The Director General

Maisons-Alfort, 22 November 2010

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

regarding the safety of yam (*Dioscorea*) alcohol extracts in food supplements

CONTEXT OF THE REQUEST

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) issued a formal internal request on 19 October 2010 for an opinion regarding the safety of yam alcohol extracts in food supplements.

1. CONTEXT

In the European Community, food supplements are defined as foodstuffs whose purpose is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, lozenges, tablets, pills and other similar forms, individual packets of powder, ampoules of liquids, drop-dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities (French Decree 2006/352 of 20 March 2006, Chapter 1 Article 1).

At the present time, the composition and marketing of food supplements are regulated by French Decree no. 2006-3524 and the French Order of 9 May 2006 which correspond to the transposition of Directive no. 2002/46/EC into French law.

This decree defines the substances that are authorised in the manufacture of food supplements: nutrients (vitamins and minerals), substances with a nutritional or physiological purpose, plants and plant preparations. It stipulates the establishment of positive lists for these substances by ministerial order.

The decree introduces various marketing procedures aimed at the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) to comply with European Community obligations, and particularly those related to the free movement of goods.

The French Order of 9 May 2006 contains the positive list of vitamins and minerals from European Directive no. 2002/46/EC and adds maximum consumption doses. In its 2007 and 2008 opinions (AFSSA, 2007), ANSES stressed that positive lists of plants, algae and fungi could not guarantee the safety of products. Indeed, safe consumption of plants and plant products relies on tradition, assurance of the plant's botanical identity, knowledge of its composition, and in the case of extracts, knowledge of the compounds carried away by the extraction method.

The French Act on Regional Health Governance (*Loi Hôpital, Patients, Santé et Territoires* 2009-879) of 21 July 2009 entrusted ANSES with "implementation of the vigilance system for novel foods, food supplements, foods to which substances have been added for nutritional or physiological purposes and products intended for particular nutritional uses". In the framework of this national nutritional vigilance system, eleven declarations of adverse effects likely to be related to the

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consumption of two products from the same range of food supplements were brought to ANSES' attention.

Imputability of the food supplement in the occurrence of the adverse effect was determined in accordance with the methods used in pharmacovigilance and cosmetovigilance and the method that is currently under development for nutritional vigilance. These methods are based on clinical analysis, chronology and bibliographic data on the substances used in the final product. The data are taken into consideration as a whole to obtain an imputability score ranging from 'excluded' to 'very likely' (Bégaud *et al.*, 1985; AFSSAPS, 2009).

After examining the records using these three methods, the technical committee on nutritional vigilance was unable to exclude a link between food supplement consumption and the reported adverse effects in ten cases. Six of these ten cases required hospitalisation. Details of the ten declarations are given in the annex. There were six cases of liver damage, one case of acute pancreatitis, one case with decreased urination frequency and volume, and two cases with no specific adverse effects.

The six cases of liver damage were cholestatic and/or cytolytic. The liver damage occurred, for the cases where the information was available, two to five weeks after the subject began consuming the food supplement, but the daily dose consumed was unknown. In the six cases, the food supplement was combined with another food supplement or drug treatments. The clinical course was spontaneously favourable when the food supplement and drug treatments were discontinued.

The technical committee on nutritional vigilance, after discussions with the DGCCRF, analysis of the food supplements' composition, submission of production files by the manufacturers and examination of the files by pharmacognosists, considered that, if a link were to be established between the observed effects and consumption of these food supplements, the risk assessment should focus on the yam (*Dioscorea*) alcohol extract found in both products. In fact, while the yam species used in these food supplements was declared to be *Dioscorea opposita* L., the analysis of the production data was unable to determine the species actually used, the extraction method or the chemical composition of the final product.

The first food supplement contained 50 mg of yam alcohol extract per tablet and the manufacturer's recommended dose was three tablets per day for two to three months; the second food supplement contained 350 mg of yam alcohol extract per tablet with a recommended dose of two tablets per day for at least three months.

ANSES therefore issued an internal request to identify and characterise the safety of yam alcohol extracts in food supplements.

2. EXPERT ASSESSMENT METHOD

The collective expert assessment was undertaken on the basis of pharmacognosist reports, current knowledge of this plant family and the data supplied by the manufacturer on the method used to obtain yam extracts for this food supplement. The opinion was discussed by the Expert Committee on 'Human Nutrition', which met on 4 November 2010, and was validated by electronic transmission.

3. DISCUSSION

The French Agency for Food, Environmental and Occupational Health & Safety's discussion is based on the opinion of the Expert Committee on 'Human Nutrition', the main points of which are presented below.

3.1.1. Characterisation of the plant

The *Dioscorea* genus from the *Dioscoreaceae* family contains hundreds of species (n=630) (Mabberley, 2006) which go by the common name of yam.

They are extremely difficult to identify. In fact, their identification relies on the morphological characteristics of the whole plant and the tuber, and on chemical profiles on which there is currently very little documentation.

Dioscorea opposita L. has a complex botanical nomenclature and numerous synonyms (*D. batatas* Decne; *D. oppositofolia* L. var. *linnaei* Prain & Burkill; 'Chinese yam'). The book "Materia Medica", in its discussion of *Dioscorea opposita* L., describes changes in the Chinese name, the existence of several local variants and possible adulteration with Manioc (Bensky *et al.*, 2004).

3.1.2. Cultivation

Many *Dioscorea* species are cultivated in tropical or subtropical areas for their tuber's high starch content.

Dioscorea opposita L. is cultivated in China, Japan, Korea, the Philippines and Brazil.

3.1.3. Use

In general, the food species of *Dioscorea,* which are acrid and potentially toxic in their raw state, must be washed and then boiled, with the water discarded at the end of cooking before consumption (Dietrich Frohne, 2009) (tradition of consuming peeled and cooked yams).

The EFSA scientific cooperation working group on botanicals and botanical preparations (EFSA, 2009) mentions the possibility of consuming the *Dioscorea opposita* L. tuber raw. According to expert statements, for this local Chinese consumption method, the scraped whole tuber is plunged briefly into a vinegar solution to neutralise the calcium oxalate crystals under the skin. No publication, however, confirm this practice.

3.1.4. Chemical composition of the tubers

The chemical composition of the tuber varies from one *Dioscorea* species to another. A compound can be found in one species and not in another, or can be found at widely differing levels depending on the species. Each *Dioscorea* species has its own chemical profile but prior determination of these profiles, which would be required to identify the species, is highly imperfect at the present time.

The literature sets out the following chemical compounds that have been found in *Dioscorea* tubers:

- Some species from Central America, South Africa and Asia bio-synthesise alkaloids that result from nicotinic acid metabolism (Bruneton, 2009), including dioscorin. The presence of these alkaloids suggests that consumption of these tubers should be approached with caution.

- Numerous species contain terpene derivatives, including varying quantities of steroidal saponins. These compounds are likely to cause bitterness in some yam tubers (Bhandari & Kawabata, 2005). They have been used as reaction intermediates in the semisynthesis of steroidal hormones when their concentration has exceeded 2%. While the synthesis of steroidal hormones from steroidal saponins is chemically possible, this transformation in the human body has not been described to date (Bruneton, 2009).
  Nothing is known about the safety of these saponins, especially when they are concentrated and consumed over long periods (Bruneton, 2009).

- Some species contain potentially toxic cyanogenic glycosides (Bhandari & Kawabata, 2005; Dietrich Frohne, 2009).

- Some species contain calcium oxalate raphides in the tuber's sub-epidermal layer, and especially *D. opposita* L, associated with perforations, irritation and inflammation of the mucous membranes (Bhandari & Kawabata, 2005; Dietrich Frohne, 2009; EFSA, 2009).

- Some species contain potentially toxic histamine compounds (Bhandari & Kawabata, 2005).

- Lastly, the yam contains vitamins and minerals.

These compounds are likely to be found in the alcoholic phase of extraction.

3.1.5.Toxicity

Some *Dioscorea* species are known to be toxic. The EFSA scientific cooperation working group on botanicals and botanical preparations mentions hepatotoxicity for alcohol extracts of *D. bulbifera* (EFSA, 2009). However, the chemical profiles linked to this toxicity have not been precisely determined.

In humans, one case of hepatotoxicity requiring admission to the Accident & Emergency department was reported by the Berlin poison control centre in Germany in 1996 (Dietrich Frohne, 2009) following consumption of *Dioscorea bulbifera* L. cooked as food. The ingested dose was not specified.

In animals, five studies have objectively demonstrated hepatotoxicity in rodents that consumed *D. bulbifera* L or *D. villosa*. (Su *et al.*, 2003; Tan *et al.*, 2003; Chen *et al.*, 2006; Wojcikowski *et al.*, 2008; Wang *et al.*, 2010).
Two of these five studies also reported nephrotoxicity associated with consumption of *D. bulbifera* or *D. villosa* (Su *et al.*, 2003; Wojcikowski *et al.*, 2008). Wojcikowski *et al.,* 2008 even recommend avoiding long-term consumption of *D. villosa*, especially in patients with a history of kidney damage or suffering from a disease that requires nephrotoxic medication.
The quality of these studies (description of the extraction method, chemical characterisation of the extract used, information about the administered doses, number of animals studied) is highly heterogeneous.
Hepatotoxicity in mice was observed with a dose starting at 160 mg/kg/day for 14 days in a study that gave a NOAEL of 80 mg/kg/day (Wang *et al.,* 2010) and hepatotoxicity and nephrotoxicity in rats were observed in another study at a dose of 790 mg/kg/day for 28 days (Wojcikowski *et al.*, 2008). Because the published data are incomplete, particularly regarding the origin and composition of the extract used, these effects cannot exclusively be attributed to *Dioscorea bulbifera*. Moreover, it should be noted that genotoxicity data are available neither for the *Dioscorea* extract (*bulbifera* and *villosa*) nor for diosgenin.

Lastly, one *in vitro* study highlighted the toxicity of *D. villosa* on renal cell lines (Wohlmuth *et al.*, 2008), while another study examining the development of Medaka fish larvae showed liver inflammation (Rakotobe *et al.*, 2010).

3.1.6.Extract traceability

The analysis of data on yams shows that any industrial dossier that accompanies a product containing a yam alcohol extract should confirm the identity of the yam species used in the product and take into consideration the steps involved in preparing the yams (peeling, washing, cooking and extraction). Lastly, before using these yam extracts, producers should develop methods for their chemical characterisation.

4. CONCLUSION

The French Agency for Food, Environmental and Occupational Health & Safety, in the framework of the nutritional vigilance system, registered eleven declarations of adverse effects in people who had consumed food supplements containing yam extracts. The causal link between the food supplement and the reported adverse effects cannot be excluded in ten cases, including six cases of hepatotoxicity.

There are more than 600 yam species, some of which are toxic (hepatotoxicity, nephrotoxicity). Their identification relies on morphological characteristics of the whole plant and the tuber; the chromatographic profiles of the tubers' chemical compositions vary by species but have been imperfectly defined to date.

Food species of *Dioscorea* are, according to traditional consumption methods, washed and then boiled and the water is discarded at the end of cooking before consumption.

Alcohol extraction from a yam tuber, a production method that is very different from traditional tuber consumption, can produce and concentrate toxic compounds that may be present in certain species.

In this context, the safe use of a yam alcohol extract could be guaranteed only if the following were precisely known:

- the identity of the botanical species used (morphological characteristics and chemical profiles, taking into account risks of confusion and adulteration with other species);
- the extraction process used and the chemical characterisation of the alcohol extract obtained;
- the entire production chain's traceability.

Owing to the lack of data on yams, the French Agency for Food, Environmental and Occupational Health & Safety considers that studies need to be undertaken to define the chemical profiles of tubers, the composition of extracts and their toxicity.

It recommends that these data (chemical profiles of tubers, composition of extracts and toxicity) be acquired as quickly as possible by manufacturers of products containing yam extracts that are already on the market and, above all, before others are placed on the market.

The Director General

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KEYWORDS

Keywords: food supplement, yam, nutritional vigilance, hepatotoxicity, nephrotoxicity

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ANNEX

Table I: Details of cases with this food supplement brought to the attention of ANSES

Year reported	Gender, age	Consumption duration	Time after which the adverse effect occurred	Reported adverse effect	Additional tests performed	Confusion factors	Clinical course
2009	F, 59 years	30 days	38 days after beginning consumption, i.e. 8 days after discontinuing the food supplement	Cytolytic hepatitis	Increased liver enzymes No hepatocellular insufficiency Etiological assessment negative	Crohn's disease treated since 2003	**Hospitalisation** Improvement of symptoms during hospitalisation
2009	F, 44 years	28 days	28 days after beginning consumption of the food supplement	Hepatitis	Increased liver enzymes Serological tests, ultrasound and immune assessment normal	Consumption of another food supplement	Clinical course favourable with the discontinuation of food supplements
2009	F, 60 years	Consumption until hospitalisation. Food supplements discontinued due to the clinical presentation	No information	Cytolytic and cholestatic hepatitis with jaundice	Increased liver enzymes Non-medicine related etiologies discarded Liver biopsy in favour of a toxic origin	History of allergies Hypothyroid treated for 28 years Consumption of another food supplement	**Hospitalisation** Clinical course favourable with discontinuation of food supplements and drug treatment The diagnosis of autoimmune hepatitis was accepted during a 2nd episode a few months later
2009	F, 59 years	No information	No information	Cytolytic hepatitis	Increased liver enzymes from the start of hospitalisation Viral serological tests negative	Effect observed in the context of deliberate drug intoxication	**Hospitalisation** Clinical course favourable
2008	F, 36 years	12 days	13 days after beginning consumption of the food supplement	Cholestatic hepatitis with jaundice	Increased liver enzymes and bilirubin Serological tests and immune assessment negative Liver biopsy showing inflammatory hepatic parenchyma	Polymedication Consumption of another food supplement	**Hospitalisation** Clinical course favourable with discontinuation of food supplements and drug treatment

Year reported	Gender, age	Consumption duration	Time after which the adverse effect occurred	Reported adverse effect	Additional tests performed	Confusion factors	Clinical course
2006	F, 47 years	8 days	28 days after beginning consumption, i.e. 20 days after discontinuing the food supplement	Cytolytic and cholestatic hepatitis with jaundice	Increased liver enzymes and bilirubin Serological tests and immune assessment negative Liver biopsy in favour of a toxic origin	Patient taking an oral contraceptive	**Hospitalisation** Clinical course spontaneously favourable with hospitalisation
2009	F, 68 years	15 days	15 days after the start of consumption of the food supplement	Acute pancreatitis	Very high amylasemia and lipasemia Echo-endoscopy normal Biliary, alcoholic, infectious, parasitic and metabolic etiologies discarded	Blood pressure treated for 10 years	**Hospitalisation** Clinical course favourable with discontinuation of the food supplement and drug treatment
2009	F, 49 years	4 months	1.5 months after the start of consumption of the food supplement	Decreased urination frequency and volume	Reduced creatinine clearance	None	Clinical course spontaneously favourable with discontinuation of the food supplement
2009	F, 51 years	3 days	2nd day after the start of consumption of the food supplement	Discomfort, asthenia, nausea, dizziness	None	Consumption of another food supplement	No information about the clinical course Food supplements discontinued the day after symptom onset
2006	F, 52 years	3 days	From the start of consumption of the food supplement ¾ hour after taking each tablet	Headache, nosebleed and leg pain	None	Treatment of arthritis pain for several years	Clinical course immediately favourable with discontinuation of the food supplement