The Director General

Maisons-Alfort, 7 November 2016

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

on the risks associated with the consumption of food supplements for athletes seeking to develop muscle or reduce body fat

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring 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 the necessary information concerning these risks as well as the requisite expertise 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 published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 7 November 2016 shall prevail.

On 2 January 2014, ANSES issued an internal request to conduct an expert appraisal on the following issue: risks associated with the consumption of food supplements for athletes seeking to develop muscle or reduce body fat.

1. BACKGROUND AND PURPOSE OF THE REQUEST

■ Context

In France, food supplements are regulated by Decree No. 2006-352 of 20 March 2006. The vitamin and mineral substances that can be used in the manufacture of food supplements are listed by the Ministerial Order of 9 May 2006. Regarding other substances, in order to be incorporated in food supplements, they must not be subject to any prohibition by the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF). Regulation (EC) No 1925/2006 provides for a positive list of the ingredients that can be used in their composition to be compiled progressively. The list of plants authorised in food supplements is laid down by the Decree of 24 June 2014, which indicates the conditions of their use. These regulatory provisions mainly aim to manage the health risks associated with the consumption of food supplements.
European Commission Regulation (EC) No 1924/2006 harmonises the rules concerning the use of nutrition or health claims. This Regulation is based on the principle of positive lists; only the claims appearing on the European lists can be used.

Lastly, food supplements must be notified to the DGCCRF before they can be placed on the market.

Between the establishment of the national nutrivigilance scheme in 2009 and 16 February 2016, forty-nine reports of adverse effects likely to be associated with the consumption of food supplements for athletes[1] were brought to the attention of ANSES. Seventeen of these reports contained enough information to be analysed for their causality. The adverse effects reported in these cases are primarily cardiovascular (tachycardia, arrhythmia and stroke) and psychological (anxiety disorders and nervousness). Causality was considered likely in eight cases of adverse effects.

Although this type of food supplement is traditionally used by bodybuilders, its consumption has been expanding in other sports disciplines. Moreover, since it appears that this practice is increasingly being encouraged on forums and by products that are highly visible on the Internet, it is highly unlikely that consumers will benefit from medical supervision or the advice of healthcare professionals. In this context, ANSES issued an internal request with a view to assessing the risks associated with the consumption of food supplements for athletes seeking to develop muscle or reduce body fat.

■ Subject of the request

The internal request focused on the adverse effects of food supplements consumed by athletes seeking to increase muscle mass or reduce body fat. In the present report, the generic term "food supplements for athletes" will encompass **exclusively** food supplements seeking to develop muscle mass or reduce body fat. Consequently, energy bars, recovery products or pre-competition snacks that can be consumed in the framework of physical activity are not covered by this internal request.

In addition, food supplements presented as able to reduce body fat may also be used by consumers wishing to lose weight in a context other than sport. Regarding food supplements seeking to reduce body fat (often called "fat burners"), only those intended specifically for athletes are concerned by this internal request.

Lastly, the objective of the report is to assess the health risks and not the possible effectiveness of food supplements for athletes.

2. ORGANISATION OF THE EXPERT APPRAISAL AND METHOD

■ 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)".

The issues being appraised fall within the scope of the Expert Committee (CES) on "Human Nutrition". ANSES entrusted the expert appraisal to external rapporteurs and to the Working Group (WG) on "Nutrivigilance". The methodological and scientific aspects of the work were presented to the CES on 9 June 2016. It was adopted by the CES at its meeting on 6 July 2016.

[1] Food supplements seeking to develop muscle or reduce body fat.

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).

Method

The forty-nine reports of adverse effects likely to be associated with the consumption of food supplements for athletes were collected in the framework of the nutrivigilance scheme. Seventeen reports underwent a causality analysis, carried out using the method developed by ANSES (2011), while the others were regarded as inadmissible due to a lack of information (product not clearly identified, dates of consumption unknown, etc.). The substances mentioned in this report are the ingredients presented as having an action on muscle mass or body fat and fulfilling one of the following conditions:

- included in the composition of food supplements that have been the subject of admissible nutrivigilance reports;
- considered, by the specialists consulted in the framework of the request, as being potentially consumed by athletes.

The products concerned by the present Opinion are thus not solely confined to food supplements that comply with French regulations, but also include products presented as food supplements and sold illegally on the Internet or in sports centres.

On the other hand, the present Opinion does not address the risks associated with the misuse of drugs for the purposes of muscle development or body fat reduction in a sporting context.

The Toxicovigilance Coordination Committee (CCTV) was consulted in order to produce a summary of the cases notified to the poison control centres. This summary gave rise to a report (CCTV 2015).

ANSES contacted the health agencies in various European countries, Canada and the United States to obtain any insights they may have gained from surveillance and expertise on the safety of food supplements for athletes. The answers received are included in the WG's expert appraisal report.

Lastly, several stakeholders were consulted by ANSES in the framework of this internal request:

- the French Federation of Weightlifting, Weight Training, Athletic Strength and Bodybuilding (FFHMFAC);
- the French Society for Exercise and Sport Medicine (SFMES);
- the French Association of Dieticians-Nutritionists (AFDN).

These organisations were invited to respond to questions posed by the Agency and to bring to its attention any information considered useful in the framework of the assessment of the risks associated with the consumption of food supplements for athletes. The accounts of these hearings are included in the WG's expert appraisal report.

3. ANALYSIS AND CONCLUSIONS OF THE CES AND THE WG

The discussion and conclusions presented below summarise the collective expert appraisal report of the WG on "Nutrivigilance" and the CES on "Human Nutrition".

Composition of food supplements for athletes

Many substances may be included in the composition of food supplements for athletes. They are often presented as able to increase muscle mass or reduce body fat. Some of these substances are included on the list of prohibited substances produced by the World Anti-Doping Agency and revised every year, and are adopted in France annually by a decree of the Ministry of Sports. However, although the food supplements containing these substances are not authorised for sale, it is still possible to obtain them, in particular on the Internet. Some athletes may thus consume prohibited substances, sometimes unknowingly if the food supplement has been adulterated, i.e. when a substance has been added by the manufacturer but is not mentioned on the labelling. This risk has been identified in particular for food supplements claiming to have an action on muscle mass or body fat (Geyer *et al.* 2008; Geyer *et al.* 2004).

- Non-prohibited substances
    - Substances aiming to increase muscle mass

Proteins and amino acids and metabolites:

Proteins: two types of proteins are consumed: whey proteins and caseins, which represent the two main protein fractions in milk. The claims "protein contributes to a growth in muscle mass" and "protein contributes to the maintenance of muscle mass" have been authorised and are included in Commission Regulation (EC) No 432/2012.

Branched-chain amino acids (BCAA): these include leucine, isoleucine and valine. These are essential amino acids, i.e. they must be provided by food.

Glutamine: glutamine is an amino acid produced predominantly by the skeletal muscles (Bowtell *et al.* 1999; Golden *et al.* 1982). It is one of the so-called conditionally-essential amino acids, since it may be produced by the body in insufficient quantities in certain specific situations (states of acute aggression for example), when it then has to be provided by food.

β-hydroxy-β-methylbutyrate (HMB) and α-ketoisocaproate: β-hydroxy-β-methylbutyrate or HMB is a metabolite from the catabolism of leucine. Leucine is converted to α-ketoisocaproate, which in turn is metabolised partly to HMB.

L-tyrosine: L-tyrosine is an α-amino acid which is not strictly essential, since it is synthesised from phenylalanine. It is involved in the synthesis of catecholamines: adrenaline, noradrenaline, dopamine and L-DOPA. It is also a precursor of melanin and thyroid hormones (Berg *et al.* 2013; EFSA 2013b).

β-alanine: β-alanine is a β-amino acid produced *in vivo* by the degradation of dihydrouracil and carnosine and metabolised to acetic acid. It is a constituent of certain peptides such as carnosine and anserine, as well as of pantothenic acid (vitamin B5), itself a constituent of coenzyme A (Trexler *et al.* 2015).

Arginine: arginine is an α-amino acid classified among the conditionally-essential amino acids. Arginine is synthesised from citrulline, itself derived from glutamic acid or glutamine. The endogenous synthesis of arginine is mainly renal. Arginine enables the synthesis of nitric oxide (NO) by NO synthase (Lorin *et al.* 2014); it is also a precursor of creatine (Berg *et al*. 2013; EFSA 2016b).

Creatine: Creatine is an endogenous derivative of amino acids. It is synthesised by the kidney, the liver and the pancreas from three amino acids: arginine, glycine and methionine (Juhn and Tarnopolsky 1998; Tarnopolsky and Beal 2001; Walker 1979). The claim "creatine increases

physical performance in successive bursts of short-term, high intensity exercise" has been authorised and is included in Commission Regulation (EC) No 432/2012.

DHEA:

DHEA is a particular case of a substance that is not prohibited in food supplements, and is described later with the anabolic androgenic steroids.

Plant extracts:

Tribulus terrestris: *Tribulus terrestris* L. (bindii, puncture vine) is an annual creeping plant, belonging to the Zygophyllaceae family. A large number of compounds have been identified in the species, including saponins, flavonoids and alkaloids (Chhatre *et al.* 2014; Kang *et al.* 2014).

Plants of the genus *Smilax*: the extracts included in the composition of some food supplements for athletes come from *Smilax aristolochiifolia* Mill. (syn. *Smilax medica* Schltdl. & Cham.), *Smilax officinalis* Kunth. and *Smilax ornata* Lem. (syn. *Smilax regelii* Killip & C.V.Morton) (King *et al.* 2012). These extracts are particularly rich in sterols and steroidal saponins (Bucci 2000).

Minerals:

Vanadium: vanadium is a mineral that is poorly absorbed in the duodenum (less than 1%) after transformation into vanadyl (V^{4+}) in the stomach (Arnaud 2001). It is transported in the bloodstream by transferrin. It accumulates primarily in the kidney and to a lesser degree in the liver, bones and spleen (Korbecki *et al.* 2012).

Chromium: chromium is a mineral that is often formulated as chromium picolinate due to its greater bioavailability (Hasten *et al.* 1992).

- Substances aiming to reduce body fat

Regular practitioners of weight training or strength training are interested in substances that claim to be able to melt body fat. These substances are presented as "*fat burners*".

L-carnitine:

Carnitine is a quaternary amine. The only isomer with biological activity is L-carnitine (Hathcock and Shao 2006).

Choline:

Choline is provided by food or synthesised endogenously. It plays a functional and structural role in cells, and in particular is a precursor of acetylcholine and phospholipids. It is also involved in lipoprotein metabolism and is part of the group of methyl donors (Penry and Manore 2008; Zeisel 2015).

2-phenylethylamine (PEA):

2-phenylethylamine or β-phenylethylamine (PEA) constitutes the basic structure of a family of neuroactive compounds, in particular certain endogenous neurotransmitters such as dopamine,

adrenaline and noradrenaline (Passmore and Robson 1970). In the mammalian brain, endogenous production of PEA is very low (a few nanograms per gram of nervous tissue). The synthesis of PEA results from the decarboxylation of phenylalanine by the dopaminergic neurons. The presence of PEA has been identified in certain foods, in particular cocoa (Chaytor *et al.* 1975).

Plant extracts:

Cissus quadrangularis: *Cissus quadrangularis* L. is an edible plant belonging to the Vitaceae family. The extracts from this plant in particular contain vitamin C and substances related to anabolic steroids (Potu *et al.* 2009).

Coleus forskohlii: *Coleus forskohlii* (Willd.) Briq., synonym *Plectranthus forskohlii* Willd., belongs to the family of Lamiaceae. The name *Plectranthus barbatus* Andrews is sometimes used to designate *Coleus forskohlii*. This plant produces diterpenes, including forskolin (Alasbahi and Melzig 2010).

Garcinia cambogia: the current valid name of the Malabar tamarind is *Garcinia gummi-gutta* (L.) Roxb. (Clusiaceae). It is most often known by the name *G. cambogia* [Gaertn.] Desr. Extracts from the pericarp of the fruit of this species contain (-)-hydroxycitric acid or HCA (2*S*, 3*S*-hydroxycitric acid) (Semwal *et al.* 2015).

Magnolia officinalis: The bark of *Magnolia officinalis* Rehder & E.H.Wilson (Magnoliaceae) is described by monographs in the Chinese and European pharmacopoeias. The main active ingredients identified are lignans (magnolol, honokiol) (Yan *et al.* 2013).

Substances extracted from plants:

Evodiamine: evodiamine is one of the main alkaloids from the evodia fruit (*Evodia ruticarpa* (A. Juss) Hook.f. & Thomson, Rutaceae).

Caffeine: caffeine or 1,3,7-trimethylxanthine belongs to the family of methylxanthines. It is found in more than sixty plants, including coffee, tea, kola nut, guarana and maté; coffee and tea are the main food vectors. Caffeine may also be produced by chemical synthesis (ANSES, 2013a; Heckman *et al*. 2010).

Theobromine: theobromine is a methylxanthine found in the fruit of the cacao tree and in smaller quantities in tea leaves (Hicks *et al.* 1996), guarana seeds (Weckerle *et al.* 2003), maté leaves (Cardozo Jr *et al.* 2007) and kola nuts (Burdock *et al.* 2009).

***P*-synephrine**: *p*-synephrine is an alkaloid naturally present in several species of the genus *Citrus*, in particular *Citrus* x *aurantium* L. or bitter orange, used in food supplements (ANSES 2014).

Raspberry ketone: "raspberry ketone" (4-(4-hydroxyphenyl)butan-2-one) is one of the main aromatic compounds of the fruit of the raspberry (*Rubus idaeus* L., Rosaceae) (Beekwilder *et al.* 2007).

- Prohibited substances

Although certain substances are prohibited from sale in France, their presence has been identified in some food supplements liable to be consumed in France. Their presence constitutes fraud, and the food supplements that contain them are regarded as adulterated. The development of transnational orders via the Internet increases the possibility of ordering and importing foodstuffs that do not comply with French regulations.

The adulteration of foodstuffs may expose an athlete to a positive drug test. One of the actions in the area of doping prevention involves ensuring that people practising a physical and sporting activity do not ingest doping substances, especially through food supplements and other foodstuffs intended for athletes. The French standard NF V 94-001 (AFNOR 2012) has described the requirements relating to the development and manufacture of food supplements and other foodstuffs for athletes that are free from substances prohibited by the UNESCO International Convention against Doping in Sport (transposed into French law by Act No. 2007-129 of 31 January 2007 and ratified by Decree No. 007- 503 of 2 April).

- Substances aiming to increase muscle mass

Anabolic androgenic steroids: the precursors of anabolic steroids (prohormones) are androgenic precursors which, after ingestion, are transformed into derivatives of testosterone (King *et al.* 2012). Although available on the Internet, these substances are prohibited from sale in many countries and are *a fortiori* prohibited in athletes likely to be subject to drug tests. These substances include androstenedione and androstenediol (Brown *et al.* 2006). Dehydroepiandrosterone (DHEA) is a particular case since it is included on the list of doping substances of the World Anti-Doping Agency (WADA)[2], but is not prohibited in food supplements (Webb *et al.* 2006).

- Substance aiming to increase muscle mass and reduce body fat

Clenbuterol: clenbuterol is a β-adrenergic receptor agonist used as bronchodilator medication for veterinary purposes and misused by some athletes for its supposed anabolic and lipolytic effects. This product is included on the list of doping substances (Prather *et al.* 1995).

- Substances aiming to reduce body fat

Ephedrine, pseudoephedrine and phenylpropanolamine: ephedrine and its analogues, pseudoephedrine and phenylpropanolamine, are alkaloids used by certain athletes, and extracted from plants of the genus *Ephedra* (Ephedraceae), in particular *Ephedra sinica* Stapf. also known as *Ma huang* (Bergeron *et al.* 2010). They are structurally similar to amphetamines (Magkos and Kavouras 2004).
Despite the ban on the marketing of ephedrine in France and many other countries, this substance is still used and is found in food supplements for athletes practising weight training. Pseudoephedrine and phenylpropanolamine are decongestants of the upper airways; pseudoephedrine is still found in commercial French pharmaceutical products while phenylpropanolamine has been withdrawn.

Sibutramine: sibutramine was a drug indicated in the treatment of obesity and overweight, whose marketing authorisation (MA) was suspended in 2010 following the results of a long-term study that identified an increased risk of cardiovascular complications. It had been on the list of substances prohibited in competition for athletes since 2006. Despite these bans, it is found in food

[2] WADA's prohibited list aims to include as many known substances and methods as possible that meet two of the following three criteria:
a. The substance or method has the potential to enhance or enhances sports performance
b. The substance or method presents an actual or potential health risk to the athlete
c. The use of the substance or the method violates the spirit of sport (as defined in the World Anti-Doping Code).

supplements targeting weight loss, sometimes without its presence being mentioned on the labelling (Muller *et al.* 2009).

1,3-dimethylamine (DMAA): 1,3-dimethylamylamine (DMAA), also called methylhexanamine, is a stimulant molecularly similar to a sympathomimetic, tuaminoheptane, both of which have been included since 2010 on the list of substances prohibited in competition. It was used in decongestant sprays for nasal mucosa. Although prohibited from sale, it is still found in food supplements (mainly on the American market), in which it acts as an anorectic stimulant.

2,4-dinitrophenol (2,4-DNP): 2,4-dinitrophenol (2,4-DNP) had historically been used as a dye, preservative, herbicide and explosive since the 1930s. In 1938, the emergence of cases of adverse effects (mainly cataracts but also fatalities) led the American authorities to prohibit its prescription (Yen and Ewald 2012).

■ Adverse effects of food supplements for athletes

- Cases from nutrivigilance

The nutrivigilance scheme collected forty-nine reports of adverse effects likely to be associated with the consumption of food supplements for athletes, between its establishment in 2009 and 16 February 2016. Seventeen of these reports contained enough information to be analysed for their causality, which was considered likely in eight of the cases.

The adverse effects reported were mainly cardiovascular and less frequently psychological, hepatic, renal, neurological, dermatological, gastroenterological, etc.

- Literature data and mechanistic elements likely to explain the reported adverse effects

The experts conducted a review of the bibliography on the adverse effects of substances in food supplements for athletes seeking to develop muscle mass or reduce body fat. Only the adverse effects of greatest concern were addressed.

o Cardiovascular effects

<u>Non-prohibited substances</u>

Caffeine alone

Caffeine has adrenergic effects and probably potentiates the effects of other stimulants by increasing the levels of cAMP (cyclic adenosine monophosphate). It can thus cause agitation, tremors and arrhythmia (Dhar *et al.* 2005). Tachycardia is a classic symptom of caffeine intoxication. These effects appear to be even more marked where consumption is high and the consumer is 'naive', i.e., unaccustomed to caffeine consumption, which is often the case with the adolescent population (ANSES, 2013a).

Caffeine associated with *p*-synephrine

The health effects of caffeine combined with synephrine were discussed in an opinion issued by ANSES (2014) on the risks associated with the presence in food supplements of *p*-synephrine or ingredients obtained from *Citrus* spp. fruits containing this substance. In the conclusions of the opinion, ANSES recommends avoiding combining *p*-synephrine with caffeine or preparations containing it, as the association of these substances induces a risk of occurrence of cardiovascular

events. In addition, the slight vasoconstrictor effects exercised by the synephrine acting on the α1-adrenergic receptors (Hibino *et al.* 2009) are exacerbated by the association with caffeine (Hansen *et al.* 2012).

Evodiamine
The adverse effects of evodiamine may be related to its ability to activate the capsaicin receptor underlying its positive inotropic and chronotropic actions (Kobayashi *et al.* 2001; Shoji *et al.* 1986) and vasodilator actions (risk of hypotension) (Kobayashi *et al.* 2000).

Prohibited substances

DMAA
DMAA, which has the same stimulating action as ephedrine, has adverse cardiovascular effects similar to those observed with stimulants such as ephedrine and amphetamines. The adverse effects reported in the literature are myocardial infarction, arrhythmia and cardiomyopathies (BfR 2012; Cohen 2012; Karnatovskaia *et al.* 2015). The study by Bloomer *et al.* (2011) suggests that the increase in blood pressure caused by DMAA is dose-dependent.

In general, stimulants are arrhythmogenic. To the extent that physical exercise can also lead to arrhythmia, the risk of the onset of rhythm disorders is increased with the concomitant use of stimulant(s). In addition, physical exercise enhances the thermogenic effect of stimulants, which increases the risk of occurrence of exercise-induced hyperthermia (Hatton *et al.* 2014).

Ephedrine alkaloids
Ephedrine and its analogues exert an indirect sympathomimetic action by stimulating the release of a neurotransmitter, noradrenaline (or norepinephrine) (Cannon and Nedergaard 2004). The adverse effects of ephedrine alkaloids are directly related to this action. In addition to their hypertension effects, they cause vasoconstriction and coronary vasospasm that are more pronounced in individuals with an increase in vagal tone, such as athletes. Many cases of adverse effects associated with these substances have been reported, in particular myocardial infarction in otherwise healthy young individuals, arrhythmia in pregnant women, tachycardia, palpitations, stroke, transient ischemic attacks and sudden death due to brain haemorrhage. The cases of myocardial infarction can be explained by vasoconstriction and hypercoagulability induced by the alkaloids extracted from the ephedra plant that reduce the intake of oxygen in the coronary artery network (Andraws *et al.* 2005; EFSA 2013a).

Caffeine combined with prohibited sympathomimetic substances
Caffeine can also cause adverse effects when it is associated with other substances; one case of stroke was reported with caffeine combined with ephedrine (Vahedi *et al.* 2000). One case of cerebral haemorrhage was also reported in a young man aged 21 years who consumed DMAA and caffeine (Gee *et al.* 2010).

The concomitant consumption of caffeine and other substances with sympathomimetic activity (ephedrine, *p*-synephrine, DMAA, clenbuterol, sibutramine) potentiates their specific effects and may increase the tachycardia effects of caffeine. This assumption has been widely confirmed with ephedrine (Greenway *et al.* 2004; Vukovich *et al.* 2005) and may be extended to other sympathomimetic substances.

Anabolic androgenic steroids

Anabolic androgenic steroids can affect the cardiovascular system (Basaria 2010). Gårevik *et al.* (2012) showed that after intramuscular administration of testosterone, blood concentrations of total cholesterol were higher. This rise is due to an increase in expression of the gene encoding the enzyme responsible for cholesterol synthesis, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, thus increasing the concentrations of LDL cholesterol. Therefore, taking anabolic androgenic steroids can promote the development of coronary artery disease (Hatton *et al.* 2014).

Anabolic androgenic steroids can also cause cardiomyopathy with left ventricular hypertrophy. These effects contribute to increasing the risk of sudden death (Nascimento and Medei 2011). The cardiomyopathies induced by anabolic androgenic steroids depend on the type and dose of steroid administered, and seem to be at least partially reversible on cessation of consumption and with drug treatment (Momaya *et al.* 2015; Rothman *et al.* 2011).

Anabolic steroid supplementation leads to an increase in plasma homocysteine concentrations. Homocysteine is a derivative of methionine metabolism and a risk marker for atherosclerosis and atherothrombosis. The consumption of anabolic substances by bodybuilding practitioners increases homocysteinaemia and increases the risk of thromboembolic events (Ebenbichler *et al.* 2001; Graham *et al.* 2006).

2,4-DNP

The toxicity of 2,4-DNP is directly related to its mechanism of action: by decoupling mitochondrial oxidative phosphorylation, it cancels the electrochemical proton gradient, preventing the functioning of ATP synthetase. It results in cells being unable to produce ATP and an increase in thermogenesis. The acute toxicity of 2,4-DNP can result in hyperthermia, tachycardia, excessive sweating and cardiac arrest. In addition, 2,4-DNP causes potassium retention in the kidneys, leading to hyperkalaemia. Deaths identified as being caused by 2,4-DNP have emerged in the past fifteen years. The lowest lethal dose reported is 300 mg/d for six weeks. With acute consumption, the lowest lethal dose reported is 2.3 g. The time between consumption and death is on average 14 h (Grundlingh *et al.* 2011; Yen and Ewald 2012).

Clenbuterol

Clenbuterol, which is a β_2-adrenergic agonist (Li *et al.* 2012), has adverse effects associated with its sympathomimetic activity. The cases of adverse effects reported are essentially tachycardia, hypokalemia, myocardial ischemia and palpitations, sometimes accompanied by agitation and anxiety (Spiller *et al.* 2013).

Sibutramine

Sibutramine is a serotonin and noradrenaline re-uptake inhibitor (Bray and Greenway 2007). Its adverse effects result from its sympathomimetic action. It increases blood pressure and heart rate, which is why, when it was authorised, it was contraindicated in patients with a history of cardiovascular disease (Florentin *et al.* 2008).

- Neuropsychiatric effects

<u>Non-prohibited substances</u>

Caffeine

Caffeine has many biochemical targets in the central nervous system, such as the GABA receptors and adenosine A_1 and A_{2A} receptors. Taking caffeine may thus induce psycho-behavioural disorders including nervousness, irritability, anxiety and even panic attacks or psychotic phenomena, including hallucinations. Single doses of 300 or 400 mg of caffeine can induce phenomena of mental tension and anxiety, all the more so if the subject is in a stressful environment. It seems that the mental complications are mainly present in subjects who previously suffered from a psychiatric condition, in particular chronic generalised anxiety disorders and panic attacks. In such subjects after acute caffeine consumption, symptoms such as nervousness, anxiety disorders or even distress, nausea, palpitations and tremors can appear. Chronic use of high doses of caffeine (i.e. higher than 300 mg/d) may increase the risk of experiencing hallucinations, in particular in situations of stress (ANSES, 2013a).

PEA

In a review article, Wolf and Mosnaim (1983) indicate that endogenous PEA can play a role in the aetiology of various neuropsychiatric disorders such as schizophrenia, Parkinson's disease or certain aggressive behaviours. In addition, Beck *et al.* (1998) report three cases of hospitalisation occurring in 1973, in drug addicts who unintentionally consumed PEA instead of amphetamines. The following adverse effects were reported: anxiety, tachycardia, nausea and vomiting.

β-alanine

The only known adverse effect is the onset of paraesthesia, observed for doses equal to or greater than 8 g/d (Harris *et al.* 2006). This paraesthesia is directly correlated to plasma levels of β-alanine and appears to be due to a direct effect of β-alanine on the peripheral sensory receptors (Artioli *et al.* 2010; Décombaz *et al.* 2012). Generally, a reduction in dose or the use of delayed-release forms helps remove them and they disappear within 60 to 90 minutes of consumption (Stellingwerff *et al.* 2012).

Prohibited substances

Amphetamine-type stimulants

Amphetamine-type stimulants may be neurotoxic depending on the dose and the duration of consumption. They can cause agitation, nervousness, hallucinations and acute paranoia similar to that observed in patients with schizophrenia. In addition, the cognitive functions of amphetamine users are impaired. The acute administration of amphetamines increases the level of extracellular dopamine and affects other neurotransmitters (serotonin, norepinephrine and glutamate) by interacting with their transporters. Repeated administration in rodents (rats and guinea pigs) and non-human primates decreases concentrations of dopamine and its metabolites in the striatum (Nordahl *et al.* 2003).

Amphetamines act in mitochondria by inhibiting the activity of the enzymes involved in the Krebs cycle and inhibiting the mitochondrial respiratory chain. The decline in the mitochondrial proton gradient causes a decrease in the intracellular concentration of ATP and generates oxidative stress. This leads to the release of proteins involved in apoptosis and, eventually, to cell death (Barbosa *et al.* 2015).

In general, these stimulants can cause convulsions and lead to a dependency (Momaya *et al.* 2015). However, there is still little documentation on the mechanistic link between these substances and these effects.

Anabolic androgenic steroids
Kanayama *et al.* (2013) conducted cognitive tests in weightlifters divided into two groups: one made up of users of anabolic androgenic steroids and the other of non-users. Visual memory was significantly reduced in users of anabolic androgenic steroids, compared to non-users. In addition, within the group of users, visual memory was more affected in long-duration users (more than two years) than in low users (at least eight weeks).
This deficiency may be the symptomatic manifestation of the neurotoxicity of anabolic androgenic steroids at high doses. Indeed, doses higher than physiological concentrations decrease the viability of neuronal cells by triggering their apoptosis (Estrada *et al.* 2006). Furthermore, taking high doses of anabolic steroids has been associated with neurobehavioural changes such as irritability, aggressiveness and suicidal tendencies (Estrada *et al.* 2006; Su *et al.* 1993; Yates *et al.* 1992). Villalba *et al.* (1999) showed in rats that the regular administration of androstenedione induced hypertrophy of the brain areas that control aggressiveness.

2,4-DNP
Behavioural disorders such as confusion or agitation, as well as convulsions and peripheral neuritis have been reported following consumption of 2,4-DNP (Grundlingh *et al.* 2011). The mechanism of action involved in the manifestation of these adverse effects remains unknown.

- Hepatic effects

Non-prohibited substances

Garcinia cambogia
From the pharmacological point of view, publications concerning *Garcinia cambogia* are rather contradictory, mentioning hepatoprotective and anti-inflammatory effects (Semwal *et al.* 2015) as well as pro-inflammatory and hepatic fibrosis-inducing effects (Kim *et al.* 2013). These last observations have been challenged and, according to Clouatre and Preuss (2013), the discrepancy in these results is related to the type of salt used and the degree of lactonisation of hydroxycitric acid, which is the compound suspected of being primarily responsible for the toxicity of *Garcinia cambogia*.

Proteins, amino acids and creatine
One case of hepatitis was reported in an adolescent aged 17 years, three months after beginning the consumption of three products for athletes seeking to develop muscle (Avelar-Escobar *et al.* 2012). These products contained creatine, amino acids and L-carnitine with whey proteins. From an aetiological point of view, the toxic cause was the most likely. The patient received treatment (colestyramine, ursodeoxycholic acid), stopped taking the products and recovered fully after one month. The authors concluded that the causality of the products for athletes was likely. Nevertheless, it was not possible to identify the substance responsible for the hepatotoxicity.
A similar case was reported in a man aged 27 years who consumed creatine eight to nine months before the onset of jaundice, as well as whey proteins four weeks before development of the symptoms (Whitt *et al.* 2008). The mechanism of action involved in the manifestation of these adverse effects remains unknown.

Prohibited substances

DMAA

Foley *et al.* (2014) reported seven cases of liver damage (jaundice or increase in transaminases) occurring in consumers of products containing DMAA: three women aged 24, 28 and 45 years and four men aged 19, 23 and 28 years. In two cases, the patients had to receive a liver transplant. The others experienced a favourable progression when they ceased taking the products. For these seven cases, the toxic cause was the most likely, as the other aetiologies (viral, auto-immune, etc.) had been ruled out. In one of the self-limiting cases, the patient had also consumed food supplements containing proteins and creatine. The mechanism of action involved in the manifestation of these adverse effects remains unknown.

Anabolic androgenic steroids

Anabolic androgenic steroids can cause hepatotoxicity, in particular hepatic adenomas and hepatocellular carcinomas (Dhar *et al.* 2005). However, the frequency of liver damage induced by anabolic androgenic steroids is probably overestimated because of the fact that the increase in transaminases can also be due to rhabdomyolysis resulting from intense physical activity (Pope *et al.* 2014).

Sibutramine

A single case of hepatotoxicity with sibutramine has been reported in the literature. A woman aged 47 years presented with a hepatic fibrosis two weeks after starting treatment with sibutramine. The liver biopsy was consistent with a toxic cause and further examinations excluded the other possible aetiologies. The patient recovered after stopping the sibutramine (Florentin *et al.* 2008). The mechanism of action involved in the manifestation of this adverse effect remains unknown.

- Renal effects

Non-prohibited substances

Creatine

Koshy *et al.* (1999) reported the case of a previously healthy man aged 20 years, who had been taking 20 g/d of creatine in four separate doses. Four weeks later, the patient presented with flank pain, as well as nausea and vomiting that had been progressing for four days. He had not taken any other products (medication or other food supplements). He ceased taking the creatine. The kidney biopsy revealed interstitial nephritis. The patient recovered within an unspecified period.

Many studies have shown no kidney damage after taking creatine. However, these studies were conducted on small numbers of subjects or over short durations, which does not rule out the possibility of long-term effects of creatine.

The ingestion of creatine causes an increase in serum creatinine, since creatine is spontaneously and irreversibly converted into creatinine. Therefore, a falsely positive diagnosis of kidney damage may be made in an individual consuming creatine when only blood tests are taken into consideration (Lugaresi *et al.* 2013).

The current state of knowledge is insufficient to be able to assert that creatine has a deleterious effect on the kidneys in the long term. Nevertheless, several cases of aggravation of renal pathologies have been described in the literature and attributed to the consumption of creatine (Pritchard and Kalra 1998).

Garcinia cambogia
Li and Bordelon (2011) reported the case of an obese woman aged 38 years who presented with a nephropathy. For the past year she had been taking a food supplement containing 500 mg/day of hydroxycitric acid, five days a week. Her renal function returned to normal after seven days of hospitalisation. The authors identified the food supplement as being most likely responsible for the nephropathy. The mechanism of action involved in the manifestation of these adverse effects remains unknown.

Prohibited substance

2,4-DNP
Tubular necrosis in connection with the consumption of 2,4-DNP has been reported (Grundlingh *et al.* 2011). The mechanism of action involved in the manifestation of these adverse effects remains unknown.

- Dermal effects

Non-prohibited substance

Theobromine
Tognetti *et al.* (2011) reported the case of a woman aged 34 years with an erythema multiforme type rash, which developed after 12 days of consuming a pilosella tincture and a slimming and thermogenic preparation, consisting of clorazepate dipotassium, theobromine, pseudoephedrine hydrochloride and dehydrocholic acid. The authors believe that the clorazepate dipotassium and theobromine were the two compounds most likely to have triggered the rash. The mechanism of action involved in the manifestation of this adverse effect remains unknown.

Prohibited substances

Sibutramine
Dermatological conditions such as urticaria, petechiae and moderate rash have been described in subjects who had consumed sibutramine. A woman aged 24 years presented with necrotising vasculitis after three months of treatment with sibutramine at a dose of 10 mg/d. She recovered on cessation of the product but again presented with this adverse effect after resuming the sibutramine. The second vasculitis also resolved on cessation of the treatment (Ha *et al.* 2011). A woman aged 19 years presented with bullous dermatosis on the third day of taking sibutramine. The patient was not taking any other products, and recovered on treatment with prednisolone (Goh *et al.* 2003). The mechanism of action involved in the manifestation of these adverse effects remains unknown.

2,4-DNP
Cases of urticaria, angioedema and severe exfoliative dermatitis have been reported following consumption of 2,4-DNP (Grundlingh *et al.* 2011). The mechanism of action involved in the manifestation of these adverse effects remains unknown.

- Other effects

Non-prohibited substances

Creatine

In the literature, a link has been reported – controversially – between the consumption of creatine and the emergence of muscle cramps. These effects, which can occur spontaneously during physical activity, can also be due to an increase in intracellular osmotic load induced by the creatine and exacerbated by dehydration (Dhar *et al.* 2005; Williams and David Branch 1998).

In a report on the assessment of the risks presented by creatine to the consumer, the assumption made was that chronic ingestion of creatine associated with the decrease in renal excretion that takes place during sports training could lead to the conversion of creatine into methylamine, itself converted into formaldehyde, both of which are toxic substances (AFSSA 2001). Poortmans *et al.* (2005) confirmed this assumption by collecting the urine of healthy athletes before and after the consumption of 21 g/d of creatine for 14 days. They revealed a significant increase in urinary concentrations of methylamine (6.41 ± 1.45 mg/d) and formaldehyde (290.4 ± 66.3 µg/d) after consumption of creatine. However, the urinary excretion of methylamine in these subjects was lower than the average value of urinary methylamine (11 mg/d) measured by Mitchell and Zhang (2001) in healthy adults. These results are therefore difficult to interpret.

A case of asthma attack thirty minutes after an intense workout and consumption of creatine was reported in a man aged 43 years (Roukens *et al.* 2013). The patient had a history of allergic asthma, occasionally requiring treatment with salbutamol. On arriving at the hospital, he was unconscious and in respiratory arrest. He had a rash over his whole body. The blood tests carried out revealed metabolic acidosis. Despite the treatment put in place, the patient progressed to a state of cerebral death. The autopsy revealed a severe pulmonary oedema, with eosinophil and neutrophil infiltration in the alveolar tissue, consistent with fatal allergic asthma. This is the only case reported in the literature of asthma occurring after the consumption of creatine. Exertion is, however, liable to cause death by severe asthma attack in athletes, irrespective of the consumption of creatine.

Caffeine alone

Caffeine blocks the adenosine receptors in the central nervous system and adenosine acts on the release of dopamine, which is involved in thermoregulation. Therefore, the consumption of caffeine during physical exercise, especially when practised in hot conditions, may cause a deregulation of dopaminergic signalling and increase the risk of hyperthermia (ANSES, 2013a). Other mechanisms of action have been proposed to explain the adverse effect of caffeine on the risk of hyperthermia during exertion (Zheng and Hasegawa 2015, 2016).

Prohibited substances

Anabolic androgenic steroids

Anabolic androgenic steroids are responsible for the emergence of diffuse acne, especially on the back and the chest, in consumers of these types of products (Hatton *et al.* 2014). In addition, they cause various adverse effects according to sex. In men, they can be responsible for the emergence of gynecomastia, hypertrophy of the gonads or infertility. These substances also expose consumers to the risk of prostate pathologies. Indeed, the increase in dihydrotestosterone by the prohormones leads to an increase in the circulating concentration of oestrogens (by aromatisation of testosterone to oestrone and oestradiol), which translates to a high risk of benign hypertrophy or cancer of the prostate (Castell *et al.* 2009). In women, they may cause masculinisation, hirsutism or amenorrhoea (Basaria 2010; Dhar *et al.* 2005).

Anabolic steroids or precursors can also cause premature closure of epiphyseal plates through an increase in osteoblast activity, with cessation of statural growth (Leder *et al.* 2000).

- Conclusions relating to the analysis of the adverse effects reported in the literature

Several adverse effects have been reported in the literature in humans and are associated with substances likely to be found in food supplements for athletes seeking to develop muscle mass or reduce body fat. The available information is not always sufficient to establish with certainty a causal link between the consumption of a substance and the emergence of a symptom. In addition, the absence of data in the literature on the potential adverse effects of a substance in humans does not guarantee its safety.

The main substances presented as having effects on the development of muscle mass or the melting of body fat have adverse effects that may sometimes be serious, mainly cardiovascular, neuropsychiatric, hepatic and renal. These substances are however very often combined in the same food supplement or combined by consumers themselves, which makes it difficult to determine the respective causalities. Certain substances have known potentiating effects when they are combined (synephrine and caffeine, for example) but it is highly likely that the interactions between substances are even more complex, explaining some of the adverse effects observed.

- Data from experimental toxicology

Certain substances have not been the subject of specific studies in humans, or have not been associated with adverse effects likely to be related to their consumption, according to the data available in the literature. Nevertheless, there are some data from animal studies. These data have made it possible to determine doses below which the substances do not cause adverse effects.

Creatine

EFSA (2004) considers that a dose below 3 g/d of creatine does not cause an adverse effect.

Chromium

EFSA (2014a, 2014b) has not set a tolerable upper intake level for chromium, given the few studies reporting adverse effects in animals or humans, but considers that there is no risk to health below the dose of 300 µg/kg bw/d, derived from a NOAEL (No observed adverse effect level) from a chronic toxicity study in rats.

Choline

There is no upper intake level established for choline in Europe. However, the Organisation for Economic Cooperation and Development (OECD) has set a limit of 3500 mg/d for adults (EFSA 2011a).

Vanadium

An upper intake level of 100 µg/d has been proposed for vanadium for the general population (Arnaud 2001). EFSA believes that the exposure of adults who are high consumers of food supplements containing vanadium may reach 3 µg/kg bw/d, a dose higher than the upper intake level (EFSA 2008).

In addition, kidney damage was observed in rats exposed to vanadium, although no NOAEL could be established from among the available studies (EFSA 2008). De La Torre *et al.* (1999) showed that the nephrotoxicity induced by vanadium was more marked in adult rats than in young rats, suggesting age-dependent toxicity.

Caffeine

Caffeine has a diuretic action. The consumption of caffeine increases the urinary excretion of calcium, magnesium, potassium, sodium and chlorine. This diuretic effect is lower in regular consumers of caffeine. As adenosine has a renal-protective effect, its antagonism by caffeine has led to caffeine being suspected of renal toxicity. A study conducted *in vivo* in obese and diabetic rats showed an increase in proteinuria, intrarenal resistance and accelerated degradation of renal function with the administration of caffeine (ANSES, 2013a).

EFSA has estimated that below 400 mg/d, caffeine does not have adverse effects in the general population (EFSA 2015). Similarly, ANSES considers that below 400 mg/d in adults, caffeine does not have general toxicity, adverse cardiovascular effects, adverse effects on bone health or calcium balance (for a calcium intake greater than 800 mg/d), nor does it modify behaviour, or the incidence of cancer or effects on male fertility. However, caffeine may cause an increase in anxiety from 210 mg/d (ANSES, 2013a).

Theobromine

In rats, the oral administration of 150 mg/d of theobromine for seven weeks induced testicular toxicity, with necrosis of spermatogenic cells (Gans 1984). Administered at a dose of 6 mg/d in feed to mice during gestation and lactation, it induced a significant decrease in the weight of embryos and a decrease in tissue angiogenic activity (Chorostowska-Wynimko *et al.* 2004).

Coleus forskohlii

Virgona *et al.* (2013) showed dose-dependent hepatotoxicity of *Coleus forskohlii* in a study conducted in mice administered 0.005%, 0.05%, 0.5% and 5% of *Coleus forskohlii* extract containing 10% forskolin. Indeed, the relative liver weights and the liver enzymes (AST, ALT and ALP) were significantly increased in the mice fed 0.5% and 5% of *Coleus forskohlii* extract for one week, compared to the control group. Histological observations revealed necrosis, hepatocyte hypertrophy and fatty liver. These different effects were not however observed with pure forskolin. Toxicology data on forskolin are rare. The median lethal oral dose (LD50) is 2 g/kg (Huerta *et al.* 2010).

Cissus quadrangularis

A study of the subchronic toxicity of an extract of *Cissus quadrangularis*, administered to rats by gavage, led to a NOAEL of 2500 mg/kg bw/d being determined (Kothari *et al.* 2011).

PEA

Rauscher-Gabernig *et al.* (2010) estimated that a dose of PEA lower than 5 mg in a single administration did not cause adverse effects in a healthy individual.

Raspberry ketone

A NOAEL of 70 mg/kg bw/d has been proposed on the basis of liver abnormalities (significant increase in liver weight and liver enzymes) observed in rats in a subchronic study (Bredsdorff *et al.* 2015).

Exposed populations

- Identification of consumers of food supplements seeking to develop muscle mass or reduce body fat

There are a variety of reasons why athletes use food supplements: to prevent or correct a nutritional intake perceived as inadequate (without this necessarily being substantiated), to obtain a macro- or micronutrient intake in a form that is easier to use than natural foods, to improve physical performance or increase the effects of training, or to adopt the behaviour of most top-level athletes with regard to food supplements (Maughan *et al.* 2007). In addition, athletes may consume food supplements intended to reduce body fat when they are seeking to lose weight, whether or not this is associated with a dietary weight-loss practice that itself presents a risk to health (ANSES 2010).

o Prevalence of consumption in the different disciplines

It is very difficult to assess the prevalence of consumption of food supplements during physical training or the practice of sport. This difficulty is due to large differences in the performance level of practitioners, the physiological objectives they set themselves and their individual approach to achieving them. The level of food supplement consumption very likely varies in the different disciplines.

There seems to be a culture of food supplement consumption in different sports disciplines, but data from surveys in large cohorts of athletes are lacking. However, the consumption of food supplements appears to be more common in strength/power disciplines where the development of muscle mass is required. The tests carried out as part of the fight against doping are more often positive for anabolic androgenic steroids in the following disciplines: bodybuilding, athletic strength, weightlifting, boxing and kickboxing (Pope *et al.* 2014).

Athletics disciplines have been widely studied and the prevalence of food supplement consumption was assessed at 45% in the strength disciplines at the 2004 Olympic Games in Athens (Tsitsimpikou *et al.* 2009). A survey on the prevalence of food supplement consumption in top-level athletes between 2005 and 2007 estimated this consumption to be 76% in sprint events and 91% in endurance events (Maughan *et al.* 2007).

A survey of French military personnel deployed in Afghanistan between April and September 2012 yielded 1391 exploitable questionnaires. The prevalence of consumption of food supplements deemed necessary for physical preparation was 20% (Dubecq *et al.* 2014).

o Profile of consumers

Among top-level athletes, the prevalence of food supplement consumption is lower in athletes under the age of 18 – between 2 and 47% – than in older athletes – from 41 to 99% (Erdman *et al.* 2007). Furthermore, the use of substances to increase muscle mass has been extended in recent years to non-athletes or athletes of all levels, in particular adolescents (Darvishi *et al.* 2013;

Eisenberg *et al.* 2012). This consumption concerns around 25% of adolescents enrolled in sports clubs (Šterlinko Grm *et al.* 2012).

- Identification of vulnerable populations
    - Children and adolescents

Anabolic androgenic steroids: children and adolescents constitute an at-risk population with these types of products because anabolic androgenic steroids may suppress growth and cause infertility (Dhar *et al.* 2005; Leder *et al.* 2000).

Caffeine: children and adolescents are a population with low coffee consumption and are therefore less exposed to caffeine than the adult population, which increases their susceptibility to its effects when consumption is increased (through beverages or food supplements). They are at an increased risk of adverse effects compared to adults, for the same amount of caffeine ingested. The consumption of caffeine in children and adolescents is liable to cause sleep disorders, resulting in fatigue and daytime drowsiness, whereas physical activity should have the effect of improving sleep quality (ANSES 2016). In addition, poor-quality sleep affects cognitive abilities and school performance. A chronic sleep debt has been associated with the occurrence of somatic pathologies (hypertension, cardiovascular disease, diabetes and obesity) and psychiatric disorders (anxiety, depression). A sleep debt, as well as early consumption of psychoactive substances such as caffeine, can promote progression towards addictive behaviour (ANSES, 2013a).
In its Opinion of 2015, EFSA considered that consumption of 3 mg/kg bw/d did not cause adverse effects in children and adolescents (EFSA 2015). Nevertheless, ANSES (2013a) believes that food supplements containing caffeine should be avoided in children and adolescents, considering that this substance causes an increase in anxiety from 2.5 mg/kg bw/d and that the development of tolerance and withdrawal symptoms appears from the dose of 1 mg/kg bw/d in this population.

    - Pregnant or breastfeeding women

Ephedrine: ephedrine crosses the placental barrier and passes into breast milk (Andraws *et al.* 2005). It is therefore recommended that pregnant or breastfeeding women avoid consuming food supplements containing ephedrine or its derivatives.

Caffeine: caffeine consumption should be limited in pregnant or breastfeeding women. This recommendation is based mainly on the possibility of a risk of intrauterine growth restriction related to the consumption of caffeine during pregnancy (ANSES, 2013a).

Garcinia cambogia: the *PDR for Nutritional Supplements* advises against the use of (-)-hydroxycitric acid in pregnant women (Hendler and Rorvik 2008).

Coleus forskohlii: the traditional use of *Coleus forskohlii* as an abortifacient, and a study in rats that shows an anti-implantation effect means that consumption of this plant is not advised in women who are pregnant or trying to become pregnant (Almeida and Lemonica 2000). In addition, forskolin delayed the normal meiotic progress of human oocytes in an *in vitro* fertility test (Shu *et al.* 2008).

    - Pre-existing pathologies

<u>Neuropsychiatric disorders:</u>

Caffeine: some studies mention the fact that caffeine can, at doses above 400 mg/kg, lower the seizure threshold in rats and also decrease the effectiveness of antiepileptic treatments, even at

lower doses (Chrościńska-Krawczyk *et al.* 2011). The proconvulsant activity of methylxanthines such as caffeine could be explained by their adenosine-antagonistic action, which has an anticonvulsant effect on the brain. Therapies aiming to increase adenosine concentrations in the brain are effective at reducing the occurrence of epileptic seizures while adenosine-receptor antagonists such as methylxanthines generally increase this risk. However, the relationship between caffeine consumption and the emergence of a *de novo* epileptic seizure has not been clearly established, so at this stage, it primarily seems that high caffeine consumption increases the risk of the occurrence of seizures for subjects diagnosed with epilepsy or predisposed individuals (ANSES, 2013a).

Sibutramine: neuropsychiatric manifestations (paranoid delusions, panic attacks, etc.) have been described after taking sibutramine; these were resolved on cessation of the product or with antipsychotic treatment. Sibutramine has also been associated with memory disorders. These adverse effects are rare and most often reported in patients already suffering from psychiatric disorders (Florentin *et al.* 2008).

Garcinia cambogia: the *PDR for Nutritional Supplements* advises against the use of (-)-hydroxycitric acid in people with dementia syndromes, including Alzheimer's disease, and advises caution in diabetics (Hendler and Rorvik 2008).

PEA: According to Sandler *et al.* (1974), the administration of 3 mg of PEA to people subject to migraines following chocolate consumption triggers a migraine in half of the people treated.

History of cardiovascular diseases:

Caffeine and *p*-synephrine: the risk of occurrence of vascular accidents associated with the combination of *p*-synephrine and caffeine is greater in patients treated for hypertension, heart disease or depression. This combination also induces a potential risk of high blood pressure in subjects who are overweight, with cardiovascular risk factors and practising regular physical exercise as part of their therapeutic care (ANSES 2014).

Sibutramine: sibutramine increases blood pressure and heart rate. When it was authorised as a drug, hypertensive patients were advised to regularly check their blood pressure during treatment. Even though it is now prohibited from sale, whether in drugs or in food supplements, subjects with a history of cardiovascular disease should be alerted to the risks incurred if they were to procure the product illegally (Florentin *et al.* 2008).

L-arginine: taking arginine as a food supplement is contraindicated in people with a history of myocardial infarction. Indeed, in a prospective study in patients having had a heart attack, it seems to have increased the risk of death (Schulman *et al.* 2006). Canadian law obliges manufacturers of food supplements to mention this precaution on the packaging of L-arginine. In addition, the hypotensive and vasodilator effects of arginine may add to those of antihypertensive and vasodilator drugs.

Kidney diseases:

Proteins and creatine: taking proteins and creatine seems to aggravate the impairment of renal function (Combe and Aparicio 1993; Pritchard and Kalra 1998). Indeed, it seems that the excessive consumption of protein causes renal hypertrophy and vasodilation, as well as an increase in the glomerular filtration rate leading to a decline in protein reabsorption and therefore to proteinuria. This process is accelerated when other factors are present, namely, a renal pathology, a nephrectomy or diabetes (Brenner *et al.* 1982). In subjects without any history of impaired renal function, the possible adverse consequences for the kidney of excessive consumption of dietary

proteins have not been demonstrated (Antonio *et al.* 2016; Martin *et al.* 2005; Poortmans and Dellalieux 2000). Accordingly, taking protein or creatine supplements should be avoided in patients with renal diseases, diabetes or having undergone nephrectomy (Brenner *et al.* 1982; Martin *et al.* 2005). In these subjects, urinary analyses should be carried out regularly to monitor proteinuria. The rate of urinary excretion of albumin must be lower than 20 µg/min for urine collected after 20 h of physical inactivity (Poortmans and Francaux 2000).

Liver diseases:

Caffeine: in people with liver damage, caffeine metabolism is slowed down and its adverse effects are potentially increased (hypertension, cardiac arrhythmia, psychiatric disorders, urinary and faecal incontinence, kidney failure, oesophagitis and gastro-oesophageal reflux) (ANSES, 2013a).

- Adult subjects susceptible to the effects of caffeine

There is interindividual variability in the responses to caffeine. This variability is mainly related to the genotype of individuals, their physiological or pathological condition, caffeine consumption habits and co-exposure factors such as smoking or taking medication.
The polymorphism of the gene of the 1A2 isozyme of cytochrome P450 involved in hepatic metabolism of caffeine is a major source of variation in the pharmacokinetics of caffeine and helps distinguish between "extensive metabolisers" and "poor metabolisers". The latter are more numerous in the population (55%) and more susceptible to the effects of caffeine. As well as this polymorphism, there is that of the adenosine A_{2A} receptors of the central nervous system, which can also be responsible for differences in susceptibility to the effects of caffeine on sleep and anxiety (ANSES, 2013a).

- Identification of risk situations
  - Association of multiple ingredients contained in the food supplements

The consumption of food supplements intended to increase muscle mass or to reduce body fat poses a risk of interactions between components for the following reasons:
- these supplements are themselves composed of multiple ingredients;
- the users of these food supplements generally consume several products concomitantly.

These interactions can lead in particular to a substance's adverse effects being potentiated by the presence of another substance (caffeine and synephrine, caffeine and ephedrine, caffeine and DMAA). They are complex and poorly documented. In the current state of knowledge, the consequences on endogenous production of steroid hormones and on endocrine balance of the consumption of several prohormones or compounds presumed to be prohormones, found in some food supplements, are unknown.

- Association of food supplements with other products

The association of food supplements for athletes seeking to develop muscle with painkillers or stimulants risks delaying the perception of fatigue. Training will then be longer or more intense, which increases the risk of the onset of musculoskeletal disorders (Pope *et al.* 2014) and overtraining syndrome.

Some food supplements consumed concomitantly with medication pose a risk of drug interactions. Indeed, the substances contained in these food supplements may increase or inhibit the effect of the medication concerned:

- Combining ephedrine alkaloids and drugs with an adrenergic action, such as bupropion or monoamine oxidase inhibitors (MAOIs), should be avoided. Cases of myocardial infarction have been described with this combination (Andraws *et al.* 2005).
- DMAA is a potent inhibitor of cytochrome P450 2D6 (CYP2D6), responsible for the metabolism of 25% of drugs including many β-adrenergic receptor antagonists and antiarrhythmics. If these drugs are administered, the inhibition of the CYP2D6 could cause loss of drug efficacy or, conversely, overdose or even toxicity (Liu and Santillo 2015).
- The major alkaloids of *Evodia*, evodiamine and rutaecarpine, have modulatory effects on metabolising enzymes, in particular cytochromes P450 CYP3A4, CYP1A2 and CYP1A1 (Ueng *et al.* 2002; Wen *et al.* 2014; Zhang *et al.* 2016), which metabolise many drugs.
- In human hepatocytes *in vitro*, honokiol is an inhibitor of cytochromes P450 CYP1A2, CYP2C8, CYP2C9 and CYP2C19 (Jeong *et al.* 2013) and magnolol is an inhibitor of CYP1A and CYP2C (Kim *et al.* 2016), raising suspicions of pharmacokinetic interactions between extracts of *Magnolia officinalis* and drugs that are metabolised by these cytochromes. Homma *et al.* (1993) have suggested an interaction between magnolol and prednisolone.
- An inhibition of serotonin re-uptake was observed *in vitro* in the cortical cells of rats exposed to hydroxycitric acid (Semwal *et al.* 2015). An interaction can therefore be suspected between hydroxycitric acid and drug treatment comprising a serotonin re-uptake inhibitor.

4. RECOMMENDATIONS OF THE CES AND THE WG

The nutrivigilance scheme collected forty-nine reports of adverse effects likely to be associated with the consumption of food supplements for athletes, between 2009 and February 2016. The most frequently reported adverse effects were cardiovascular. Among the seventeen admissible cases, eight cases described adverse effects for which the causality of the food supplements was considered likely.

The compositions of the food supplements for athletes indicate a great diversity of ingredients. Some ingredients are presented as increasing muscle mass: milk proteins, branched-chain amino acids, glutamine, β-hydroxy-β-methylbutyrate (HMB), α-ketoisocaproate, L-tyrosine, β-alanine, L-arginine, creatine, plant extracts (*Tribulus terrestris* and species of the genus *Smilax*) and minerals (vanadium and chromium picolinate). Others are presented as reducing body fat: L-carnitine, choline, 2-phenylethylamine, extracts of *Cissus quadrangularis*, *Coleus forskohlii*, *Garcinia cambogia*, *Magnolia officinalis* and substances extracted from plants: caffeine, theobromine, evodiamine, *p*-synephrine and raspberry ketone.
Only two of these substances (proteins and creatine) benefit from claims that have been authorised in Europe relating to muscle mass or physical capacity.

Anabolic androgenic steroids (androstenedione and androstenediol), clenbuterol, ephedrine, pseudoephedrine, phenylpropanolamine, sibutramine, 1,3-dimethylamylamine (DMAA) and 2,4-dinitrophenol (2,4-DNP) are prohibited in food supplements and their presence constitutes fraud. The development of sales on the Internet increases the risk of exposing consumers to substances that are prohibited under French regulations.

The consumption of certain food supplements that have been adulterated poses health risks and the risk of positive results in drug tests.

Some ingredients in food supplements for athletes seeking to develop muscle mass or reduce body fat are described in the scientific literature as having adverse effects that may sometimes be serious, mainly cardiovascular, neuropsychiatric, hepatic and renal.

The risk of adverse effects following the consumption of this type of product may be even greater, as some consumers tend to take the product at higher doses than those recommended by the manufacturer (thus creating a risk of overdose) or take several products concomitantly. Certain substances have known potentiating effects when they are combined (synephrine and caffeine, for example). Plant extracts may reduce or increase the effects of drugs taken concomitantly.

This study did not consider the doses of substances nor the levels of consumption of food supplements. The effects of these doses may vary from one individual to another.

On the basis of these observations, the Working Group on "Nutrivigilance" and the Expert Committee on "Human Nutrition" are issuing the following recommendations relating to the consumption of food supplements in the framework of the practice of sport:

- Food supplements seeking to develop muscle mass or reduce body fat are not advised in subjects with cardiovascular risk factors or suffering from heart disease, impaired kidney or liver function or neuropsychiatric disorders.
- Food supplements seeking to develop muscle mass or reduce body fat are not recommended for use by children, adolescents and pregnant or breastfeeding women.
- The consumption of food supplements containing caffeine should be avoided before and during any sporting activity.

- The consumption of food supplements containing caffeine should be avoided by subjects susceptible to the effects of this substance.
- The concomitant consumption of several food supplements or their combined consumption with medicinal products should be avoided.
- Sports managers should, through their training, be capable of informing athletes about the risks associated with the consumption of certain food supplements.
- The consumption objectives of the food supplements should be discussed with a healthcare professional.
- The individual's doctor and pharmacist should be informed that he/she is taking food supplements.
- Athletes should pay close attention to the composition of the products consumed and favour products complying with AFNOR standard NF V 94-001 (July 2012).
- Consumers and sales intermediaries should favour supply channels with the best oversight by the public authorities (compliance with French regulations, traceability and identification of the manufacturer).

Moreover, the plant extracts used by athletes have been little studied and there is a lack of bibliographic data on the metabolism of their constituents and their potential long-term toxicity. The Working Group on "Nutrivigilance" and the Expert Committee on "Human Nutrition" believe that additional studies on the fate in the body and the long-term toxicity of the plant extracts and certain substances found in food supplements for athletes would be useful.

In general terms, the CES reiterates that:

- the consumption objectives of the food supplements should be discussed with a healthcare professional;
- the individual's doctor and pharmacist should be informed that he/she is taking food supplements;
- additional studies on the interactions between the multiple ingredients found in the same food supplement would be useful.

Lastly, improved international cooperation on the monitoring of adverse effects associated with the consumption of food supplements for athletes should be implemented.

5. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety adopts the recommendations of the Working Group on "Nutrivigilance" and the Expert Committee on “Human Nutrition”.

The reports of adverse effects associated with food supplements for athletes who wish to increase their muscle mass or to reduce their body fat have led ANSES to assess the risks associated with the consumption of these supplements. The severity of some of these effects associated with the widespread consumption of these types of product and the great diversity of ingredients that they contain has prompted ANSES to draw the attention of the athletes concerned to the health risks induced by these practices.
In order to reduce these risks, it calls for consumers to take care to ensure that these food supplements are compatible with their nutritional status, state of health and the objectives sought. Indeed, the inter- and intra-individual variabilities require personalised advice to be obtained from a healthcare professional, where applicable in cooperation with the trainer or the fitness coach, and with reference to the training periods and loads.

With this objective in mind, ANSES considers it necessary for healthcare professionals to have obtained initial and continuing training in the field of nutrition, including sport nutrition. It insists on the need for a multidisciplinary approach in the area of training and nutrition of the athlete. It appears, in fact, that the constraints related to training loads and the search for performance are leading to dietary behaviours liable – in addition to the risks of negative performance in the long term – to expose practitioners to risks to their health. Accordingly, the multidisciplinary approach should involve both sports managers (coaches, sports educators, etc.) and healthcare professionals (dieticians, physicians, pharmacists, etc.), in order to help the practitioner identify his/her own specific needs and reduce any risk associated with inappropriate practices.

In addition, and more specifically when seeking to modify body composition and depending on the methods used, people practising sport should be informed of firstly, the risks associated with the consumption of pharmacologically active products and secondly, the health risks intrinsically associated with nutritional manipulation leading to weight-loss without medical supervision. ANSES reiterates that weight loss practices are not without danger and that the risk of appearance of harmful consequences of varying severity on health should not be neglected. This remains true in the framework of the practice of sport.

ANSES stresses the fact that any claimed effects on performance, even if they are scientifically based, do not in any way rule out the health risk and that their evaluations came from unrelated assessments. Thus, for example, the claims relating to caffeine for increasing stamina performance and stamina capacity have been validated by EFSA but are not currently authorised by the European Commission in view of the identified health risks.

ANSES emphasises that among the large number of substances consumed, only a few are authorised to claim properties relating to one of the benefits sought (muscle gain, fat burning). The expected benefits from the consumption of food supplements containing substances whose effectiveness has not been scientifically demonstrated therefore remain extremely hypothetical, *a fortiori* in view of the risks incurred.

Concerning the development of sales *via* the Internet, which increases the risk of exposing the consumer to substances that are prohibited under French regulations, ANSES recommends that consumers pay close attention to the composition of the products and favour firstly products complying with AFNOR standard NF V 94-001 (July 2012) and secondly supply channels with the best oversight by the public authorities (compliance with French regulations, traceability and

identification of the manufacturer). Purchases on the Internet *de facto* increase the athlete's exposure to the consumption of adulterated food supplements, liable to cause effects on health and lead to positive anti-doping tests.

Through the hearings that it conducted, ANSES has found that the consumption of these supplements is a widespread and even cultural practice in these sports and in the places where they are practised. Thus, ANSES calls for effective information for practitioners, especially younger athletes, stressing the risks of taking such supplements in view of the relatively few benefits expected. In this context, ANSES also recommends that the public authorities conduct a debate on the appropriateness of selling these products at sites where sports are practised given the widespread nature of their consumption.

Moreover, because of the lack of available studies noted during the course of the expert appraisal, ANSES considers it would be useful to undertake:

- consumption surveys;
- additional studies on the interactions between the multiple ingredients found in the same food supplement;
- additional studies on the fate in the body and the long-term toxicity of the plant extracts and certain substances found in food supplements for athletes.

Lastly, ANSES emphasises the value of setting up a joint international project on the monitoring of adverse effects associated with the consumption of food supplements.

In this context, ANSES advises against the consumption of these food supplements by children, adolescents, pregnant or breastfeeding women, people with cardiovascular risk factors or suffering from heart disease, impaired kidney or liver function, or neuropsychiatric disorders. It also calls on other consumers to avoid combining several of these products, or consuming them concomitantly with drugs without the advice of a healthcare professional.

In addition, ANSES reminds healthcare professionals of the need to report to its nutrivigilance scheme any adverse effects likely to be related to the consumption of food supplements for athletes about which they become aware.

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KEYWORDS

Effets indésirables, nutrivigilance, compléments alimentaires, développement musculaire, perte de masse grasse, brûleurs de graisse, sportifs

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