Health assessment of sports and weight loss products containing synephrine and caffeine

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Synephrine is a plant constituent which is found, for example, in bitter oranges (*Citrus aurantium*) and which is chemically related to ephedrine. Some products offered as sports and weight loss supplements contain synephrine in the form of added bitter orange extract. Often such products contain caffeine and other active ingredients. They are sometimes referred to by manufacturers as food supplements or dietetic foods. The Federal Institute for Risk Assessment (BfR) has assessed the risks posed by such products.

In terms of the quantities of the substances synephrine – usually in the form of bitter orange extract – and caffeine added to the various supplements, there are very big differences between various products. Both substances influence the cardiovascular system. If synephrine and caffeine are taken at the same time, these effects can mutually reinforce each other. This notably applies to their potential to increase heart frequency, possibly even in cases of cardiac arrhythmia, as well as to their potential to increase blood pressure. Other active ingredients contained in bitter orange extracts may also enhance cardiovascular effects of synephrine.

BfR recommends that quantities of health-relevant components of *Citrus aurantium* ingested through such products should be limited to the intake levels from conventional foods such as oranges and bitter oranges. For synephrine, this means that no more than 6.7 milligrams per day should be consumed in the form of a food supplement. This quantity of synephrine represents the intake via conventional foods with maximum contents of synephrine for average consumers. This would ensure even for frequent consumers that their total intake of synephrine from both conventional foods and food supplements does not exceed 25.7 milligrams.

For some products currently available on the market, there are, due to the dosage levels, sufficient reasons to suspect that the products do not meet the requirements of Regulation No. 14 (EC) 178/2002. For this reason, they are classified as unsafe. This takes into account that the target group of the food supplement are persons who, as a result of physical exertion, already put an increased strain on their cardiovascular system which may be further exacerbated if they are overweight.

1 Subject of the assessment

The BfR has assessed the risks of sports and weight loss products containing synephrine and caffeine.

The BfR does not assess whether the claims of the various manufacturers regarding the desired effects of their products are true or misleading.

2 Result

With some sports and weight loss products, caffeine and synephrine are ingested in bolus doses which clearly exceed the single doses which are or were used for these substances in

single-agent drugs. (Bolus means that the daily dose is taken all at once as opposed to being spread out into smaller individual doses throughout the day.) Due to known synergetic effects, it is to be assumed that the substances caffeine and synephrine mutually reinforce each other in their cardiovascular effects. This applies to both, to substances' potential for increasing the heart frequency (for example by triggering cardiac arrhythmia) and their blood pressure-increasing properties. Additional enhancements of these effects through other phenylethylamines with sympathomimetic activity contained in *Citrus aurantium* extracts are possible. Some relatively high-dose combination products are – due to their potential for unwanted cardiovascular effects such as increasing heart frequency (for example by triggering cardiac arrhythmia) and also due to the their blood pressure-increasing properties – not safe in terms of Article 14 of Regulation (EC) No. 178/2002. This safety assessment takes into account that the target group of the product are persons who, on account of physical exertion, already put an increased strain on their cardiovascular system which may be further exacerbated if they are overweight.

Other products containing synephrine and caffeine contain lower quantities of these substances. However, the daily synephrine intake is clearly above the average daily intake via conventional foods if average consumption is assumed and, under certain circumstances, even higher than the daily intake of frequent consumers. The individual doses which are or were used in single-agent drugs are almost reached for synephrine. This means that the potential for unwanted cardiovascular effects is relatively lower compared to the above-mentioned products containing higher doses. As a matter of principle, however, the BfR has reservations about the use of substances in pharmacologically effective concentrations in food.

In addition, the following must be pointed out: some of these products not only consist of a combination of synephrine and caffeine with the risks involved described above but contain additional plant extracts which are not characterised in detail. However, since they too contain relevant active ingredients, they should all be included in assessments of such products.

For reasons of health protection, labels on such combination products with pharmacologically effective doses of synephrine and caffeine should inform the consumer that these products can lead to an increase in blood pressure and pulse rate and that they are therefore not suitable for persons with existing hypertension, who are overweight or suffer from other cardiovascular illnesses. The label should also warn consumers that if they are taking other medication, they should consult their doctor, that caution should be taken in connection with intensive exercise and that such products are unsuitable for pregnant and breastfeeding women as well as children.

3 Statement of Reasons

3.1 Risk Assessment

For the individual plant extracts which are frequently used in food supplements and similar products together with orange peel extract information is available neither on their qualitative and quantitative chemical composition nor the botanical source used (e.g. variety, parts of the plant, their degree of comminution). Nor is any information provided on the manufacturing process (extracting agent, duration and temperature). This makes risk assessment difficult, since additional unwanted effects may not be detected.

The intake of *Citrus aurantium* extracts which are not subjected to sufficient tests to establish their safety is acceptable in food supplements and related products only for quantities which correspond to the conventional intake of bitter orange peels in food. This assessment is conducted in line with the corresponding EFSA guidance document (EFSA 2009a). The quantities of the substances of *Citrus aurantium* extracts that are relevant for human health and ingested through such products should remain within the range of the intake from conventional foods. As regards synephrine which is the subject of this discussion, this means that the maximum intake should be 6.7 milligrams of synephrine[1] per day for food supplements (this corresponds to the intake via conventional foods with maximum synephrine levels for average consumers). This level guarantees that the total intake from traditional foods and food supplements does not, for a vast majority of consumers, exceed the value of 25.7 milligram of synephrine per day (corresponds to the intake of synephrine via conventional foods with maximum synephrine levels for frequent consumers).

3.1.1 Agent

Citrus aurantium extract, botanical origin and active ingredients

Citrus aurantium L. (synonyms: bitter orange, sour orange, Seville orange) belongs to the family of Rutaceae. The blossoms (bitter orange blossom) or peels (bitter orange peels) are the parts of the plant that are used for pharmaceutical purposes. The plant variety is *Citrus aurantium* L. ssp. *aurantium* (synonym: *Citrus aurantium* L. ssp. *amara*) (EUAB 7, 2011). The plant is native to South East Asia. Other varieties of *Citrus aurantium* L. are known to exist.

A review drawn up as part of the National Toxicology Program (NTP) assumes that in food supplements claiming to support weight reduction for the most part probably dry extracts from unripe fruit of *Citrus aurantium* L. ssp. *aurantium* produced with alcohol water mixtures are used. However, they are wrongly marketed as bitter orange peel extracts. These extracts are usually standardised for their synephrine content (e.g. 6 % or 10 % of synephrine). Some are additionally standardised for further biogenic amines such as octopamine, hordenine, N-methyltyramine and tyramine. Whole dried unripe fruits are known in Traditional Chinese Medicine as “Zhi Shi” and are sold as bulk ware (NTP/NIEHS 2004, Blumenthal 2005). *Citrus aurantium* extracts with synephrine contents of up to 95 % are also said to be commercially available (EFSA, 2009b).

Bitter orange peel (Aurantii amari epicarpium et mesocarpium) is described in the European Pharmacopoeia, 7th edition, as the dried epicarp and mesocarp from which partly the white, spongy tissue of the mesocarp and endocarp has been removed of the ripe fruit of *Citrus aurantium* L. ssp. *aurantium* (EUAB 7, 2011). The bitter orange peel tincture (Aurantii amari epicarpii et mesocarpii tinctura) is produced, using a suitable process, from 1 part of the freshly pulverised drug and 5 parts of ethanol 70 % (V/V) (EUAB 7, 2011).

According to the current monograph in the commentary on the European Pharmacopoeia (EUAB commentary, 2011), bitter orange peel contains 1 to over 2.5 % essential oil which largely (up to over 90 %) consists of (R)-(+)-limonene. In addition, numerous other monoterpenes are contained therein (hydrocarbons, alcohols, esters, aldehydes) as well as an aromatypical range of non-terpenoid aldehydes such as octanal, nonanal, decanal and similar substances. The drug additionally contains coumarin derivatives (e.g. meranzin) and

[1] This is defined as overall synephrine, consisting of natural (-)-synephrine and (+)-synephrine possibly formed dduring the manufacturing process.

methoxyflavone. It also contains the bitter tasting flavonoids naringin, neohesperidin and neoeriocitrin as well as non-bitter tasting flavonoids such as rutoside, hesperidin, eriocitrin and more highly methoxylated flavones (sinensetin, nobiletin, tangeritin and others). Bitter orange extracts also contain significant amounts of pectin and low quantities of carotenoids.

The main components in the peels of unripe fruit of *Citrus aurantium* L. ssp. *aurantium* are naringin and hesperidin, whereas the main component of the fruit pulp is umbelliferone (NTP/NIEHS 2004).

In addition, adrenergic phenylethylamine and phenylethanolamine derivatives have been detected in the fruits of bitter oranges and their peels. They are classified as biogenic amines and in some cases are designated as protoalkaloids. Apart from synephrine, as a main component, octopamine, hordenine, N-methyltyramine and tyramine have been detected (Avula et al., 2005; Nelson et al., 2007; Chizzali et al., 2011; Pelatti et al., 2002; Percy et al., 2010). For example, Nelson and colleagues detected, in three different bitter orange standard reference materials (pulverised dried unripe fruit, extract from ripe fruit and a mixture of commercially available bitter orange-containing food supplements), the following by means of a sensitive LC/MS/MS method: 8.8 to 77.5 mg/g of synephrine, 0.12 to 0.84 mg/g of octopamine, 0.18 to 4.35 mg/g of N-methyltyramine, 0.05 to 0.76 mg/g of tyramine and 0.01 to 0.02 mg/g of hordenine. The highest protoalkaloid contents were measured in bitter orange extract (Nelson et al., 2007).

Synephrine

Synephrine (synonyms: p-synephrine, oxedrine) is 1-(4-Hydroxyphenyl)-2-(methylamino)ethanol. Synephrine exists in 2 enantiomeric forms, firstly, (-)-synephrine (synonyms: l-synephrine, R-(-)-synephrine) which is naturally contained in the fruit of *Citrus aurantium* L. ssp. *aurantium* and (+)-synephrine (synonyms: d-synephrine, S-(+)-synephrine) which does not naturally occur in these fruits. However, during processing, (+)-synephrine can be formed from (-)-synephrine, depending on the conditions. High temperature influences in an acidic or alkaline environment lead, for example, to racemisation. On the basis of receptor binding studies it is assumed that (-)-synephrine is biologically about twice as active as the racemate (Stohs and Preuss, 2012). For synthetically produced racemate in the form of tartrate, a pharmaceutical application is described as a cardiovascular drug to treat low blood pressure (Martindale, 2011). Such a drug is currently no longer on the market in Germany. In dried plant material (peel, ripe / unripe fruit) 0.25 to 0.35 % of synephrine are contained as a rule, though the values vary between 0.1 and 2.0 % (Avula et al., 2005; Avula et al., 2007; Pellati et al., 2002; Pellati and Benvenuti, 2007a; Santana et al., 2008). In addition, unripe fruit contains somewhat higher quantities of synephrine than ripe fruit (NTP/NIEHS 2004, Blumenthal 2005).

In older literature, “m-synephrine” (phenylephrine) is sometimes given as a natural component of *Citrus aurantium* L. ssp. *aurantium* mixtures. However, this designation has been proven to be wrong. It appears that the sample material tested was corrupted due to the addition of artificial phenylephrine (Blumenthal 2005; NTP/NIEHS 2004; Santana et al., 2008; Stohs and Preuss 2012).

It is unclear what parts of the plant of what *Citrus aurantium* variety were used as the base material for *Citrus aurantium* extracts in the products under discussion. Similarly, it is not known what manufacturing process (extracting agent, duration, and temperature) was used, nor is it known whether they were added as a liquid or dry extract. Moreover, no information

is available on what enantiomers of the synephrine[2] were present in the products and whether or in what concentrations other biogenic amines such as octopamine, hordenine, N-methyltyramine and tyramine were contained in the two mixtures as a result of the use of the *Citrus aurantium* extract. For the rest, no data is available on residues from pesticides or agents to treat the peel of citrus fruit in the mixtures.

3.1.2 Hazard Potential

3.1.2.1 Existing monographs on *Citrus aurantium* extract

In 2004, NTP/NIEHS wrote a systematic overview of all toxicological data on bitter orange extract and its components available at the time. However, this overview does not include a risk assessment (NTP/NIEHS, 2004).

In 2009, a task force of the European Food Safety Agency (EFSA) conducted a preliminary risk assessment of the hydroalcoholic extract of *Citrus aurantium* (6 % synephrine). The conclusions drawn in this assessment included the following (EFSA, 2009b):

According to reports, bitter orange extracts contained in food supplements such as weight loss pills were enriched with *p*-synephrine, typically to concentrations of 6 – 10 % (although extracts with a concentration of 95 % *p*-synephrine are documented). This means a significant increase in intake compared to historical values, and this is attributable to the intended levels in food supplements.
It is concluded that a conventional use of bitter orange in food is safe. In contrast, to be able to assess the safety of bitter orange products with a *p*-synephrine content of over 6 %, additional data would be required…. The task force states that in accordance with the recommendations made as part of other assessments, additional data are needed if the maximum synephrine intake is estimated to be exceeded by over 20 mg per day (Belgian Arrêté Royal 29/8/1997 – Annex List 3 and subsequent regulations). The average exposure to *p*-synephrine would probably not exceed the mentioned value of 20 mg *p*-synephrine per day in case of a balanced diet including occasional consumption of citrus fruit and juices.

A health assessment of *Citrus aurantium* peel, synephrine, octopamine and caffeine by the Canadian Health Authority is also available (Health Canada, 2011a). Health Canada accepts a maximum daily dose of 50 mg of synephrine as a single active ingredient in a product for healthy adults. In combination with caffeine, the Canadian Health Authority lays down a maximum daily dose of 40 mg of synephrine and 320 mg of caffeine for health adults. In addition, all synephrine-containing products must contain the following warnings: “Contraindicated for children, pregnant and breastfeeding women; do not take if you are undergoing treatment with blood pressure-increasing or lowering drugs, thyroid medication, sympathomimetics or monoamine oxidase inhibitors”. Products which in addition to synephrine and caffeine also contain other adrenergic substances such as octopamine must be subjected to a risk assessment on a case-by-case basis. For octopamine as a single substance too, a maximum daily dose of 50 mg is accepted for healthy adults. Furthermore, it is assumed that the peel of *Citrus aurantium* does not pose a health risk for consumers in the doses in which it is typically used in plant-based foods and drugs.

[2] Hereinafter, the designations “(-)-synephrine”, “(+)-synephrine“, or “(±)-synephrine” are used, if it possible to distinguish between the individual enantinomers or the racemate, otherwise the term “synephrine” will be used.

3.1.2.2 Toxicological Studies

Unless stated otherwise, the studies cited below do not provide, in relation to the given synephrine contents of the administered *Citrus aurantium* extracts, any information on the proportions of naturally contained (-)-synephrine and any (+)-synephrine that may have been formed during the extraction process. If elevated levels of the latter were contained in the administered extract, this would lead to the wrong conclusion on a too high effective dose, when the indicated doses of synephrine are equated with those for (-)-synephrine. For administered synthetic synephrine too, stereochemical information is missing. According to Stohs and Preuss (2012) it is to be assumed that in this case the racemate is present.

3.1.2.2.1 Acute Toxicity

Oral administration of *Citrus aurantium* extract (no information on synephrine content, parts of plant: fruit, rind): LD50 of 477 mg/kg·bw for mouse (Parra et al., 2001).

Acute oral administration of *Citrus aurantium* extract (unripe fruits were extracted with 50 % methanol; 2.5 % synephrine, 300-5000 mg/kg body weight (bw) on mice (albino CF1, male) caused a reduction in locomotor activity (from 1000 mg/kg bw). Administration of 150-2000 mg/kg bw of synephrine (no information on stereochemistry given) in the same study (from 300 mg/kg bw) led to piloerection, gasping for breath, salivation, exophthalmos and a reduction in locomotor activity. The above-mentioned effects were reversible and detectable for 3-4 hours. The authors concluded that the observed toxic effects were attributable to androgenic stimulation by the *Citrus aurantium* extract and synephrine (Arbo et al., 2008).

3.1.2.2.2 Toxicity in Case of Repeated Administration

Oral administration (once a day) of hydroalcoholic extracts from *Citrus aurantium* fruits which have been standardised for 4 % and 6 % synephrine in doses of each 0; 2.5; 5; 10 or 20 mg/kg bw/d to male Sprague-Dawley rats for the duration of 15 days (8 animals per group) caused a significant and dose-dependent reduction of food intake and body weight increase (Calapai et al., 1999). In addition, increased mortality was observed for all substance groups (up to 50 % for the highest dosage of 20 mg/kg bw/d, extract with 6 % synephrine, corresponding to 1.2 mg of synephrine/kg bw/d). No blood pressure changes were detected. However, an increased dosage-dependent occurrence of ventricular arrhythmias with QRS complex extension was documented in the 20 mg/kg bw group (administration of 0.8 and 1.2 mg of synephrine/kg bw/d). There is no description of a no-observed-adverse-effect-level (NOAEL).

Groups of 9-10 mice (albino CF1, ♂) were orally given *Citrus aurantium* extract (7.5 % synephrine) in doses of 400, 2000 and 4000 mg/kg bw/d and synephrine (M.P.Biomedical, USA), in doses of 30 and 300 mg/kg bw/d for 28 days (Arbo et al., 2009b). In the clinical tests, no unwanted effects were detected and no substance-related death occurred. The biochemical and haematological parameters were also in the normal range. In the animals treated with synephrine (30 and 300 mg/kg bw/d), a statistically significant reduction in bodyweight increase was observed. A significant reduction in intercellular GSH concentrations was found in animals treated with 4000 mg/kg bw of *Citrus aurantium* extract and 30 and 300 mg/kg bw/d of synephrine. In addition, an inhibition of glutathione peroxidase activity was found in animals with 400 and 4000 mg /kg bw of *Citrus aurantium* extract and 30 and 300 mg/kg bw/d of synephrine. However, no change in malondialdehyde concentrations was found.

The physiological effects (impact on blood pressure, temperature, heart frequency, QT interval) of two different *Citrus aurantium* extracts (containing 6 % and 90 % synephrine) on rats (Sprague-Dawley, ♀) were studied in a 28-day study as part of the National Toxicology Program (Hansen et al., 2012). HPLC/MS and GC/MA analyses showed that the 6% extract contained 7.25 % synephrine, 0.63 % hordenine, 0.1 % octopamine and 0.09 % tyramine, whereas the 90% extract contained 95.0 % synephrine, 0.05 % hordenine, 0.39 % octopamine and 0.02 % tyramine. Groups of 13-14 animals were orally given two different *Citrus aurantium* extracts, so that the synephrine doses administered with extracts amounted to 10 and 50 mg/kg bw/d. In addition, 25 mg/kg bw/d of caffeine were administered at the same time to parallel dosage groups, since most dietetic products based on *Citrus aurantium* also contain caffeine. The study was carried out in accordance with 21 CFR Part 58 (Good Laboratory Practice for Nonclinical Laboratory Studies). No differences were detected with regard to bodyweight or survival rate between the control and substance groups. Both in animals treated with synephrine (95% extract) and *Citrus aurantium* extract, an increase in blood pressure (for all doses) and heart frequency (for all doses except for 50 mg/kg bw/d of synephrine) was detected. The study found clearly more significant effects for the extract than the pure substance which suggests that other plant components have an impact on the physiological parameters. Both heart frequency and blood pressure increase were more pronounced, when caffeine was administered at the same time. This means that NOAEL could not be inferred.

3.1.2.2.3 Genotoxicity

No data are available on the genotoxicity of *Citrus aurantium*.

For synephrine (racemate) (120 µM to 21.53 mM) no genotoxic effects were observed in L5178Y mouse lymphoma cells (McGregor et al., 1988).

3.1.2.2.4 Developmental and Reproductive Toxicity

The studies on developmental toxicity were conducted in rats with *Citrus aurantium* extracts (containing 6 % and 90 % synephrine) as part of the National Toxicology Program of the USA (Hansen et al., 2011). Both the HPLC/MS and GC/MA analysis showed that the 6% extract contained 7.25 % synephrine, 0.63 % hordenine, 0.1 % octopamine and 0.09 % tyramine, whereas the 90% extract contained 95.0 % synephrine, 0.05 % hordenine, 0.39 % octopamine and 0.02 % tyramine. Female Sprague-Dawley rats (25 animals per group) were given by gavage doses of 10, 25, 50 or 100 mg synephrine/kg bw/d from day 3 to day 20 of pregnancy. The tested extracts showed no teratogenic or embryotoxic effects. The study was conducted in accordance with 21 CFR Part 58 (Good Laboratory Practice for Nonclinical Laboratory Studies).

3.1.2.2.5 Mode of Action

Synephrine has structural characteristics in common with the body's own adrenoceptor agonists, the catecholamines epinephrine (adrenaline) and norepinephrine (noradrenaline) which exert their effects via α_1-, α_2-, β_1-, β_2-, and β_3 adrenoceptors. The similarity consists in phenylethylamine as the shared basic structure. In the periphery, adrenoceptor agonists mimic the effects of the sympathicus. For this reason, they are also called direct sympathomimetics which include, apart from synephrine, octopamine, hordenine and phenylephrine ("m-synephrine"). The various substances differ in their affinity and selectivity to bind to the receptors. *In vitro* studies with human and animal cells and tissues showed, for example, that the adrenergic activity of synephrine and even octopamine is magnitudes

lower than that of norepinephrine (Aktories et al., 2009; Brown et al., 1988; Hwa and Perez, 1996; Jordan et al., 1987; Ma et al., 2010). A structural similarity with adrenoceptor agonists also exists for indirect sympathomimetics which, in contrast to the direct sympathomimetics, typically do not have the OH substitution on the aromatic ring. Unlike the direct sympathomimetics, they are characterised by a psychostimulating central nervous effect component (e.g. ephedrine, amphetamine) (Aktories et al., 2009).

The stimulation of α_1- and α_2 adrenoreceptors on the blood vessels leads to vasoconstriction and that of β_1 adrenoreceptors on the heart tcardiovascular contractility and increased heart frequency (positive inotrope, chronotrope, dromotrope and lusitrope effects). The β_2 adrenoreceptors are found, for example in the smooth muscles of the blood vessels, the bronchi and the uterus where their stimulation leads to relaxation. β_3 adrenoreceptors are found in the adipocytes of white and brown fat tissue. Their activation leads to an increase in lipolysis.

The synephrine effect on α_1- and α_2 adrenoreceptors is much weaker than that of phenylephrine. However, both are in turn weaker in their effect than norepinephrine: phenylephrine is 4 to 150 times less active than norepinephrine, whereas synephrine is 30 to 1000 times less active than norepinephrine, depending on the tissue in which the receptor is located. In all cases, (-)-enantiomers show higher activity by an order of magnitude of 1-3 compared to (+)-enantiomers (Brown et al., 1988). Additional studies have confirmed that synephrine binds to human α_{1A}-, α_{2A}- and α_{2C}-adrenoreceptor subtypes with comparatively lower affinity and that it has, as a partial agonist, an effect on the α_{1A}- adrenoreceptors (EC_{50}=4 µM, maximum response at 100 µM) and also as a partial low-affinity antagonist on α_{2A}- and α_{2C}- adrenoreceptors (Airriess et al., 1997; Ma et al., 2010).

On β_1- adrenoreceptors, the following sequence of activities of (-)-enantiomers was found for the tested phenylethylamines (activity decrease compared to norepinephrine given in brackets): norepinephrine > phenylephrine (100-fold) > octopamine (6000-fold) > synephrine (40000-fold). The (+)-stereoisomers were less active by 1-2 orders of magnitude than the corresponding (-) stereoisomers. On the β_2 -adrenoreceptors (trachea of guinea pigs), (-)-stereoisomers of synephrine, octopamine and phenylephrine were more than 10000-fold less active than norepinephrine. The (+)-stereoisomers did not have any visible effect here (Jordan et al., 1987).

According to Stohs et al. (2011a), synephrine primarily has an effect on the β_3-adrenoreceptors which leads to an increase in thermogenesis and lipolysis but has no influence on blood pressure. It is assumed that synephrine exerts its weight-reducing effect through stimulation of β_3-adrenoreceptors with a subsequent acceleration of fat metabolism (Stohs et al., 2011a; Stohs et al., 2011b). Carpene et al. (1999) studied different biogenic amines for their β_3-adrenoreceptor-conveyed lipolytic effect in adipocytes in the white fat tissue of rats, hamsters, dogs, guinea pigs and humans. Synephrine showed itself to be a low-affinity β_3-agonist. In contrast, octopamine was a highly potent β_3-activator in rats, hamsters and dogs. However, octopmine was hardly efficient for guinea pigs and humans. In addition, a more recent study on isolated rat livers showed that synephrine-induced adrenergic effects on liver metabolism (such as an increase in glycogenolysis, glycolysis, oxygen consumption etc.) cannot be influenced by β_3-adreno receptor-specific antagonists (Peixoto et al., 2012).

From the available studies, Stohs and Preuss (2012) conclude that the biological activity of (-)-synephrine is roughly twice that of the racemate. In addition, the receptor binding studies clearly show that the phenylephrine (*m*-synephrine) which is found as a corruption in *Citrus*

aurantium mixtures has a clearly higher activity on α- and β-adreno receptors than synephrine (Brown et al., 1988; Jordan et al., 1987).

3.1.2.3 Pharmacological investigations, clinical studies, reports on unwanted effects

3.1.2.3.1 *Citrus aurantium* extracts

The studies cited below do not contain any information on the quantities of naturally contained (-)-synephrine, nor the (+)-synephrine that may have formed during extract production. If larger quantities of the latter were available in the administered extract, this would lead, if the indicated synephrine doses were equated with those for (-)-synephrine, to the wrong inference of an overly high effective dose.

A study by Bui et al. (2006) (randomised, double-blind, placebo-controlled, 2-arm crossover study with a 1-week washout period) was conducted on 13 normotensive test persons (aged 22-29). They were given a one-off dose of 900 mg of a *Citrus aurantium* extract (standardised to 6 % synephrine, equivalent to 54 mg of synephrine). In the course of 6 hours during which hourly measurements were taken, they showed, compared to placebo, an increase in systolic blood pressure (hours 1-5) as well as the pulse rate (hours 2-5). The hour 3 measurement of the systolic blood pressure under verum was around 7.3 $\pm$ 4.6 mm Hg higher than under placebo. The pulse rate was up to 4.2 $\pm$ 4.5 beats per minute higher than under placebo. The diastolic blood pressure (hours 4-5) decreased both under placebo and under verum over time, though the fall was comparatively more pronounced under placebo than under verum. The development profile described in the publication suggests that all measured effects were reversible within a few hours after the measurements were taken. No other unwanted effects were reported. The authors of the study point out that the study results cannot be transferred to overweight and elderly persons or to persons suffering from additional illnesses or who have taken medication regularly. They advise caution with the use of supplements containing *Citrus aurantium* extracts with synephrine.

The study Haller et al. (2005) (randomised, double-blind, placebo-controlled, 3-arm crossover study with a 1-week washout period) investigated 10 normotensive test persons (mean age 27 years). They were given a one-off dose of mixtures containing the following active ingredients:

- Single-component-product containing *citrus aurantium* with 46.9 mg of synephrine in the form of 3 pills
- Multi-component-product containing *Citrus aurantium* with 5.5 mg of synephrine, 5.7 mg of octopamine and 239.2 mg of caffeine (as well as other active ingredients not described in quantitative terms such as vitamins, minerals, green tea extract, grape seed extract etc.) in the form of 2 capsules,
- placebo.

In the course of 12 hours after ingestion of the product (2), the result showed, after a maximum of two hours compared to the placebo, a difference in systolic blood pressure of 9.6 $\pm$ 6.2 mm Hg and also a difference in diastolic blood pressure of 9.1 $\pm$ 7.8 mm Hg. For product (1), no blood pressure increase was observed. Compared to placebo, the pulse rate after verum administration rose between 11 and 17 beats per minute over the time period measured. This means that after a one-off dose of 46.9 mg of synephrine from *Citrus aurantium* extract, no increase in blood pressure occurred. However, a combination of 5.5

mg of synephrine, 5.7 mg of octopamine and 239.2 mg of caffeine (as well as other active ingredients) did lead to an increase. No other unwanted effects were reported. The authors of the study state that on the basis of monotherapy with synephrine from *Citrus aurantium* alone, no increase in blood pressure in the investigated dose, though an increase in pulse rate was detectable. However, they also say that even significantly lower doses of synephrine led to stimulation of the cardiovascular system including an increase in blood pressure and pulse rate, if synephrine is combination with caffeine and other substances was given. The authors advise caution with the use of such products and recommend that the blood pressure is taken regularly in case such products are taken. In addition, they recommend that persons with existing hypertension or other cardiovascular problems and persons suffering from health impairments which may get worse as a result of sympathomimetic stimulation refrain from taking such products.

A follow-up study conducted by Haller et. al. (2008) (randomised, double-blind, placebo-controlled, 3-arm crossover study) investigated 10 normotensive test persons in their young adulthood. They were given a one-off dose of a product with 21 mg of synephrine from *Citrus aurantium* and 304 mg of caffeine from green tea leaves, guarana and caffeine addition as well as other active ingredients not described in quantitative terms (vitamins, quercetin, naringin, bioflavonoids, additional plant extracts from ginger, cocoa, wasabi, cayenne, willow bark, catuaba). The effects were then compared with those in test persons who had received placebo. Administration of the verum was tested under resting conditions and while test persons were doing physical exercise of moderate intensity. In contrast, the placebo was tested under conditions of physical exertion of moderate intensity only. Measurements were taken over a period of 12 hours following administration of the products. The result showed a diastolic blood pressure under verum of 71.7 $\pm$ 8.7 mm Hg, and under placebo it was 63.0 $\pm$ 4.9 mm Hg (maximum difference after 3 hours). Differences in systolic blood pressure were not considered to be significant by the authors. Nor was any relevant difference found between the groups in terms of pulse rate. The authors of the study qualify the findings by saying that the results of the study cannot be transferred to supplements of other compositions or to persons without controlled exercise training. They also emphasise that the results do not apply to patients who are overweight or suffering from hypertension.

A study undertaken by Min et al. (2005) (randomised, double-blind, placebo-controlled, crossover study with a washout period of at least 7 days) investigated 18 healthy test persons (average age 25 years). They were given a one-off dose of *Citrus aurantium* extract (450 mg extract, standardised to 6 % synephrine, equivalent to 27 mg synephrine; according to the authors, it was standardised to m-synephrine or p-synephrine). The results were then compared to those of a comparative placebo group. The main criteria were the length of the QT interval in the electrocardiogram (ECG) and the blood pressure over a time span of 8 hours following ingestion of the test products (baseline and hours 1, 3, 5 and 8). The result demonstrated that neither the length of the QT interval in the ECG nor the systolic and diastolic blood pressure differed significantly after administration of 27 mg of synephrine compared to placebo. Numerically too, hardly any differences were detected. Apart from headaches in both groups, no further unwanted effects worth mentioning were observed in either group. The authors of the study qualify their findings by saying it is unlikely that they can be transferred to other *Citrus aurantium* products, and they call for more detailed studies on the safety of the tested product after administration of multiple doses. In addition, the authors stated that the results do not apply to a situation where synephrine is taken at the same time as caffeine.

In a study published by Stohs et al. (2011c), a one-off dose of *Citrus aurantium* extract with a quantity of 50 mg of synephrine was, in addition to other products, given to 10 test persons

who were then compared to a placebo group. The main criterion was, among other things, the blood pressure for which, as stated above, no relevant group differences were found. However, the measurement was taken as early as 75 minutes after the product was taken and no more measurements were taken thereafter. Since the blood pressure effects observed by Bui et al. (2006) and Haller et. al. (2005 and 2008) only occurred at later points in time in the course of the study, (maximum after hour 2 and 3), the study by Stohs et al., (2011c) cannot make any further contribution to the issue.

In the study of Seifert et al. (2011) (randomised, double-blind, placebo-controlled, crossover study with a 1-week washout period), a product in the form of a capsule with 13 mg of synephrine (*Citrus aurantium* extract with 6 % synephrine), 176 mg of caffeine in the form of guarana extract and 55.5 mg of green tea extract was given to slightly overweight test persons (n=23; average age 25 years; average BMI 27). The results were then compared to those of a placebo group. Additional active ingredients of the verum product were: bee pollen 1 mg, willow bark powder 1 mg, Panax ginseng root 2 mg, *Garcinia cambogia* extract 2 mg, vanadium 0,15 µg. The development of blood pressure and pulse rate were the main criteria of the study. On the test day, study participants were given one capsule of the verum or placebo three times spread out over the day. The next day, they were given one capsule of verum or placebo, i.e. each person received a total of four capsules with a total of 52 mg of synephrine and 704 mg of caffeine (test day: 39 mg of synephrine and 528 mg of caffeine; following day: 13 mg of synephrine and 176 mg of caffeine). The pulse rate as well as the systolic and diastolic blood pressure were measured as the baseline, i.e. before ingestion and 72-75 minutes after ingestion of the fourth dose (i.e. on the second day). The result did not show any relevant group differences with regard to either the pulse rate or systolic and diastolic blood pressure of test persons. No unwanted effects were observed. The study authors qualified their findings by saying that further studies on the long-term consumption of the verum product would be required as part of a weight loss programme and that at present they are missing. In analogy to the study by Stohs et al. (2011c), the question arises whether Seifert et al. (2011) took blood pressure measurements at the right point in time, since the observed maximum blood pressure effects in Bui et al. (2006) and also Haller et. al. (2005 and 2008) were found to be hour 2 and hour 3 after ingestion. This means that it is unclear overall what contribution the study Seifert et al., 2011 can make to the risk assessment.

Case Reports

A series of case reports are available showing that synephrine from *Citrus aurantium* extract caused unwanted cardiovascular side effects. The following publications are given as examples:

Thomas et al. (2009) reported on a previously healthy 24-year-old man who was a bodybuilder and took a combination product containing, among other things, synephrine from *Citrus aurantium* extract for three weeks with the aim of losing weight. The dosage and combination of the product is not mentioned and unknown to the authors of this assessment. On the day of the event, the product was taken together with an energy drink containing caffeine. Immediately after sports training, the patient suffered a heart attack as a result of acute occlusion of a coronary artery due to thrombus formation. The authors state that a causal relationship with the synephrine intake is possible, although they concede that uncertainties exist.

Stephensen und Sarlay (2009) reported a case of ventricular fibrillation in a 27-year old woman who was otherwise healthy. It occurred during sports training and in connection with the ingestion of a synephrine-containing combination product taken for the purpose of weight

loss. For a period of 2 weeks, the woman had taken a daily dose of 100 mg of synephrine together with 400 mg of caffeine as combination partner. The authors regarded a causal relationship between ingestion of the product and the subsequent incident as likely and advise against taking synephrine-containing products.

Firenzuoli et al. (2005) described a 53-year-old patient with long-standing but well-controlled therapy with Thyroxine. She was given a dose of 500 mg of *Citrus aurantium* extract with 30 mg of synephrine for the purpose of losing weight. Already on the day the product was first taken, the patient suffered from persistent tachycardia which required emergency treatment. One month later, after ingestion of the same product with 30 mg of synephrine the patient again suffered from tachycardia and cardiac arrhythmia which required treatment. The authors assumed a causal relationship between the synephrine intake and the symptoms experienced.

Holmes und Tavee (2008) reported on a 36-year-old previously healthy woman. She suffered from vasospasm of the left medium brain artery with neurological failure and impairment of the upper right extremity, language as well as the right half of her face. The patient had been taking a synephrine-containing product which also contained caffeine for the purpose of losing weight for a few months prior to the incident. The precise composition and dosage of the product was not given. The authors assumed a causal relationship between the synephrine intake and the symptoms experienced.

Moaddeb et al. (2011) reported on a 34-year-old woman who took a combination product containing *Citrus aurantium* extract (standardised for synephrine), guarana and caffeine. After two weeks of taking the product, she required emergency treatment in a clinic due to acute arterial hypertension (blood pressure 234/130 mm Hg and 236/143 mm Hg) and headaches, dizziness and impaired vision. Neither the precise product composition nor the doses of the individual combination partners are given. The authors regarded a causal relationship between the intake of synephrine and the symptoms experienced as likely.

Despite the fact that the reports sometimes contain insufficient information and that further questions regarding the classification of the case reports remain unanswered, the case reports given as examples indicate that *Citrus aurantium* extract with synephrine on its own and in particular in combination with caffeine can potentially cause unwanted cardiovascular effects. The important aspect here is that in some case reports the authors believed that there was a causal relationship between the consumption of *Citrus aurantium* extracts and the occurrence of severe symptoms which can be life-threatening and that the unwanted effects observed in connection with *Citrus aurantium* extract exposure were in some cases confirmed by means of re-exposure.

3.1.2.3.2 Synthetic Synephrine as a Pharmaceutical Product

Pharmacokinetics

According to studies conducted by Hengstmann und Aulepp (1978) in 10 patients, radioactively marked synephrine was quickly reabsorbed following oral administration, with peak concentrations in the blood occurring between 1 and 2 hours after ingestion. The biological half-life after oral administration was 2 hours and bioavailability 22 %. The orally administered substance was excreted as was the case with intravenous application, mainly

via urine (approximately 80 % reappearance) of which only 2.5 % was found in an unchanged state.

Pharmacodynamics

For the synthetically produced racemate consisting of (-)-synephrine and (+)-synephrine in the form of the tartrate, a medicinal use as a cardiovascular drug used for patients suffering from low blood pressure is described (Martindale, 2011). The recommended single dose for the treatment of low blood pressure is 100-150 mg of the tartrate, which can be given 3 times a day. Referring to the racemic base (±)-synephrine, this is equivalent to a single dose of 69 – 103.5 mg and a daily dose of 207 – 310.6 mg. If one follows Stohs und Preuss (2012) in assuming that the effect of the racemate is attributable solely to the (-)-synephrine portion, this is equivalent to a single dose of 34.5 - 52 mg of (-)-synephrine and a daily dose of 103.5 - 155 mg of (-)-synephrine which are said to have blood pressure-increasing effects in patients suffering from hypotension. An corresponding German drug which is no longer available in the German market mentions hypertension, sclerotic vessels, coronary heart disease and tachycardia arrhythmia as contraindications (Rote Liste, 1995).

3.1.3 Exposure

The available literature does not document whether the substance concerned is the pharmacologically more active and naturally occurring (-)-synephrine or (+)-synephrine. Matolli et al. 2005, Avula et al. 2007 and Uckoo et al. 2011 do not differentiate between the two but instead simply talk of synephrine. Wheaton & Stewart 1965 and Arbo et al. 2008 describe that the substance concerned is “p-synephrine”, but do not provide any information on the (+)- or (-)-variety. Only Kusu et al. 1996 differentiated between (-)-synephrine and (+)-synephrine. Exposure assessment was based on the higher (-)-synephrine content.

3.1.3.1 Data Sources

The National Nutrition Survey II (NVS II) of the Max Rubner Institute (MRI) was used as database for the consumption of synephrine-containing foods. The NVS II is currently the most up-to-date representative study on consumption in the German population. The survey which interviewed about 20,000 persons aged between 14 and 80 on their nutrition behaviour using three different dietary assessment methods (dietary history, 24h recall and weighed record) was conducted all over Germany between 2005 and 2006 (MRI 2008).

The analyses of food consumption data were based on data from the “Dietary History” interviews, which were collected by means of the programme “DISHES 05”. The “Dietary History” method was used to interview 15371 persons and retrospectively record their typical consumption over the previous four weeks. It provides good estimates of the long-term intake of substances if foods are grouped into categories or of regularly consumed food. For foods which are only eaten rarely and which are not part of daily nutrition, the data collection period of 4 weeks and the limited accuracy with regard to single food items can lead to an intake underestimation.

3.1.3.2 Synephrine Contents in Foods

Synephrine is an alkaloid found in citrus fruit which is not only contained in the peel of unripe bitter oranges (*Citrus aurantium*), but is also ingested, for example, via sweet oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*) and clementines (*Citrus Clementina*) and their juices (Matolli et al. 2005, Kusu et al. 1996, Uckoo et al. 2011). Synephrine differs widely in content which on the one hand depends on the diameter (the larger the fruit the lower the concentration) and the degree of ripeness of the fruit (the riper the fruit the lower the

concentration) (Arbo et al. 2008, Pelatti, Benvenuti 2007). In addition, the synephrine concentrations are different in different fruit compartments. Thus the concentration in the peel of unripe bitter oranges is 0.056 %, in Albedo (inner layer of the peel) it is 0.028 % while in the flesh it amounts to 0.019 % (Arbo et al. 2008).

The fruit of the bitter orange tree is very sour and bitter, meaning that it is not directly eaten but rather processed into marmalades and syrup. From the dried peel, the baking ingredient candied orange peel is made. The fruit peel and fruit juice of the bitter orange is also used to make liquors such as Cointreau and Curacao. No data on the synephrine contents of these foods and drinks are available, meaning that exposure assessment refer to synephrine intake from citrus fruit as a whole and their preparations.

In order to present the variability of synephrine contents that exists in the literature, **Table 1** gives an overview of the analysed synephrine contents in different types of citrus fruit. The data marked in bold are subsequently used for the exposure assessment. Within one type of food or drink (e.g. orange juice), the minimum and maximum value established in the research are used as a basis for intake levels, so that a range of exposure can be given. A minimum synephrine content of 0.00365 g/kg detected in one orange juice was published by Avula et al. 2007. As maximum value, the quantity of 0.08517 g/kg (Uckoo et al. 2011) measured in another orange juice was used. As regards lemons, synephrine was found only in unripe fruits. These contents were therefore used as a worst-case scenario in exposure assessment.

Table 1: Overview of different synephrine concentrations in different types of citrus fruit in g/kg

Food (drink)	Number of samples	converted to auf g/kg			Source
		Min	MV	Max	
Orange juice of different types	1 (single sample)	0.015 g/kg		0.027 g/kg	Wheaton & Stewart, 1965
Orange juice of different types C. sinensis	3 batches	0.0103 g/kg		0.033 g/kg	Matolli et al., 2005
Orange juice of different types (including C. aurantium)	no information given	**0.00365 g/kg**		0.06065 g/kg	Avula et al., 2007
Orange juice, C. sinensis (Valencia)	no information given		0.0233 g/kg		Kusu et al., 1996
Orange juice C. sinensis (Marrs sweet orange)	3 repetitions, no information given on number of samples		**0.08517 g/kg**		Uckoo et al, 2011
Mandarin juice C. reticulata (Cleopatra)	1 (single sample)		**0.280 g/kg**		Wheaton & Stewart, 1965
Satsumasaft C. unshiu mikan	no information given		**0.0355 g/kg**		Kusu et al., 1996
Mandarin juice C. unshiu	n=30	0.0545 g/kg		0.1602 g/kg	Dragull etl al. 2008
Clementine juice C.clementina tan.	3 repetitions, no information on the number of samples		0.114 g/kg		Uckoo et al, 2011
Tangerine juice	no information given		0.06066 g/kg		Avula et al., 2007
Mandarin juice C. reticulata	3 repetitions, no information on the number of samples		0.078 g/kg		Uckoo et al, 2011
Satsuma fruit C. unshiu (ripe)	no information given		**0.03 g/kg**		Kusu et al., 1996
Mandarin fruit C. deliciosa (unripe)		0.77 g/kg		1.97 g/kg	Arbo et al., 2008
Satsuma marmalade C. unshiu	no information given		**0.121 g/kg**		Kusu et al., 1996
Bitter orange marmalade C. aurantium	13	**0.0009 g/kg**		0.051 g/kg	Avula et al., 2007
Orange fruit C. sinensis (unripe)	no information given	0.62 g/kg		0.99 g/kg	Arbo et al., 2008
Orange fruit C. sinensis (ripe)	3 batches	**0.013 g/kg**		**0.035 g/kg**	Matolli et al., 2005
Lemon fruit C. limon (unripe)	no information given	**0.37 g/kg**		**0.45 g/kg**	Arbo et al., 2008
Lemon juice, (Meyer)	1 (single sample)		**0.002 g/kg**		Wheaton & Stewart, 1965
Lemon juice	no information given		**0 g/kg**		Avula et al., 2007
Lemon juice (Meyer Lemon)	3 repetitions, no information on the number of samples		0.00275 g/kg		Uckoo et al, 2011

The available data on the synephrine content of citrus fruit is characterised by uncertainties. Concentration of synephrine is subject to natural fluctuation and the number of analysed samples is very small.

3.1.3.3 Consumption of Synephrine-containing Products

As described under 3.1.3.2, synephrine is contained as an active ingredient in various citrus fruits and is absorbed when such fruit is eaten. The analyses of NVS II are limited to the following foods and drinks: oranges, orange juice, mandarins, mandarin juice, lemons, lemon juice and marmalades. According to definition of terms of Directive 2001/113/EC on jam, jelly, marmalade and chestnut puree intended for human consumption, marmalades are "products made from citrus fruit". Equivalent products made from other fruit, such as cherries, are called jams. Due to the average consumption quantities calculated for marmalades, jam of 11.5 g/d (all respondents) it is assumed that this consumption quantity does not refer to marmalades made from citrus fruit only but also to jams made from other fruit. This means that an overestimation of the consumption of marmalades has occurred which concur with evaluations of NVS II on sweet spreads. NVS II states that the consumption of sweet spreads is 19 g/d for men and 17 g/d for women. However, apart from fruit spreads, the NVS II evaluation also classifies as sweet spreads marmalades and jams as well as honey, syrup, cocoa and nut-containing spreads (MRI 2008). Due to these uncertainties regarding marmalade consumption, underestimation due to a lack of data on other synephrine-containing foods plays a subordinate role. In consequence, it is assumed that synephrine intake tends to be over rather than underestimated. **Table 2** and **Table 3** give an overview of the consumption quantities for citrus fruit and marmalade in g/d.

Table 2: Average (monthly average) consumption quantities for citrus fruit and marmalade per day in g/d (Basis: all respondents)

		Total	Male	Female	14-18 years	19-24 years	25-34 years	35-50 years	51-64 years	65-80 years
Oranges	N	15,371	7,613	7,758	1,058	1,286	2,201	4,737	3,169	2,921
	MW	21.3	18.7	23.8	12.0	13.9	17.0	19.4	28.9	26.1
	P95	145.0	120.0	145.0	63.1	72.3	103.6	124.3	145.0	145.0
Orange juice	N	15.371	7.613	7.758	1.058	1.286	2.201	4.737	3.169	2.921
	MW	48.5	56.0	41.1	102.0	82.3	59.4	41.1	38.6	28.4
	P95	257.1	300.0	214.3	470.0	420.0	300.1	214.5	200.0	170.1
Mandarins, clementines	N	15,371	7,613	7,758	1,058	1,286	2,201	4,737	3,169	2,921
	MW	8.6	7.4	9.8	7.3	5.9	7.7	8.8	10.0	9.2
	P95	45.8	40.0	58.0	40.0	34.3	45.7	48.6	60.0	48.8
Mandarin juice	N	15,371	7,613	7,758	1,058	1,286	2,201	4,737	3,169	2,921
	MW	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
	P95	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lemon	N	15,371	7,613	7,758	1,058	1,286	2,201	4,737	3,169	2,921
	MW	0.3	0.3	0.3	0.2	0.1	0.2	0.3	0.3	0,4
	P95	0.6	0.7	0.6	0.4	0.6	0.6	0.6	0.6	0,7
Lemon juice	N	15,371	7,613	7,758	1,058	1,286	2,201	4,737	3,169	2.921
	MW	0.9	0.9	0.9	0.6	0.7	0.8	0.9	1.0	1,1
	P95	2.7	2.5	2.8	2.0	1.9	2.3	2.4	3.1	3,6
Jam, marmalade	N	15,371	7,613	7,758	1,058	1,286	2,201	4,737	3,169	2.921
	MW	11.5	11.8	11.3	7.2	6.4	8.3	9.9	13.2	18,6
	P95	45.0	50.0	40.0	35.0	35.0	35.0	40.0	50.0	60,0

Table 3: Average (monthly average) consumption quantities for citrus fruit and marmalade per day in g/d (basis: only consumers)

		Total	Male	Female	14-18 years	19-24 years	25-34 years	35-50 years	51-64 years	65-80 years
Oranges	N	5,404	2,631	2,773	287	377	721	1,666	1,296	1,058
	MW	60.6	54.2	66.7	44.3	47.4	51.8	55.1	70.6	72.2
	P95	192.9	168.8	225.0	152.7	145.2	168.8	168.8	225.0	225.0
Orange juice	N	8,908	4,478	4,430	692	821	1,393	2,867	1,803	1,332
	MW	83.6	95.2	71.9	156.0	129.0	93.9	68.0	67.8	62.3
	P95	357.2	425.7	300.6	600.0	614.3	470.8	300.3	300.0	291.4
Mandarins, clementines	N	5,619	2,638	2,980	328	392	675	1,663	1,238	1,322
	MW	23.5	21.3	25.5	23.6	19.4	25.0	25.0	25.5	20.4
	P95	85.7	82.3	97.1	80.9	80.0	100.0	100.0	120.0	80.0
Mandarin juice	N	4	2	2	0	0	0	0	2	1
	MW	25.8	56.8	5.4	194.3	.	.	.	5.4	17.9
	P95	194.3	194.3	5.4	194.3	.	.	.	5.4	17.9
Lemon	N	7,707	3<824	3<883	403	485	1,003	2,374	1,822	1,620
	MW	0.6	0.6	0.6	0.6	0.3	0.5	0.5	0.6	0.7
	P95	1.0	1.0	1.0	0.9	1.1	1.1	0.9	0.9	1.0
Lemon juice	N	14,658	7,264	7,394	975	1,209	2,085	4,565	3,040	2,784
	MW	1.0	0.9	1.0	0.7	0.7	0.8	1.0	1.0	1.2
	P95	2.8	2.6	2.9	2.0	2.0	2.3	2.4	3.2	3.7
Jam, marmalade	N	9,372	4,287	5,084	509	527	1,204	2,838	2,093	2,201
	MW	18.9	20.9	17.2	14.9	15.6	15.1	16.6	20.0	24.7
	P95	55.7	70.0	50.0	50.0	51.8	50.0	50.0	60.0	70.0

3.1.3.4 Exposure assessment

If a person on their training days takes the recommended dose of 3-6 capsules of a food supplement containing 200 mg of *Citrus aurantium* per capsule and 50 mg of caffeine per capsule before they start their training, this means that their total daily intake can range between 37.5 and 75 mg of synephrine. If they take the recommended dose of 3 times 2 capsules on training-free days, this can lead to a daily intake of synephrine of 75 mg. However, the dosage recommendation “before training” is based on the assumption that training is limited to one session a day. Since it must be taken into account, however, that some people complete several training sessions per day, it is to be assumed that intake of such food supplements, in line with this training pattern, increases accordingly. The synephrine intake in conjunction with caffeine could therefore be several times higher than intended. For this reason, it is to be expected that synephrine intake via food supplements can be significantly higher than via conventional foods. The comparison of exposure via citrus fruit on the one hand and via food supplements containing *Citrus aurantium* on the other hand is based on one training session per day.

Table 4 and **Table 5** show exposure assessment for individual foods, for example oranges and mandarin juice and also the sum total of exposure from all foods. The various consumption quantities are assigned a minimum content of synephrine in one column and a maximum content in the other column to provide a possible range of exposure. **Table 4** shows synephrine intake taking into account all respondents, whereas **Table 5** gives an

overview of synephrine intake among consumers only. In **Table 4**, the total intake, taking into consideration all respondents, amounts to between an average of 0.82 mg/d if an assumption is made that all foods have minimal synephrine contents and 6.66 mg/d, if it is assumed that all foods have maximum synephrine contents. For frequent consumers (P 95) this results in an analogous range between 3.49 and 25.67 mg/d.

Table 4: Synephrine intake per day (mg/d) under the assumption that all foods contain minimum / maximum levels of synephrine (basis: all respondents)

		Total consumption in g/d	Synephrine intake in reference to minimum contents	Synephrine intake in reference to maximum contents
Oranges	N	15371		
	MW	21.3	0.3	0.7
	P95	145.0	1.9	5.1
Orange juice	N	15371		
	MW	48.5	0.2	4.1
	P95	257.1	0.9	21.9
Mandarins, clementines	N	15371		
	MW	8.6	0.3	0.3
	P95	45.8	1.4	1.4
Mandarin juice	N	15371		
	MW	< 0.1	< 0.1	< 0.1
	P95	0	0	0
Lemon	N	15371		
	MW	0.3	0.1	0.1
	P95	0.6	0.2	0.3
Lemon juice	N	15371		
	MW	0.9	0	< 0.1
	P95	2.7	0	< 0.1
Jam, marmalade	N	15371		
	MW	11.5	< 0.1	1.4
	P95	45.0	< 0.1	5.4
Total intake	**For average consumption**		**0.82**	**6.66**
	For high consumption		**3.49**	**25.67**

In **Table 5**, total exposure for medium consumption quantities for consumers averages between 0.88 mg/d under the assumption that all foods contain minimal synephrine levels and 6.73 mg/d if it is assumed that all foods contain maximum synephrine quantities. For frequent users[3] the analogous intake range is between 3.62 und 25.82 mg/d. The intake quantities of synephrine of all respondents are very similar to the intake quantities of consumers. For rarely eaten foods, the intake amount of all respondents would be lower compared to consumers, but this does not apply to these frequently consumed products.

[3] The value is calculated as 95 percentile of the sum for all food categories based on all persons who have eaten at least one of the summed products.

Table 5: Synephrine intake (mg/d) under the assumption that all foods contain minimum / maximum levels of synephrine (basis: consumers only)

		Total consumption in g/d	Synephrine exposure in reference to minimum contents	Synephrine exposure in reference to maximum contents
Oranges	N	5404		
	MW	60.6	0.8	2.1
	P95	192.9	2.5	6.7
Orange juice	N	8908		
	MW	83.6	0.3	7.1
	P95	357.2	1.3	30.4
Mandarins, clementines	N	5619		
	MW	23.5	0.7	0.7
	P95	85.7	2.6	2.6
Mandarin juice	N	4		
	MW	25.8	0.9	7.2
	P95	194.3	6.9	54.4
Lemon	N	7707		
	MW	0.6	0.2	0.3
	P95	1.0	0.4	0.4
Lemon juice	N	14658		
	MW	1.0	0	< 0.1
	P95	2.8	0	< 0.1
Jam, marmalade	N	9372		
	MW	18.9	< 0.1	2.3
	P95	55.7	0.1	6.7
Total exposure	**For average consumption**		**0.88**	**6.73**
	For high consumption		**3.62**	**25.82**

In order to give an estimate of the additionally possible exposure via products containing bitter orange extract, cake and pastries containing candied orange peel, lemon peel products and orange liquors were analysed separately. The consumption quantity for baked goods with candied orange peel amounts to 2.5 g/d on average (basis: consumers only). On the basis of the itemised recipes of the 24-h recall of NVS II it is assumed that the candied orange peel added to these baked goods amounts to 9% which would correspond to 0.225 g/d of candied orange peel. In a study by Arbo et al., a synephrine content of 0.056 % was measured in unripe peel (Arbo et al. 2008). This corresponds to an additional intake of 0.126 mg/d of synephrine and would not markedly change the exposure assessment described above. Furthermore, consumption levels for citrus peel products were evaluated. Under citrus peel products were summarised candied orange peel, candied lemon peel, lemon powder, lemon peel and grated orange peel for which the mean consumption eaten by average consumers amounts to 0.02 g/d or 0.1 g/d for frequent consumers (all respondents). If only consumers are considered, the mean consumption is 0.1 g/d and 0.3 g/d (P 95) respectively. Since no content data is available for lemon peel, the synephrine levels contained in the peel of unripe bitter oranges of 0.056 % are used as a basis. If the mean consumption of consumers of 0.1 g/d is considered, this corresponds to an additional synephrine intake amounting to 0.056 mg/d and would not change the exposure assessment.

The average consumption of orange liquors is 3.6 g/d (basis: only consumers). The synephrine content of orange liquors is not known, meaning that such products cannot be taken into account in exposure assessment.

The synephrine intake levels from conventional foods should, according to information of the EFSA guidance, not be exceeded by synephrine intake via traditional foods (EFSA 2009a). For the intake quantity from conventional foods, the average daily consumption of the population is used as a basis. The exposure assessment shows that the total synephrine intake from conventional foods amounts to 6.66 mg/d for average consumers, if maximum synephrine levels are assumed (s. **Table 4**). This means that the synephrine intake levels from some of the available sports and weight loss products described here are higher than the average daily synephrine intake from conventional foods.

3.1.4 Discussion, Risk Assessment and Conclusion

Some of the synephrine-containing products available on the market which are labelled by the manufacturer as *food supplements* or *dietetic food*, are non-standardised plant-based products with a complex structure which in terms of their chemical composition and possible contaminations are very inadequately characterised and which have been assessed neither from a toxicological nor a clinical viewpoint. Apart from caffeine with its known stimulating effects on the central nervous system which, can be accompanied by unwanted cardiovascular effects (e.g. Fachinformation, 2008 BfR, 2009), quantitative analysis data based on official studies only exist for synephrine as a direct sympathomimetic active ingredient contained in *Citrus aurantium* extracts. The focus of the risk assessment presented here is therefore on the use of *Citrus aurantium* extracts in the offered sports and weight loss products, especially in combination with caffeine. These products have now replaced ephedra-containing food supplements in the North American market, due to a ban on the latter following reports on serious unwanted effects (Health Canada, 2011). The (-)-ephedrine contained in *Ephedra* is a directly active sympathomimetic substance which, in contrast to (-)-synephrine also has a psychostimulating effect on the central nervous system.

What parts of which *Citrus aurantium* variety are chosen as the base material for the *Citrus aurantium* extracts used in products is not known. Similarly little is known about the manufacturing process (extracting agent, duration, temperature). Nor is any information available on whether extracts are added as liquid or dry extracts. Due to these analysis findings, it is known that some products contain a daily dose of synephrine of more than 30 mg, this content being attributable to the *Citrus aurantium* extracts. The question whether the synephrine is only present in the form of the naturally contained (-)-synephrine or whether, for example due to the manufacturing processes, partial conversion into the pharmacologically less active (+)-enantiomer took place was not investigated. Nor do we know in what concentration additional pharmacologically effective biogenic amines such as octopamine, hordenine, N-methyltyramine and tyramine are contained as a result of the use of the *Citrus aurantium* extract. With the exception of the *Citrus aurantium* extract, no information is available on the qualitative and quantitative composition of the plant extracts used in the products.

As regards the use of *Citrus aurantium* extracts in general, both the BfR and the EFSA (EFSA, 2009a) come to the conclusion that the intake quantities from these products should, in terms of the health-relevant quantities of *Citrus aurantium* extracts that they contain, remain within the intake level ranges ingested via conventional foods for persons with average consumption habits. In other words, such products should not lead to a significant increase of the levels of these substances typically absorbed from conventional food. In

respect of the synephrine studied in detail here this means that no more than approximately 6.7 mg of synephrine[4] per day should be ingested with a food supplement. The reason for this is that according to the exposure estimation conducted the total synephrine intake via conventional foods is 6.7 mg/d from conventional foods for average consumers if based on maximum contents. This guarantees that for up to a relatively high intake of up to 19 mg of synephrine per day from conventional foods, the synephrine intake from both conventional foods and food supplements does not exceed the value of 25.7 mg of synephrine per day (total synephrine intake from conventional foods based on maximum synephrine contents for frequent consumers; see **Table 4**). This means that *Citrus aurantium* extracts could, depending on their synephrine concentration, be added to food supplements in quantities which correspond to a daily intake of a maximum of 6.7 mg of synephrine.

The daily synephrine intake calculated in accordance with the consumption recommendation is, on account of some products offered in the food industry, significantly higher than the average daily synephrine intake under the assumption of average consumption of foods which naturally contain synephrine. Even the synephrine intake of frequent consumers of the relevant foods is below the intake of the daily dose of some products.

For the assessment of synephrine intakes from *Citrus aurantium* extracts, data from *in vitro* experiments, animal experiments and human studies are available showing that the sympathomimetic effects on the cardiovascular system are most relevant for risk assessment. This overview does not discuss the results of animal experimental studies, since the dosages chosen were so high that no NOAEL values could be inferred, meaning that they are hardly meaningful compared to the human data. Hansen et al. (2012) conducted a 28-day study as part of the NTP programme with two *Citrus aurantium* extracts. One of them was selectively enriched synephrine with comparatively low quantities of octopamine, hordenine and tyramine, whereas the other extract had comparatively higher levels of these biogenic amines relative to the synephrine concentration. From this study, it can be inferred, however, that components contained in the *Citrus aurantium* extracts other than synephrine contribute to the cardiovascular effects.

As regards human data, it must be emphasised that only clinical studies with a one-off intake of synephrine from *Citrus aurantium* extracts are available (with the exception of the study by Seifert et al., 2011 which, however, is hampered by severe methodological flaws). However, there is a lack of suitable studies based on repeated and long-term intake of *Citrus aurantium* with synephrine. This means that these studies particularly lend themselves to assessing acute effects. In addition, in the available studies, the number of study participants (e.g. 13 test persons per study arm) is relatively small, and only young, healthy normotensive persons were included in the studies. Last but not least, the cited studies did not specify the contents of naturally contained (-)-synephrine, nor the quantities of (+)-synephrine that may have formed in the course of the extraction process. If the latter was contained to a higher degree in the extract applied in the studies and the indicated synephrine doses are equated with those of (-)-synephrine, this would lead to the inference of an erroneously high effective those. This false conclusion would in turn lead to an underestimation of the risk.

The clinical studies on humans using *Citrus aurantium* extracts containing synephrine show a potentially activating effect of synephrine on the cardiovascular system with an increase in arterial blood pressure and pulse rate. This potential relates to clinically relevant sympathomimetic effects which are reinforced in combination with the substance caffeine.

[4] This value refers to total synephrine consisting of natural (-)-synephrine and (+)-synephrine that may have formed from it during the manufacturing process.

Following a one-off dose of 54 mg of synephrine in *Citrus aurantium* extract, healthy test persons without any physical exercise showed, compared to placebo, an average increase in systolic blood pressure of 7.3 mm Hg as well as slight acceleration of the pulse rate. Following administration of *Citrus aurantium* with 46.9 mg of synephrine, the pulse rate increased though not the blood pressure measured. At 27 mg of synephrine in *Citrus aurantium* extract given as a one-off dose, there were no differences with regard to the length of the QT interval in the electrocardiogram (ECG), or in relation to systolic and diastolic blood pressure. If 239.2 mg of caffeine and 5.5 mg of synephrine (together with 5.7 mg of octopamine) were given at the same time, the systolic blood pressure rose by an average of 9.6 mm Hg and the diastolic blood pressure by an average of 9.1 mm Hg. A further study showed that a one-off dose of 21 mg of synephrine in *Citrus aurantium* extract together with 304 mg of caffeine caused an average group difference in diastolic blood pressure compared to placebo of approximately 9 mm Hg (increase under verum). In contrast, no differences were found in systolic blood pressure or pulse rate.

In the risk assessment, the results of human studies must therefore be taken into account according to which, above a single dose of 27 mg of synephrine per day, a dosage range begins where relevant cardiovascular effects are to be expected. Moreover, in combination with caffeine, these effects already begin to manifest themselves for synephrine quantities in the mid one-digit milligram range. For some products it must be taken into consideration that such intake can lead to an increase in blood pressure and pulse rate. In addition, it is to be expected that in line with the advertising focus, people who are overweight and who carry higher risks for cardiovascular disease and athletes who put increased stress on their cardiovascular system are particularly likely to take these products. This means that persons who are typically within the target group for these products can show increased sensitivity to the cardiovascular effects induced by sympathomimetic substances. It must be pointed out that the existing studies were conducted with young, healthy normotensive test persons and that persons suffering from arterial hypertension, overweight, older age, cardiovascular diseases and / or people who were on medication may be particularly sensitive to such substances.

The data relating to the use of the racemate of synephrine tartrate as a drug for treating cardiovascular complaints resulting from hypotension concur with the results from clinical studies on dose-effect-relationships for synephrine as a component of citrus aurantium extract. According to these data, a blood pressure increasing effect in individuals suffering from hypotension was and is attributed to single doses of 34.5 – 52 mg of (-)-synephrine and daily doses of 103.5 - 155 mg of (-)-synephrine. For a corresponding German drug which is not anymore available on the market in the Federal Republic of Germany, hypertension, sclerotic vessel changes, coronary heart diseases and tachycardial arrhythmia were, among others, mentioned as contraindications (Rote Liste, 1995).

Equally, the exemplary case reports indicate that combination products containing synephrine may, under certain conditions, have the potential to cause coronary damage, tachydardia and cardiac arrhythmia. These symptoms were reported about athletes and persons undergoing weight loss procedures following intake of *Citrus aurantium* extract (in combination with caffeine). The important aspect here is that in several case reports the authors either observed causal relationships between *Citrus aurantium* extract intake and the occurrence of severe problems (e.g. tachycardia requiring treatment, cardiac arrhythmia, acute arterial hypertension, cerebral vasospasm) which could be life-threatening or considered these relationships possible. In one case, the occurrence of unwanted effects in connection with *Citrus aurantium* extract exposure was even confirmed by re-exposure.

For the risk assessment, it is of particular importance that synephrine is not taken on its own but together with caffeine. This is all the more worth to be considered, as the Fachinformation (2008) for caffeine tablets (single dose: 100-200 mg) describes that caffeine has a synergetic effect in relation to the tachycardial effect of sympathomimetic substances (to which synephrine and other phenylethylamines contained in *Citrus aurantium* extracts belong) and that tachycardia is a side effect even at low pharmaceutical caffeine doses.[5] In addition, the presence of cardiac arrhythmia is contraindicated both for caffeine (in single doses over 100 mg) and for synephrine. Some sports and weight loss products available in the market contain synephrine and caffeine in doses which correspond to or even exceed the range of pharmaceutically used single doses of synephrine or caffeine containing single agent drugs.

Furthermore, it must be considered that other common foods and drinks with relatively high caffeine or synephrine contents, such as coffee and energy drinks and orange juice respectively, may additionally be consumed at the same time. This means that the acute effective doses of caffeine and synephrine are increased further (in case of a 250 ml energy drink, for example, by 80 mg of caffeine). This is not unrealistic as shown by the above description of Thomas et al. (2009): . They discuss a possible connection between a heart attack immediately following sports activity,and the intake of a combination product containing synephrine from *Citrus aurantium* extract together with an energy drink. Reference is made to the existing BfR reports on cases of cardiovascular complications in connection with sports activities and energy drink consumption (BfR, 2008 and 2009).

Overall Assessment and Conclusion

Some sports and weight loss products are geared to persons aiming to reduce their body weight and as part of their effort undertake physical exercise. Such products can contain caffeine and synephrine in pharmaceutically active doses in which they have cardiovascular effects and which significantly exceed the single doses in which they were or still are used in single agent drugs. This is all the more concerning, as it is to be expected that the two substances mutually reinforce each other's effects on the cardiovascular system. This applies to the known synergy between caffeine and sympathomimetic substances which include synephrineregarding an induction of increase in heart frequency and possibly of cardiac arrhythmia. In addition, for some products, a blood pressure-increasing effect of synephrine in combination with caffeine is to be expected. This effect already manifests itself in much lower doses. Other effect amplifications through other sympathomimetic phenylethylamines contained in *Citrus aurantium* extracts or through caffeine and synephrine intake from other foods must be additionally considered.

Since, in addition, the daily synephrine intake is higher for some of these products than the average daily intake via conventional foods and the synephrine is present in combination with caffeine, there are sufficient reasons to suspect that these products do not comply with the requirements of Art. 14. VO (EC) 178/2002 and that they can be classified as unsafe.

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[5] Additional unwanted effects of caffeine and on the central nervous system are not discussed here.

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