean	0.9	0.002	0.003
	P95	3.4	0.01	0.01
Jams	N	2624		
	Mean	0.3	0.0003	0.04
	P95	0	0	0

Table 3 provides a range of total estimated synephrine intakes at individual level in the entire adult French population. The estimates shown correspond to the average individual intakes and the intakes at the 95th percentile.

Table 3: Total intakes, means and 95th percentile, estimated from minimum and maximum synephrine concentrations (mg/day) in the entire adult French population

In the entire adult population		Synephrine intakes with minimum levels (in mg/day)	Synephrine intakes with maximum levels (in mg/day)
Total synephrine intakes	means	0.4	4.3
	95th percentile	1.5	17.7

■ In adult consumers only

Table 4 shows the consumption levels of the citrus fruits studied as well as a range of estimated synephrine intakes for each type of citrus fruit in question, in adult consumers alone.

Table 4: Consumption levels (g/day) and minimum and maximum synephrine intakes (mg/day) depending on the citrus fruits consumed in adult consumers only

In adult consumers only			Total consumption (in g/day)		Synephrine intakes with minimum levels (in mg/day)	Synephrine intakes with maximum levels (in mg/day)
Oranges	N	Rate of consumers (CI95%)	608	20.9% (19.3-22.5)		
	Mean		36.5		0.5	1.3
	P95		122.1		1.6	4.3
Orange juice	N	Rate of consumers (CI95%)	1223	47.3% (45.2-49.4)		
	Mean		85.3		0.3	7.2
	P95		250.0		0.9	21.3
Mandarins, Clementines	N	Rate of consumers (CI95%)	676	20.9% (19.3-22.5)		
	Mean		34.1		0.3	1.8
	P95		82.5		0.7	4.5
Mandarin juice	N	Rate of consumers (CI95%)	96	3.8% (3.0-4.6)		
	Mean		12.3		0.4	3.4
	P95		71.4		2.5	20.0
Lemons	N	Rate of consumers (CI95%)	233	9.1% (7.9-10.2)		
	Mean		2.4		0.9	1.1
	P95		6.8		2.5	3.0
Lemon juice	N	Rate of consumers (CI95%)	327	12.5% (11.1-13.9)		
	Mean		7.5		0.02	0.02
	P95		34.3		0.1	0.1
Jams	N	Rate of consumers (CI95%)	83	2.9% (2.2-3.6)		
	Mean		11.6		0.01	1.4
	P95		30.0		0.03	3.6

Table 5 provides total estimated synephrine intakes at individual level in adult consumers only. The estimates shown correspond to mean individual intakes and intakes at the 95th percentile.

Table 5: Total intakes, means and 95th percentile, estimated from minimum and maximum synephrine concentrations (mg/day) in adult consumers alone

In adult consumers only		Synephrine intakes with minimum levels (in mg/day)	Synephrine intakes with maximum levels (in mg/day)
Total synephrine intakes	means	0.6	6.2
	95th percentile	1.8	20.0

Comments

The total estimated intakes for the adult French population are generally lower than those shown in the BfR report for the German population, mainly due to lower consumption of oranges in France and a more precise targeting of jams consumed in the INCA 2 study.

Study limitations

Any comparison between the data presented in this support study and those available in the BfR report must take into account the following limitations. Firstly, the consumption data may not exactly cover the same types of foods and recipes in the two studies, which is the case for jams, for instance. Secondly, as the sample sizes were quite low for certain foods (mandarin juice and jams mainly), the representativeness of

the data relating to them should be treated with caution. Moreover, the concentrations used for the intake simulations for mandarins and lemon juice differ between the two studies. Lastly, the total intakes presented in the two studies should be compared with caution, since they may not have been estimated in the same way.

KEYWORDS

INCA 2, food consumption, synephrine, citrus fruits