

The Director General

Maisons-Alfort, 1 August 2019

OPINION

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

on the “safety of use of berberine-containing plants in the composition of food supplements”

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

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

Its opinions are 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 1 August 2019 shall prevail.

On 12 April 2018, ANSES received a formal request from the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) to establish the conditions most likely to guarantee the safety of use of food supplements made with plants or plant preparations containing berberine.

1. BACKGROUND AND PURPOSE OF THE REQUEST

The use of food supplements in France is regulated by Decree no. 2006-352 of 20 March 2006¹, whose Article 16 describes the principle of mutual recognition, authorising notification of the placing on the market of food supplements manufactured or marketed in another Member State of the European Union.

The DGCCRF has received numerous mutual recognition notifications for the use of plants of the *Berberis* genus in the composition of food supplements intended for the French market. Use of these plants in food supplements in Belgium is subject to compliance with a maximum daily dose of isoquinoline alkaloids (expressed as berberine) set at 10 mg. The formal request letter states that: “*The Commission for Plant Preparations, reporting to the Belgian authorities, considered that a very low limit needed to be set to prevent food supplements made with plants or plant preparations containing berberine from falling within the scope of medicinal products*”.

¹ Decree no. 2006-352 of 20 March 2006 on food supplements.

In France, the French Pharmacopoeia Commission for “Medicinal plants and essential oils”, within the French Health Products Safety Agency (ANSM), reported that alkaloids such as berberine can increase uterine contractions². In the Ministerial Order of 24 June 2014³, hereinafter referred to as the “Plants” Order, the DGCCRF indicated that isoquinoline alkaloids (berberine and palmatine) are substances to be monitored in *Phellodendron amurense* Rupr. and that “*labels shall include a warning advising pregnant women not to use them*”. However, no maximum level of isoquinoline alkaloids is proposed for food supplements made with plants or plant preparations containing berberine. Moreover, food supplements containing berberine, defined as a substance, are available on the market.

In this context, the DGCCRF asked ANSES for an Opinion regarding the conditions most likely to guarantee the safety use of food supplements made with plants or plant preparations containing berberine.

This expert appraisal first sought to investigate whether the dose of 10 mg of berberine per day established by the Belgian authorities is relevant in light of the available safety data. If not, the experts would then analyse the toxicological data currently available to determine whether they could identify a maximum daily dose applicable to food supplements made with plants or plant preparations containing berberine.

This expert appraisal took into account all foodstuffs likely to contain plants or plant preparations containing berberine and other dietary sources of berberine (as a standardised extract, additive or processing aid) that can be consumed in France. Furthermore, other substances can be found in berberine-containing plants, in particular other isoquinoline alkaloids and terpene and furan derivatives. These substances should be covered by a separate risk assessment.

2. ORGANISATION OF THE EXPERT APPRAISAL

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

It falls within the scope of the Expert Committee (CES) on “Human Nutrition”. ANSES entrusted the expert appraisal to the Working Group (WG) on “Plants”. This WG contributes to the missions of the CES on “Human Nutrition”, to which it reports, by providing it with specific scientific support in the area of pharmacognosy.

This work relied on the reports of two experts in the WG on “Plants” and on the report of a toxicologist, appointed for the appraisal of the toxicological data. In this context, the CES on “Health reference values” (HRV Committee) was asked to review the toxicological report and determine a toxicity reference value for berberine.

The methodological and scientific aspects of this work were presented to the CES on “Human Nutrition” on 6 December 2018, while the toxicological aspects were presented to the HRV Committee on 22 March 2019 and 10 May 2019. They were adopted by the CES on “Human Nutrition” at its meeting on 6 June 2019.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts’ declarations of interests are made public via the ANSES website (www.anses.fr).

The analysis of the regulatory and literature data was conducted with regard to the medicinal or nutritional status of berberine-containing plants.

ANSES’s nutrivigilance scheme was also asked to analyse reports of adverse effects suspected of being caused by the consumption of food supplements containing berberine, reports submitted by

² Document CP022015043: Meeting report no. 8 of 13 October 2015.

³ Ministerial Order of 24 June 2014 establishing the list of plants authorised in France in food supplements and the conditions of their use.

the ANSM as part of the pharmacovigilance scheme, reports submitted by ANSES's Health Monitoring & Alerts Department (DAVS), as well as information provided by health agencies in the European Union, Canada and the United States.

3. ANALYSIS OF THE WG ON "PLANTS" AND THE CES ON "HUMAN NUTRITION"

The analysis and conclusions presented below summarise the expert appraisal undertaken by the WG on "Plants" and the CES on "Human Nutrition".

3.1 Berberine and berberine-containing plants

3.1.1 Characterisation of berberine

Berberine, also called umbellatine, is an isoquinoline alkaloid belonging to the class of protoberberines. It is a quaternary ammonium salt with a bright yellow colour that is usually found in sulphate or hydrochloride form in food supplements.

Berberine is generally accompanied by the following structural analogues: palmatine, jatrorrhizine and coptisine (Figure 1).

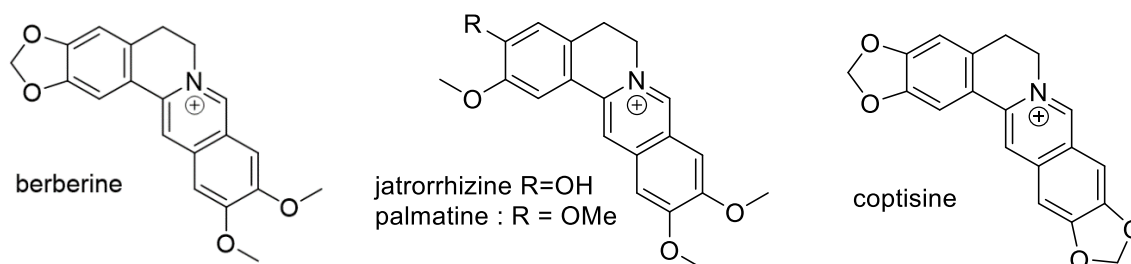


Figure 1: Chemical structure of berberine and its analogues

Underground plant parts (roots and rhizomes) contain particularly high concentrations of these alkaloids, which are also found in stems and bark. However, their levels are lower in fruits and leaves (Singh and Sharma 2018). In some species of berberine-containing plants, such as *Hydrastis canadensis* L., berberine is not the main isoquinoline alkaloid.

Berberine and protoberberines are fairly widely distributed in the following plant families: Annonaceae, Berberidaceae, Menispermaceae, Papaveraceae, Ranunculaceae and Rutaceae (Bruneton 2016, Neag *et al.* 2018). Berberine can also be synthesised (O'Neil 1983).

3.1.2 Regulatory status of berberine-containing plants

ANSES approached its European and Canadian counterparts to obtain information about the status of berberine-containing plants and their conditions of use.

Numerous berberine-containing plants are listed in the European Food Safety Authority's (EFSA) Compendium of Botanicals, a database of plants identified as containing one or more naturally occurring substances of possible concern for human health.

In France, *Phellodendron amurense* Rupr., commonly called "corktree", is the only berberine-containing plant included in the list of the "Plants" Order, for the use of its bark. The other berberine-containing plants currently authorised on the food supplements market in France appear in the list

of plants eligible for Article 15⁴ of Decree no. 2006-352, as well as in the new list of 1011 plants published on the DGCCRF website in January 2019⁵. No maximum daily dose of berberine has been defined for these plants but labels must include a warning advising pregnant women not to use these products.

In Italy⁶, an update of the list of plants authorised in food supplements includes a warning stating that *Phellodendron amurense* Rupr. should not be used by pregnant women. However, other berberine-containing plants appear on this list without any restrictions. Moreover, *Hydrastis canadensis* L. is not authorised for use in food supplements.

In Belgium⁷, berberine-containing plants are authorised in food supplements with the following recommendation: “*The recommended daily amount should not lead to an intake of isoquinoline alkaloids (expressed as berberine) exceeding 10 mg*”. However, there is no warning indicating that these products should not be used by pregnant women.

In Poland, the same restriction is applied based on the decision of the Belgian authorities.

In Germany, the bark and root of berberine-containing plants are not recommended for use in food.

In Serbia, Slovenia and Greece, berberine-containing plants (bark and root) are not authorised in food supplements.

In Sweden and Hungary, berberine-containing plants (bark and root) are included in a negative list (considered as unauthorised) of plants intended for use in food.

In Europe, the BELFRIT list⁸ includes numerous berberine-containing plants with the following statement: “*The amount of alkaloids must be determined*”.

In Canada, berberine is classified as a natural health product (NHP) in Appendix 1, Article 2 of the Natural Health Products Regulations, without any restrictions or warnings. Berberine is mentioned by the National Research Council of Canada as a way of managing high blood cholesterol.

No health claims are currently authorised in the European regulations for berberine-containing plants or for substances in the group of isoquinoline alkaloids⁹.

To summarise, berberine-containing plants are not used in food and are only used in food supplements subject to certain restrictive conditions of use.

3.2 Pharmacological properties of berberine

The information provided by the Belgian authorities regarding the data used to determine the daily limit of 10 mg of isoquinoline alkaloids (expressed as berberine equivalent) was based on data showing pharmacological activities with berberine on its own, and not with berberine-containing plant extracts.

Pharmacological studies undertaken with berberine have shown effects on the central nervous system (anticonvulsant, antidepressant, analgesic), cardiovascular system (hypotensive effects,

⁴ https://www.economie.gouv.fr/files/files/directions_services/dgccrf/secure/teleicare/Table-Plantes.pdf

⁵ Consolidated list including plants considered as traditional in conventional food and those authorised under the principle of mutual recognition: <https://www.economie.gouv.fr/dgccrf/complements-alimentaires-plantes>.

⁶ List of plants updated in September 2018: Regulation on the use of plant substances and preparations in food supplements in Italy.

⁷ Belgian Royal Decree of 29 August 1997 on the manufacture and trade of foods composed of or containing plants or plant preparations (consolidated version, February 2017).

⁸ Harmonised list of 1025 plants identified in Belgium, France and Italy as being eligible for use in food supplements. This list has no legally binding regulatory value.

⁹ Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health.

positive inotropic and negative chronotropic effects), immune system and endocrine system (hypoglycaemic and hypolipidaemic) (Imenshahidi and Hosseinzadeh 2016, Neag *et al.* 2018).

- **Cytotoxic and antimicrobial effects**

Berberine has *in vitro* cytotoxic activity at very high concentrations (10 to 80 µM) in various cell lines, with very moderate selectivity against tumour cell lines compared to healthy cell lines (Bao *et al.* 2015, Sakagami *et al.* 2007).

Berberine also has antimicrobial properties that justify traditional uses of berberine-containing plants, according to some authors (Chu *et al.* 2016, Chu *et al.* 2014, Budeyri Gokgoz *et al.* 2017).

- **Effects on the cardiovascular system**

Berberine and its derivatives have positive inotropic, negative chronotropic, antiarrhythmic and vasodilator effects (Zeng, Zeng, and Li 2003). At low plasma concentrations (< 1 µM), berberine showed endothelium-dependent aortic relaxation in isolated organ models. At higher doses (> 1 µM), it induced aortic relaxation irrespective of the endothelium (Wong 1998).

Berberine blocks the hERG potassium channel¹⁰ and inhibits its membrane expression (Rodriguez-Menchaca *et al.* 2006, Zhang *et al.* 2014). The hERG channel plays a role in cardiac repolarisation and is targeted by antiarrhythmic drugs (Yan *et al.* 2015). Berberine-induced interactions with these drugs are described in Section 3.8.

- **Effects on metabolism**

Berberine controls glucose metabolism *in vitro* and *in vivo* in rats and mice by activating AMP-activated protein kinase (AMPK) (Yin *et al.* 2002, Lee *et al.* 2006, Yin *et al.* 2008a). Its hypoglycaemic activity may also involve the RBP4¹¹-GLUT4¹² system (Zhang *et al.* 2008b) and the secretion of GLP1¹³, an intestinal hormone that stimulates insulin secretion (Lu *et al.* 2009).

As reported in a recent summary review, berberine appears to reduce serum concentrations of triglycerides, glucose and total cholesterol (Singh and Sashidhara 2017).

Berberine and its metabolites reduce lipid accumulation in HepG2 liver cells by increasing the expression of LDL receptors (Zhou *et al.* 2014).

Oral administration of berberine (75 to 300 mg/kg bw¹⁴) in diabetic rats¹⁵ limited increases in blood glucose, haemoglobin A1c¹⁶, total cholesterol, triglycerides, LDL cholesterol and apolipoprotein B and decreases in HDL cholesterol and apolipoprotein A1 by maintaining them at levels similar to those of the controls (non-diabetic rats) (Zhou *et al.* 2008). In the liver, berberine reduced the progression of hepatic symptoms observed in diabetic patients and reverted the main liver functions to levels similar to those of the controls (Zhou *et al.* 2008). It also reduced the expression of the PCSK9¹⁷ enzyme that regulates the synthesis of LDL receptors via a post-transcriptional mechanism (Cameron *et al.* 2008, Pirillo and Catapano 2015). Its action on PCSK9 has been demonstrated both *in vitro* and *in vivo* (Hunter and Hegele 2017).

3.3 Clinical data on berberine and berberine-containing plant extracts

Numerous clinical studies have been conducted with berberine (generally in the form of unspecified salts) or berberine-containing plant extracts at daily doses of 400 to 1500 mg berberine, for 10 days to three months.

¹⁰ hERG: human ether-a-go-go-related gene

¹¹ RBP4: Retinol-binding protein 4, involved in the pathogenesis of type 2 diabetes.

¹² GLUT4: Glucose transporter type 4, a protein that transports glucose from the plasma to the adipose tissue and skeletal muscle.

¹³ GLP1: Glucagon-like peptide-1.

¹⁴ bw: body weight

¹⁵ Diabetes was induced by injecting streptozotocin (35 mg/kg), and then the rats were given a high-carbohydrate and high-fat diet.

¹⁶ Glycated haemoglobin (or HbA1c) reflects glycaemic control over a period of two or three months.

¹⁷ PCSK9: Proprotein convertase subtilisin/kexin type 9.

- **Gastrointestinal infections**

Berberine has traditionally been used to treat diarrhoea, especially bacterial diarrhoea (Chen *et al.* 2014). Berberine inhibits the intestinal secretory response induced by *Shigella* enterotoxins (Sabir, Akhter, and Bhide 1978, Sack and Froehlich 1982). Its effect was demonstrated for aqueous and ethanolic extracts of *Berberis aristata* (Joshi *et al.* 2011), at a dose of 400 mg berberine per day in patients with acute diarrhoea caused by enterotoxigenic *Escherichia coli* (Rabbani *et al.* 1987). In cholera patients, the dose of 400 mg of berberine per day was less effective (Khin *et al.* 1985).

- **Parasitic diseases**

In a study undertaken with 42 children with giardiasis between the ages of five months and 14 years, berberine administered orally at the daily dose of 10 mg/kg bw for 10 days showed an acceptable therapeutic effect, comparable to that of other anti-infective drugs (metronidazole, furazolidone and quinacrine hydrochloride¹⁸), depending on the dose used (Gupte 1975).

- **Cardiovascular disorders**

A meta-analysis showed that berberine (500 to 1500 mg/day) with lifestyle intervention tended to lower blood pressure more than the lifestyle intervention alone or a placebo (Lan *et al.* 2015).

Berberine administered by the intravenous route at the dose of 2 mg/kg bw per minute, for 30 minutes, in 12 patients with heart failure, improved cardiac performance, probably due to peripheral vasodilator and positive inotropic effects. However, in four patients, *torsades de pointes* ventricular tachycardia occurred 1 to 20 hours after injection of berberine (Marin-Neto *et al.* 1988).

- **Diabetes and hyperlipidaemia**

A double-blind clinical study undertaken for three months in 36 adults with newly diagnosed type 2 diabetes showed berberine (500 mg by the oral route, three times a day) to have a hypoglycaemic effect equivalent to that of metformin (500 mg, three times a day) (Yin, Xing, and Ye 2008b).

A clinical study undertaken for three months in 48 adults with poorly controlled type 2 diabetes showed decreases in fasting and postprandial blood glucose following administration of berberine (500 mg by the oral route, three times a day), from the first week to the end of the trial. Total cholesterol and LDL cholesterol decreased significantly as well (Yin, Xing, and Ye 2008b).

A clinical study undertaken for three months in 116 patients with type 2 diabetes and dyslipidaemia treated with berberine (1000 mg/day by the oral route) versus a placebo showed decreases in fasting and postprandial blood glucose as well as a decrease in lipid parameters (triglycerides, total cholesterol and LDL cholesterol) (Zhang *et al.* 2008a).

Oral administration of 1000 mg of berberine per day for three months in 32 patients with hypercholesterolaemia reduced total serum cholesterol by 29%, triglycerides by 35% and LDL cholesterol by 25% (Kong *et al.* 2004).

A meta-analysis showed that berberine (600 to 1500 mg/day by the oral route) was associated with a statistically significant decrease in total cholesterol, triglycerides and LDL cholesterol and an increase in HDL cholesterol, in various adult populations (obese or dyslipidaemic), compared with a placebo or simvastatin (Dong *et al.* 2013).

A meta-analysis showed that treating type 2 diabetes with berberine (500 mg/day) in addition to lifestyle intervention reduced fasting and postprandial blood glucose and HbA1c levels more than the lifestyle intervention alone or a placebo (Lan *et al.* 2015). This meta-analysis also showed that, in populations with hyperlipidaemia, berberine (900 to 1500 mg/day) combined with a lipid-

¹⁸ Drugs not marketed in France.

lowering treatment was more effective at reducing the level of triglycerides and increasing the level of HDL cholesterol than a lipid-lowering treatment alone (Lan *et al.* 2015).

This result was confirmed by a recent meta-analysis of randomised clinical trials including 2147 participants. It showed that berberine used alone improved lipid profiles in a context of dyslipidaemia (Ju *et al.* 2018).

In conclusion, these clinical studies show that berberine has significant therapeutic effects in populations of treated patients, from a dose of 400 mg/day in adults. Its effects are increased when it is used in combination with reference drugs, to treat hypoglycaemia or hypolipidaemia, but the safety of these combinations has not been specifically assessed. However, numerous drug interactions identified in the literature are described in Section 3.8.

Berberine appears to be well tolerated in humans at the doses tested in clinical studies. However, intestinal disorders have been observed by some authors for the highest doses (Dong *et al.* 2013, Lan *et al.* 2015). The other adverse effects identified in the literature are described in Section 3.7.

The minimum dose of berberine of 400 mg/day used for therapeutic purposes does not rule out the possibility of lower doses having the same effects. Therefore, these clinical data do not support the daily dose of 10 mg of isoquinoline alkaloids (expressed as berberine equivalent) set by the Belgian authorities to exclude food supplements containing them from the scope of medicinal products by function.

3.4 Pharmacokinetic data on berberine

- **Absorption and bioavailability**

The bioavailability of berberine taken orally seems very low; *in vivo* studies reported less than 1% bioavailability (Liu *et al.* 2010, Chen *et al.* 2011). Its low intestinal absorption appears to be the main reason for this low oral bioavailability. Its interactions with P-glycoprotein (P-gp¹⁹) in the intestines, its hepatic metabolism and its hepatobiliary excretion are thought to be other factors explaining its low plasma concentrations (Pan *et al.* 2002, Liu *et al.* 2010, Chen *et al.* 2011, Ma *et al.* 2013, Liu *et al.* 2016).

- **Distribution**

A study on the tissue distribution of berberine and its Phase I metabolites was undertaken in rats, with oral administration of 200 mg/kg bw. Berberine was mainly distributed in the liver and kidneys and was also found in the brain. Concentrations of berberine and its metabolites in the liver were 10 to 30 times higher than those in plasma, after four hours (Tan *et al.* 2013).

- **Metabolism**

In humans, following oral administration of 1000 mg/day, berberine was metabolised by the liver, with oxidative demethylation involving CYP2D6 and CYP1A2 isoforms and then CYP3A4, CYP2E1 and CYP2C19 isoforms (Guo *et al.* 2012, Li *et al.* 2011). Four Phase I metabolites (berberrubine, thalifendine, demethyleneberberine and jatrorrhizine), accounting for 90% of total metabolites, were thus found in plasma and tissues. Plasma concentrations of these metabolites, measured four hours after a single oral administration (500 mg) in 10 healthy volunteers, were equal to or greater than concentrations of non-metabolised berberine (0.07 to 0.14 nM) (Spinozzi *et al.* 2014). These metabolites also contribute to the pharmacological activities of berberine (Liu *et al.* 2016, Yu *et al.* 2017).

¹⁹ P-gp: permeability glycoprotein

These metabolites are then glucuronoconjugated by UDP-glucuronosyltransferases (UGT1A1 and UGT2B1), primarily leading to the formation of thalifendine glucuronide (Liu *et al.* 2009). They can also be sulphoconjugated (Ye *et al.* 2009).

- **Excretion**

In *in vivo* rat models, berberine was primarily excreted by the hepatobiliary system with an enterohepatic cycle, as well as by the kidneys in metabolised form. Berberine was also excreted in unchanged form in faeces. It was only found in very low proportions in urine and bile in rats, after administration by the oral or intravenous route (Chen and Chang 1995, Tan *et al.* 2007). Demethylated metabolites of berberine, in particular berberrubine and thalifendine, were also found in urine and bile (Ma *et al.* 2013). Conjugated derivatives were hydrolysed to release Phase I metabolites that were reabsorbed by enterohepatic circulation (Tsai and Tsai 2004).

3.5 Toxicological data on berberine and berberine-containing plant extracts

The summary of toxicological data was written based on summary reports prepared by internationally recognised organisations (International Agency for Cancer Research 2016, ESCOP 2013, National Toxicology Program 2010, WHO 2009), supplemented by a review of the literature (1990-2019).

3.5.1 Acute toxicity

- **Berberine**

In vivo acute toxicity studies have shown that the toxicity of berberine alone is higher than that of plant extracts containing it.

LD₅₀²⁰ values by the oral route above 1000 mg/kg bw berberine in rats and 329 mg/kg bw berberine sulphate in mice have been reported (Haginiwa and Harada 1962, Morgan *et al.* 2005). For berberine isolated from the rhizome of *Coptis spp.*, an LD₅₀ by the oral route of 714 mg/kg bw in mice was determined (Yi *et al.* 2013).

Ingestion of berberine causes gastrointestinal disorders (nausea, vomiting, diarrhoea) in mice, cats and dogs (Rad, Rameshrad, and Hosseinzadeh 2017) as well as gastric ulcers in mice from the dose of 200 mg/kg bw (Yesilada and Kupeli 2002).

- **Berberine-containing plant extracts**

Berberis vulgaris and *Hydrastis canadensis* (rhizome extract) appear to be moderately toxic with LD₅₀ values by the oral route in mice of 520 mg/kg bw (Morgan *et al.* 2005) and 1620 mg/kg bw (ESCOP 2013), respectively. *Berberis aristata* (ethanolic extract, aqueous extract of bark) and *Coptis chinensis* Franch (methanolic extract of fibrous root) did not induce toxicity in mice after a single oral ingestion of a high dose. Their LD₅₀ values were greater than 5000 mg/kg bw (Joshi *et al.* 2011) and 7000 mg/kg bw (Ning *et al.* 2015), respectively.

3.5.2 Subacute toxicity

In vivo subacute toxicity studies undertaken with berberine-containing plant extracts enabled the liver to be identified as a target organ. After oral exposure, increases in liver weights were observed in:

- Sprague-Dawley rats exposed for 14 days to various extracts and fractions of *Berberis crataegina* containing 1.16% berberine (ethanol: 300 mg/kg, butanol: 642 mg/kg, aqueous: 472 mg/kg, chloroform-ethanol: 614 mg/kg) (Yesilada and Kupeli 2002);

²⁰ LD₅₀: Median lethal dose, i.e. the dose of substance that can kill 50% of a group of animals.

- F344/N rats exposed for two weeks, from 1560 ppm of a *Hydrastis canadensis* root powder (containing 3.45% berberine), and in B6C3F1 mice, from 25,000 ppm of this powder (National Toxicology Program 2010).

3.5.3 Subchronic and chronic toxicity

- **Berberine**

In Sprague-Dawley rats orally exposed for 90 days to berberine and its derivatives (coptisine, palmatine, epiberberine), alkaloids found in *Rhizoma coptidis*, at a dose of 156 mg/kg bw, no symptoms of poisoning and no significant effects on body weight, mortality or blood and tissue parameters were observed (Yi *et al.* 2013).

Oral administration of 75, 100 and 300 mg/kg bw/day of berberine to diabetic male Wistar rats for 16 weeks (four months) led to a reduction in total body weights and relative liver weights (Zhou *et al.* 2008).

- **Berberine-containing plant extracts**

A methanolic extract of *Rhizoma coptidis* (containing 1.20% berberine) administered orally for 90 days at the dose of 3760 mg/kg bw/day to Sprague-Dawley rats resulted in histological liver lesions (hepatocyte degeneration), associated with a significant increase in alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) liver enzymes, as well as histological lung lesions (inflammatory cell infiltrates in the lung) (NOAEL²¹ = 1880 mg/kg bw/day) (Ning *et al.* 2015). In a similar study, Sprague-Dawley rats were orally exposed to 521 mg/kg bw/day of *Rhizoma coptidis* rhizome for 90 days but did not show any signs of toxicity (Yi *et al.* 2013).

A three-month study was undertaken in F344/N rats and B6C3F1 mice fed 0, 3121, 6250, 12,500 or 50,000 ppm of *Hydrastis canadensis* root powder (containing 3.45% berberine). The corresponding average daily doses of powder used were 0.26 to 4.1 g/kg bw/day for rats and 0.6 to 10.6 g/kg bw/day for mice. The results did not show any mortality or any clinical signs due to exposure. The liver was identified as a target organ in both species of rodents, characterised by dose-dependent centrilobular hypertrophy and increases in absolute and relative liver weights from 3121 ppm in female rats and from 12,500 ppm in female mice (National Toxicology Program 2010). Increased liver weights (with no hepatic lesions) were also observed in female Sprague-Dawley rats exposed to doses of 0 to 18,400 ppm from gestation days 6 to 20 (National Toxicology Program 2003) and in female Swiss albino (CD-1) mice exposed to 0 to 50,000 ppm from gestation days 6 to 17 (National Toxicology Program 2002).

3.5.4 Reproductive and developmental toxicity

The World Health Organization (WHO) considers that *Berberis vulgaris*, *Coptis chinensis* and *Hydrastis canadensis* should not be used by pregnant and breastfeeding women (WHO 1999, 2007, 2009). The European Scientific Cooperative on Phytotherapy (ESCOP) also states that *Hydrastis canadensis* should not be used throughout pregnancy and breastfeeding (ESCOP 2013):

- in the first trimester of pregnancy due to a risk of maternal and embryofetal toxicity and teratogenic concerns;
- at the end of pregnancy due to an increased risk of kernicterus;
- in other stages of pregnancy, their short-term use is likely safe despite the observation of oxytocic effects.

²¹ NOAEL: no observed adverse effect level.

- **Berberine**

Berberine chloride dihydrate was administered through diet to female Sprague-Dawley (CD) rats (3625, 7250 and 14,500 ppm; i.e. 282, 531 and 1313 mg/kg bw/day), from gestation days 6 to 20, and to mice (3500, 5250 and 7000 ppm, i.e. 569, 841 and 1155 mg/kg bw/day), from gestation days 6 to 17 (Jahnke *et al.* 2006). In rats, maternal toxicity (reduced weight gain, feed intake and absolute and relative liver weights, NOAEL = 282 mg/kg bw/day) as well as decreased foetal body weights (NOAEL = 1313 mg/kg bw/day) were reported. In mice, increased water intake was observed in the mothers from 841 mg/kg bw/day and effects on the foetuses were observed from 569 mg/kg bw/day (significant increase in the percentage of foetuses with malformations (cleft palate, discontinuous rib)).

- **Berberine-containing plant extracts**

Reprotoxicity studies did not show any effects on reproduction or development with *Hydrastis canadensis* (National Toxicology Program 2003, 2002) or *Rhizoma coptidis* (Ning *et al.* 2015). However, berberine-containing plants induced oxytocic effects (Haginiwa and Harada 1962, McKenna and Plotnikoff 2005, ESCOP 2013).

3.5.5 Genotoxicity

The mutagenicity and genotoxicity of berberine and berberine-containing plants have been studied in numerous *in vitro* and *in vivo* tests covering all parameters: primary DNA damage (Jantova *et al.* 2006), gene mutations and chromosomal aberrations (Pasqual *et al.* 1993). The most relevant studies and/or those undertaken according to the OECD guidelines showed that *in vitro*, berberine induces primary DNA lesions via an induction mechanism that may involve the formation of reactive oxygen species and the inhibition of DNA repair by inhibiting topoisomerase II (Chen *et al.* 2013). Various gene mutation tests undertaken with bacteria (Ames test) did not find berberine or extracts of *Hydrastis canadensis* and *Rhizoma coptidis* to have any mutagenic effects (National Toxicology Program 2010). *In vivo*, an erythrocyte micronucleus test in peripheral blood undertaken following intraperitoneal injection of berberine chloride in male B6C3F1 mice did not show any genotoxic effects (National Toxicology Program 2010). Similar results were obtained with a *Rhizoma coptidis* extract in the erythrocyte micronucleus test in peripheral blood (National Toxicology Program 2010) and in the bone marrow micronucleus test in rodents treated by the oral route (Ning *et al.* 2015). However, unlike the clastogenic potential, the mutagenic potential of berberine and berberine-containing plant extracts has not been studied *in vivo* to date.

3.5.6 Carcinogenicity

In vitro, berberine has antiproliferative and cytotoxic effects on various normal and cancerous cell lines (Yi *et al.* 2013). *In vivo*, the administration of berberine reduces tumour growth and incidence in various animal models (mice and rats) of carcinogenesis (National Toxicology Program 2010).

The International Agency for Cancer Research (IARC) classified *Hydrastis canadensis* root powder as possibly carcinogenic to humans (Group 2B) (International Agency for Cancer Research 2016). In a carcinogenicity study, Dunnick *et al.* observed an increase in liver tumours (adenomas and/or hepatocellular carcinomas) in male and female rats and male mice exposed for two years to *Hydrastis canadensis* root powder. However, since this plant contains several alkaloids, these effects could not be attributed exclusively to berberine. Moreover, the authors underline that this increased tumourigenicity in rodents may have been partly due to the topoisomerase II inhibition properties of berberine or its metabolite berberrubine (National Toxicology Program 2010, Dunnick *et al.* 2011).

3.5.7 Cardiotoxicity

The data available in the literature show a cardiotoxic effect of berberine and plant extracts (*Berberis vulgaris* and *Rhizoma coptis*), via the inhibition of the hERG potassium channel which plays a critical role in cardiac repolarisation (Rad, Rameshrad, and Hosseinzadeh 2017). A study undertaken in humans showed that berberine may improve the cardiac performance of patients with heart failure but that cardiotoxic effects such as ventricular tachycardia (*torsades de pointes*) may occur (Yan *et al.* 2015). A case of cardiotoxicity is also described in Section 3.7 on the vigilance schemes.

Based on the available toxicological data, it is not possible to rule out a human health effect due to the consumption of food supplements containing berberine or berberine-containing plant extracts.

3.6 Establishment of a reference value

The nature of a TRV²² or iTV²³ (acute, subchronic, chronic) is partly determined by the duration of exposure in the toxicological studies but also by the health risk assessment needs. As a reminder, when dealing with TRVs and in line with the scenarios generally taken into account when assessing health risks in humans, ANSES distinguishes between three types of exposure durations:

- acute exposure, from 1 to 14 days;
- subchronic exposure, from 15 to 364 days;
- chronic exposure, for 365 or more days.

In light of the maximum use period of 14 days recommended by manufacturers of food supplements containing berberine, the experts chose to establish an acute reference value.

In light of the few toxicological data described above, the HRV Committee decided to establish an indicative toxicity value (iTV) for berberine. An iTV is an indicative toxicological benchmark that is less robust than a TRV; it thus has a lower confidence level but can nonetheless be used to assess a risk. Unlike a TRV, an iTV should only be used to respond to the specific situation and context that justified its establishment (ANSES 2017).

3.6.1 Choice of the critical effect

The few available studies in animals reported hepatic effects (changes in absolute and relative liver weights, hepatocellular hypertrophy). Other studies showed that these hypertrophy effects were also accompanied by histological lesions. Furthermore, pharmaco-toxicovigilance data indicate acute liver damage (cytolysis in particular). All of these data support the choice of hepatocellular hypertrophy as the critical effect.

3.6.2 Choice of the key study

Only two oral exposure studies lasting less than 14 days showed a dose-response relationship:

- the subacute toxicity study (National Toxicology Program 2010) during which F344/N rats and B6C3F1 mice (n = 5 animals/sex/group/species) were fed 0, 1560, 3121, 6250, 12,500, 25,000 or 50,000 ppm of *Hydrastis canadensis* root powder (containing 3.45% berberine) for two weeks. Significant increases in absolute and relative liver weights were observed in the rats, from 1560 ppm in males and from 6250 ppm in females, as well as in the mice, from 25,000 ppm in males and at 50,000 ppm in females. Mild to moderate hepatocellular hypertrophy (characterised by the enlargement of centrilobular hepatocytes) was observed in three male rats exposed to 25,000 ppm and in all of the rodents at 50,000 ppm.

²² TRV: toxicity reference value

²³ iTV: indicative toxicity value

- the reprotoxicity study (Jahnke *et al.* 2006) during which female Sprague-Dawley rats and female Swiss albino (CD-1) mice were fed 0, 3625, 7250 and 14,500 ppm of berberine chloride dihydrate from gestation days 6 to 20 for the rats and from gestation days 6 to 17 for the mice. Maternal toxicity was observed in the female rats, with decreases in weight gain (from 7250 ppm) and in feed intake and absolute and relative liver weights (at 14,500 ppm).

Liver weights (absolute and relative) significantly increased in the NTP study in rats and mice whereas they decreased in the study by Jahnke *et al.* (2006), undoubtedly due to an issue of feed palatability. That is why the results of the study by Jahnke *et al.* (2006) were not deemed relevant. The key study selected was therefore that of the National Toxicology Program (2010), even though it was conducted with a plant extract and not with berberine itself. Moreover, *Hydrastis canadensis* contains several alkaloids, including berberine and hydrastine, which can have different pharmacological and toxicological effects (WHO 2007).

3.6.3 Choice of the critical dose

Increases in relative liver weights have been observed both in rats and in mice, with rats appearing more susceptible than mice. Changes in absolute and relative liver weights in rats are shown in the table below.

Table 1: Changes in liver weights in rats exposed for two weeks (National Toxicology Program 2010)

Dose of <i>Hydrastis canadensis</i> root powder (ppm)	Dose of <i>Hydrastis canadensis</i> root powder (mg/kg/day)	Dose of berberine (mg/kg bw/day)	Absolute liver weight	Relative liver weight	Dose of <i>Hydrastis canadensis</i> root powder (mg/kg/day)	Dose of berberine (mg/kg/day)	Absolute liver weight	Relative liver weight
Males					Females			
0	0	0	9.63 ± 0.26	46.017 ± 0.421	0	0	6.28 ± 0.22	43.297 ± 1.032
1,560	155	5.35	10.53 ± 0.22	49.568 ± 0.869*	150	5.18	6.17 ± 0.16	43.914 ± 0.757
3,121	315	10.87	10.65 ± 0.22	50.296 ± 0.603*	290	10.01	6.62 ± 0.24	45.616 ± 0.915
6,250	630	21.74	11.76 ± 0.14*	56.021 ± 0.837*	640	22.08	6.9 ± 0.2	49.491 ± 0.616*
12,500	1,190	41.1	12.74 ± 0.32*	60.816 ± 1.378*	1,240	42.78	7.44 ± 0.22*	52.406 ± 1.081*
25,000	2,465	85.04	13.5 ± 0.38*	66.048 ± 0.754*	2,370	81.77	7.71 ± 0.1*	54.089 ± 0.8*
50,000	4,815	166.12	14.29 ± 0.74*	71.048 ± 1.455*	4,870	168.02	8.55 ± 0.31*	60.068 ± 1.475*

^a Levels in powder measured by HPLC: 3.45% berberine, 3.02% hydrastine and 0.08% canadine. Palmatine ND.

*p < 0.01

It was not possible to establish a BMD²⁴ based on these data since the model did not fulfil the validation criteria recommended by EFSA and the US EPA²⁵. The first tested dose was a LOAEL²⁶ with an effect of around 8%. In the absence of a NOAEL and given the limited number of animals, the uncertainty around the BMD was too high.

Thus, a LOAEL of 5.35 mg/kg bw/day of berberine was selected as the critical dose.

²⁴ BMD: the benchmark dose is a method for estimating the critical dose based on modelled data.

²⁵ US EPA: United States Environmental Protection Agency

²⁶ LOAEL: lowest observed adverse effect level

3.6.4 Allometric adjustment

An allometric adjustment was performed to reduce the value of the uncertainty regarding interspecies variability. A human equivalent dose (HED) was calculated, using the following equation²⁷:

$$\text{Human equivalent dose} = \text{Animal dose} \times \left(\frac{\text{Animal weight}}{\text{Human weight}} \right)^{1/4}$$

The average weight of the control rats at the end of the study was 209 g. That used for humans for the calculation was 70 kg.

Thus, the critical dose = **LOEL_{HED} = 1.25 mg/kg bw/day**

3.6.5 Choice of uncertainty factors

The iTV was calculated from the LOEL_{HED} using the following uncertainty factors (ANSES 2017):

- Inter-species variability (UF_A): 2.5. The allometric adjustment performed enabled a human equivalent dose to be calculated, using the previous equation. To account for toxicodynamic variability and residual uncertainties, an additional uncertainty factor was set at 2.5 according to WHO-IPCS recommendations (WHO-IPCS, 2005) and based on ANSES practices.
- Inter-individual variability (UF_H): 10. Compounds containing berberine are capable of inducing haemolytic anaemia (severe complications) in G6PD (glucose-6-phosphate dehydrogenase)-deficient patients due to the pro-oxidant properties of this alkaloid. It was therefore appropriate to maintain a UF_H of 10 to prevent any risk in this vulnerable sub-population (prevalence of 400 million cases worldwide and at least 250,000 in France according to the French National Authority for Health (HAS)).
- Use of a BMDL, LOEL/C or NOEL/C (UF_{B/L}): 3
- Inadequacy of the data (UF_D): 10

An overall uncertainty factor of 750 was thus used to establish the iTV.

3.6.6 Proposed value

iTV = 1.7 µg/kg bw/day

For example, for a 60 kg individual, the maximum daily dose of berberine would be 0.1 mg.

3.7 Adverse effects associated with the consumption of berberine

• Cases from the nutriviigilance scheme

Between the establishment of the nutriviigilance scheme in 2009 and the month of October 2018, ANSES received three reports of adverse effects likely to be associated with the consumption of food supplements containing berberine. They are given in the table below.

Table 2: Nutriviigilance cases registered between 2009 and 2018.

²⁷ This equation was taken from the recommendations of the US EPA (US EPA, 2006).

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Registration no.	Product name (manufacturer)	Sex and age of the consumer	- Adverse effect(s) ²⁸ - Onset time - Dose ingested per day	Level of severity of the clinical picture ²⁹	Intrinsic causality ³⁰	Comments
2012-057	Myoshock (Muscletech)	M, 34 years	- Effects: aesthenia, myalgia and tachycardia - Unknown time - Unknown dose	unknown	not analysable	concomitant consumption of the Jack3d food supplement and caffeine
2016-120	Armolid Plus (Meda Pharma)	F, 52 years	- Effects: Quincke's oedema - Unknown time - Unknown dose	3	not analysable	no associated consumption
2018-465	Berberine (Super Smart)	M, 68 years	- Effects: headaches, dizziness - Time: 2 hours - Dose: 500 mg	1	I2 (possible)	disappearance of the adverse effects on cessation of the product and after administration of a symptomatic treatment

Only one case of headaches and dizziness occurring after the consumption of berberine could be analysed by the Working Group on Nutrivigilance, which considered causality to be “possible”.

- **Cases from pharmacovigilance**

The ANSM was approached in June 2018 with a view to obtaining data on the adverse effects likely to be associated with the consumption of drugs containing berberine. A case of acute liver impairment was reported following use of the “Berberis” homeopathic medicinal product manufactured by Lehning Laboratories. Moreover, five reports (ocular pain, cold sores, rash and allergic reaction) were identified following administration of “Sedacollyre” eye drops containing berberine, which were withdrawn from the market in 2009.

- **Cases from toxicovigilance**

Alongside the cases identified by the nutrivigilance scheme, two cases of adverse effects likely to be associated with the consumption of food supplements containing berberine were registered by the French poison control centres. The first involved impaired general condition, but the data were insufficient to establish causality. The second, described in the table below, was analysed by the Working Group on Nutrivigilance. A causality score of “likely” was assigned.

Table 3: Case transmitted by the toxicovigilance scheme

Registration no.	Product name (manufacturer)	Sex and age of the consumer	- Adverse effect(s) ³¹ - Onset time - Dose ingested per day	Level of severity of the clinical picture ³²	Intrinsic causality ³³	Comments
2018-734	Equilibre Mucilyse Biofilms (Biophenix)	F, 32 years	- Effects: migraine headache, sleepiness, respiratory discomfort - Time: 1 day - Dose: 4 capsules	1	I3 (likely)	disappearance of the adverse effects on cessation of the product

- **Cases identified abroad**

- In Europe

In June 2018, ANSES approached its European counterparts with a view to obtaining more data on the adverse effects likely to be associated with the consumption of food supplements containing berberine. All of the countries that responded (Spain, Czech Republic, Estonia, Portugal, Germany, Hungary, Serbia, Switzerland, Latvia, Poland, Denmark, Slovenia, Greece, Italy and Bulgaria) indicated that they had not received any reports of adverse effects associated with this type of product.

²⁸ Adverse effect selected by the Nutrivigilance WG after the case had been analysed.

²⁹ The scale of severity in nutrivigilance goes from Level 1 (low severity) to Level 4 (death).

³⁰ The intrinsic score ranges from I0 (excluded) to I4 (very likely).

³¹ Adverse effect selected by the Nutrivigilance WG after the case had been analysed.

³² The scale of severity in nutrivigilance goes from Level 1 (low severity) to Level 4 (death).

³³ The intrinsic score ranges from I0 (excluded) to I4 (very likely).

- In North America

In Canada, data were sought using the Canada Vigilance database for the period from 1 January 1965 to 31 March 2018. One case involving a food supplement containing berberine was identified. It was that of a 64-year-old woman experiencing nausea, fatigue, jaundice and a disturbance in her liver function. She was taking other products at the same time.

In the United States, data were sought in the FDA-Medwatch database. Thirteen cases involving berberine were identified. They were mainly cases of diarrhoea and interactions with major drugs with a narrow therapeutic range (tacrolimus, cyclosporin). The drug interactions identified in the literature are described in Section 3.8.

In the United States, two patients presented with vomiting, jaundice, itching, weight loss, confusion, and increased liver enzymes and bilirubin, after one month of taking a multi-ingredient food supplement (Tricycline) containing berberine and artemisinin, among other things (FDA 2017).

Causality could not be established by ANSES for any of the cases transmitted by any of the foreign vigilance schemes, due to a lack of information.

• **Cases in humans reported in the literature**

The adverse effects most commonly reported in clinical studies have been moderate gastrointestinal reactions, including diarrhoea, constipation, abdominal pain and nausea. Headaches and hypoglycaemia have been observed in a very few cases. A meta-analysis showed that the observed side effects are more common from the dose of 900 mg of berberine per day. Many patients tolerate the adverse effects without discontinuing treatment or by reducing the dose to 600 mg/day (Lan *et al.* 2015).

The case of a 53-year-old man admitted to the emergency department for fatigue, dyspnoea upon exertion and bradycardia, six days after starting a berberine-containing product to treat hypercholesterolaemia, was reported. This case suggested that in some cases, berberine may lead to cardiac side effects, especially in hypervagotonic people (Cannillo *et al.* 2013).

3.8 Drug interactions related to berberine

Berberine is likely to cause multiple pharmacodynamic and pharmacokinetic drug interactions as shown in the following tables. According to Gupta *et al.*, the risk of pharmacological interactions has been identified as the main health risk in the general population associated with the use of berberine (Gupta, Prasad, and Aggarwal 2016).

Table 4: Possible pharmacokinetic interactions with berberine

Effect	Model	Reference
Inhibition of CYP3A4 (19 µM) (a) Decrease in the expression of CYP3A4 (5 µM, 24 h) (b)	<i>In vitro</i> , rat microsomes (a); HepG2 cell line (b)	(Feng <i>et al.</i> 2018)
Slight inhibition of CYP3A (IC ₅₀ : 400 µM)	<i>In vitro</i> , human microsomes	(Chatterjee and Franklin 2003)
Inhibition of CYP2D6, 2E1; No inhibition of CYP3A4, 1A2, 2C19	<i>In vitro</i> , human microsomes	(Chen <i>et al.</i> 2013)
Slight inhibition of CYP3A4/5 (IC ₅₀ : 473 µM)	<i>In vitro</i> , human microsomes	(Wang <i>et al.</i> 2015)
P-GP/MDR inducing effect at 32 µM	<i>In vitro</i> , murine and human hepatic carcinomas, 24 h	(Lin, Liu, Lui, <i>et al.</i> 1999)
P-GP/MDR inhibiting effect	<i>In vitro</i> , endothelial cells (bovine blood-brain barrier)	(Qiu <i>et al.</i> 2009)
Induction of CYP1A1, 2B9, 2B10; no induction of CYP1A2	<i>In vitro</i> , mouse hepatocytes, 24 h, 1-10 µM	(Chatuphonprasert <i>et al.</i> 2012)

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Induction of CYP1A1, 2B9 in diabetic mice; induction of CYP2B10 in normal and diabetic mice	Mice (normal and diabetic – streptozotocin-induced diabetes model), berberine 200 mg/kg/day, intragastric route, 14 days	(Chatuphonprasert <i>et al.</i> 2012)
Suppression of CYP3A11, CYP3A25 (levels of hepatic mRNA decreased by 68% and 87%, respectively) Induction of CYP1A2 (43% increase) Reduction in hepatic CYP3A11 and CYP2D22 activity (of 68% and 32%, respectively) No influence on the expression or activity of CYP2A4, 2B10, 2C9	Mice, berberine 300 mg/kg, intragastric route, 14 days	(Guo <i>et al.</i> 2011)

Data relating to the inhibition of CYP by a *Hydrastis canadensis* extract have been reported in clinical studies:

- approximate 40% inhibition of CYP3A4 and CYP2D6 observed in six healthy volunteers (assessment of CYP1A2, CYP2D6, CYP2E1, CYP3A4/5) after 28 days of supplementation with a *Hydrastis canadensis* extract (900 mg, three times a day), containing hydrastine (65 mg/day) and berberine (77 mg/day). However, a study with *Hydrastis canadensis* root powder (2 g/day for two weeks) in 10 patients did not show any influence on the pharmacokinetics of indinavir (800 mg, single dose) (Gurley *et al.* 2005),
- approximate 50% inhibition of CYP2D6 observed in nine healthy volunteers, with monitoring of urinary debrisoquine (5 mg) excretion, following the administration of *Hydrastis canadensis* (1 g, three times a day, i.e. 75 mg of alkaloids per day) for 15 days (Gurley *et al.* 2008).

Table 5: Pharmacokinetic drug interactions studied with berberine

Active ingredient	Level of evidence	Observation	Proposed mechanism	Reference
Caffeine (93 mg)	Clinical study: 18 healthy volunteers; berberine 900 mg/day for 14 days; compared with a placebo	No effects	No inhibition of CYP1A2	(Guo <i>et al.</i> 2012)
Carbamazepine	<i>In vivo</i> , rats, pre-treatment with berberine: 10, 30, 100 mg/kg bw, intragastric route. Twice a day, two weeks (negative control: water) + carbamazepine, intragastric route, 50 mg/kg bw, 1 h after last administration of berberine on D14	No influence on the plasma profile of carbamazepine or its metabolite, carbamazepine-10,11-epoxide, at the three tested doses of berberine.	No inhibition of CYP3A4	(Qiu <i>et al.</i> 2009)
Cyclosporin A and verapamil	<i>In vitro</i> , Caco-2 cell line; berberine: 1-100 µM	Cyclosporin and verapamil inhibited the extrusion of berberine. Berberine did not inhibit P-gp.	Berberine is a substrate of P-gp	(Zhang <i>et al.</i> 2011)
	<i>In vivo</i> , rats, pre-treatment with berberine, 14 days, intragastric route: 10, 30, 100 mg/kg bw, twice a day (negative control: water). 1 h after the last administration of berberine on D14: cyclosporin by the intragastric route, 5 mg/kg bw.	The bioavailability of cyclosporin at 30 and 100 mg/kg increased by a factor of 1.62 and 1.96 respectively, compared to the control group.	Inhibition of intestinal P-gp rather than CYP3A; modification of biliary absorption and excretion kinetics.	(Qiu <i>et al.</i> 2009)
	a) Clinical study: 52 renal-transplant recipients, treated with cyclosporin (319±91 mg/day), and administration of 600 mg of berberine per day for three months, compared with 52 patients receiving cyclosporin alone (303±80 mg/day). b) Pharmacokinetic study: Six renal-transplant recipients treated with cyclosporin (6 mg/kg/day), with administration of berberine (600 mg/day) for 12 days.	a) Between the beginning and end of treatment, 88.9% increase in levels of circulating cyclosporin in the patients receiving berberine (versus 64.5% in the control group). b) The bioavailability of cyclosporin was 34.5% higher at the end of the protocol; modification of T_{max} and C_{max} .	The effect was attributed to the inhibition of CYP3A4 by berberine in the liver and/or small intestine (Wu <i>et al.</i> 2005). The inhibiting effect on intestinal P-gp was not ruled out (Qiu <i>et al.</i> 2009).	(Wu <i>et al.</i> 2005)
Dextromethorphan (30 mg)	Clinical study: 18 healthy volunteers; berberine 900 mg/day for 14 days; compared with a placebo	Increase in circulating levels of dextromethorphan: urinary dextromethorphan/dextrorphan (its metabolite) ratio multiplied by 10.	Decrease in the activity of CYP2D6 (suggested competitive inhibition)	(Guo <i>et al.</i> 2012)
Digoxin	<i>In vivo</i> , rats, pre-treatment with berberine: single administration 1 h before the administration of digoxin; compared with repeated administration over two weeks, intragastric route: berberine 10, 30, 100 mg/kg bw, twice a day (negative control: water) Treatment with digoxin, 1 h after the last administration of berberine: intragastric route, 0.05 mg/kg, compared with the i.v. route, 0.03 mg/kg	By the intragastric route, the AUC ³⁴ for digoxin increased by 14%, 33% and 70% and by 19%, 23% and 69%, after single or repeated administration, at the doses of 10, 30 and 100 mg/kg of berberine respectively. An increase in T_{max} for digoxin was observed after a single administration of berberine. A decrease in T_{max} was observed after two weeks of treatment with 30 and 100 mg/kg of berberine.	Changes in gastrointestinal transit and motility may have been responsible for these paradoxical effects. The inhibition of P-gp appeared to affect the pharmacokinetics of digoxin.	(Qiu <i>et al.</i> 2009)
	<i>In vivo</i> , rats, oral route, berberine (30 or 100 mg/kg bw)	The AUC for digoxin increased by 33% and 70%, respectively.	Increase in the bioavailability of digoxin via the inhibition of intestinal P-gp.	(Ju <i>et al.</i> 2011).

³⁴ AUC: area under the curve. Expression of plasma concentrations over time.

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Ketoconazole	<i>In vivo</i> , rats, oral route, oral co-administration of 60 mg/kg bw of berberine with 10 mg/kg bw of ketoconazole	215% increase in the AUC for ketoconazole compared with ketoconazole alone. 173% increase in the AUC for berberine compared with berberine alone.	Proposed inhibition of CYP	(Zhou <i>et al.</i> 2012)
Losartan	<i>In vivo</i> , rats, oral route, pre-treatment with berberine 20 mg/kg; administration of losartan, oral route, 10 mg/kg after 24 h	Increase in C_{max} (155%) and the AUC (153%) for losartan, versus without pre-treatment.	Proposed inhibition of CYP3A4 and CYP2C9	(Li <i>et al.</i> 2016)
	Clinical study: 18 healthy volunteers; berberine 900 mg/day for 14 days; compared with a placebo	Doubling of the urinary losartan/E-3174 (metabolite) ratio	Decrease in the activity of CYP2C9	(Guo <i>et al.</i> 2012)
Metformin	<i>In vivo</i> , rats, i.v. route, co-administration of 10 mg/kg bw of berberine and 2 mg/kg bw of metformin	Increase in the initial plasma concentration and decrease in the volume of distribution and systemic clearance of metformin	Inhibition of the transport activity of OCT1 and OCT2 (organic cation transporters 1 and 2)	(Kwon <i>et al.</i> 2015)
Midazolam	<i>In vivo</i> , rats, berberine 20 to 100 mg/kg/day, 10 days; midazolam 20 mg/kg on D10 (or rhodamine 123 at 5 mg/kg on D10)	C_{max} and the AUC for midazolam increased by 63% and 62% at 100 mg/kg of berberine. C_{max} increased by 49% at 50 mg/kg of berberine.	Inhibition of CYP3A. The authors also identified a significant inhibition of P-gp with co-treatment with rhodamine 123.	(Xin <i>et al.</i> 2016)
	Clinical study: 18 healthy volunteers; berberine 900 mg/day for 14 days; compared with a placebo (same subjects, after four weeks of wash-out)	C_{max} , $AUC_{0-\infty}$, and AUC_{0-12} for midazolam increased by 38%, 40% and 37%, respectively. $T_{1/2}$ increased by 150%; clearance reduced by 27%.	Decrease in the activity of CYP3A4	(Guo <i>et al.</i> 2012)
Omeprazole	Clinical study: 18 healthy volunteers; berberine 900 mg/day for 14 days; compared with a placebo	No effect	No inhibition of CYP2C19	(Guo <i>et al.</i> 2012)
Paclitaxel	<i>In vitro</i> , six cancer cell lines; berberine 32 μ M, 24 h	Decrease in the cytotoxic activity of paclitaxel	Modulation of the expression of P-gp 170	(Lin, Liu, Wu, <i>et al.</i> 1999)
Statins (simvastatin and atorvastatin)	<i>In vitro</i> , rat microsomes, HepG2 cell line; berberine (10 μ M) in combination with simvastatin or atorvastatin (1-5 μ M)	Increase in the concentration of statins compared with lack of treatment with berberine	Combined inhibiting and repressing effects on CYP3A4	(Feng <i>et al.</i> 2018)
Tacrolimus	Clinical case of an adolescent patient treated with tacrolimus for nephrotic syndrome, 600 mg of berberine per day	Increase in circulating levels of tacrolimus after administration of berberine, with an increase in the renal toxicity of this drug	Probable inhibition of CYP3A4	(Hou, Han, and Fu 2013).

Interactions with tamoxifen and verapamil, with potential clinical repercussions, have also been reported (McKenna and Plotnikoff 2005, Shi *et al.* 2009). Moreover, a study indicated that berberine can displace warfarin and thiopental from their plasma protein binding sites in mice, with an increased risk of toxicity (Tan *et al.* 2002).

While the experimental data in *in vitro* and *in vivo* models are inconsistent, clinical findings indicate possible increases in the bioavailability and potential toxicity of drugs with a narrow therapeutic range as well as treatment disruption with potentially severe repercussions for patient health.

Pharmacodynamic interactions also appear to be possible:

- *In vitro*, berberine concentrations from 1.25 to 5 μM significantly reduced the anti-cancer activity of 5-fluorouracil and paclitaxel, unlike high doses ($> 10 \mu\text{M}$). This was also observed with camptothecin. This effect appeared to be due to a stress response activated by berberine in cancer cells, associated with the increased activity of the MAPK/ERK1/2 and PI3K/AKT signalling pathways (Bao *et al.* 2015).
- With macrolides (azithromycin, clarithromycin), the administration of berberine should be closely monitored due to the risk of their cardiotoxicity being enhanced by cumulative inhibitory effects on hERG channels, with a possible increase in the time required for ventricular depolarisation and repolarisation (Zhi *et al.* 2015, Schramm *et al.* 2014). A clinical case of cardiac effects occurring with berberine is reported in Section 3.7.
- *In vitro*, combining berberine with simvastatin or atorvastatin synergistically inhibited hERG channels, and a pharmacokinetic interaction involving CYP3A4 was also observed (Feng *et al.* 2018).
- Combining berberine with hypoglycaemic drugs enhances their effects in humans, inducing a potential risk of hypoglycaemia (Lan *et al.* 2015).

Considering the multiple drug interactions identified above, the consumption of food supplements containing berberine in combination with a drug treatment is not recommended, especially for drugs with a narrow therapeutic range; where appropriate, it should be discussed with a doctor or pharmacist.

3.9 Berberine-containing plants considered in food

Berberis vulgaris fruits, commonly called barberries, are used in France to make jams, soft drinks and liqueurs. However, their consumption remains anecdotal, as they are picked locally and produced on a small scale (Chauvet 2018).

In Iran and neighbouring countries, only the ripe berries of various *Berberis* species are consumed. Iran is a major producer and consumer of these dried berries. The dried fruits are mainly used as a food additive in dishes and added to rice. The ripe fruits are also used to produce jellies, syrups, jams, sauces, juices, fruit concentrates and carbonated beverages (Alemardan *et al.* 2013).

Berberine is found in all of the parts of plants of the *Berberis* genus but its concentration in fruits remains lower than that in roots and stems and much lower than that in bark. This bark is used to prepare powders and extracts for the formulation of food supplements (Imenshahidi and Hosseinzadeh 2016).

Some berberine-containing fruits, such as those of *Rollinia mucosa* (syn. *Annona mucosa*, Annonaceae) in French Guiana and *Zanthoxylum* spp. (Rutaceae) in Africa, can also be consumed in their production areas (Lim 2016).

3.10 Berberine-containing plants considered in food supplements

The berberine-containing plants currently authorised in food supplements in France, Belgium and Italy are listed in the table below. Berberine is the main alkaloid in the plant, with the exception of *Tinospora sinensis*. In some rare cases in the literature, berberine has been described in California poppy (*Eschscholzia californica* Cham.), but it is generally not found in this plant (European Medicines Agency 2015).

Table 6: Berberine-containing plants authorised in food supplements in France, Belgium or Italy.

Scientific name	Parts used	France*	Belgium**	Italy	BELFRIT***	WHO monograph	French Pharmacopoeia
<i>Berberis aquifolium</i> Pursh.	Root	X	X	X	X		
<i>Berberis aristata</i> DC.	Root	X	X	X	X		
<i>Berberis vulgaris</i> L.	Root	X	X	X	X	X	List A ³⁵ (root bark)
<i>Coptis japonica</i> (Thunb.) Makino	Root	X	X	X	X	X	
<i>Coptis teeta</i> Wall.	Root	X	X	X	X	X	List B ³⁶
<i>Coptis trifolia</i> (L.) Salisb.	Rhizome	X	X	X	X	X	
<i>Coscinium fenestratum</i> (Goetgh.) Colebr.	Root	X	X	X	X		
<i>Hydrastis canadensis</i> L.	Root/ rhizome	X	X	X		X	List A
<i>Jateorhiza palmata</i> (Lam.) Miers syn. <i>Menispermum palmatum</i> Lam.	Root	X	X		X		List B
<i>Phellodendron amurense</i> Rupr.	Bark	X	X	X	X	X	
<i>Thalictrum flavum</i> L.	Root	X	X	X	X		
<i>Tinospora sinensis</i> (Lour.) Merr.	Root, stem, leaf	X	X				

*Only *Phellodendron amurense* is included in the list in the "Plants" Order; the other plants have been taken from the new list of plants published on the DGCCRF website in January 2019.

**The limit of 10 mg of berberine per day is not indicated for *Tinospora sinensis*.

***The BELFRIT list specifies that the amount of alkaloids must be determined for these plants.

The berberine-containing food supplements available on the French market are usually formulated with standardised extracts of roots of *Berberis* species (*Berberis vulgaris* L. or *Berberis aristata* DC.). A partial extraction of the Télécare database shown in the table below was performed from the DGCCRF website³⁷.

Table 7: Examples of food supplements containing berberine (source: Télécare, August 2018)

Composition*	Recommended daily dose	Information indicated by the operator on the label	Precautions for use
Dry extract of <i>Berberis aristata</i> and red yeast rice	500 mg/day of berberine hydrochloride	Maintains normal cholesterol and triglyceride levels	Do not use if pregnant or breastfeeding. Do not exceed the recommended daily dosage.
<i>Berberis aristata</i> root extract	250 mg/day of berberine hydrochloride	Type 2 diabetes and overweight	
<i>Berberis aristata</i> root extract	1000 mg/day of berberine	Maintains normal blood glucose	
<i>Berberis aristata</i> extract	500 to 1000 mg/day of berberine	Helps control triglyceride and blood glucose levels	Not recommended for pregnant or breastfeeding women or for children under six years of age
Standardised <i>Berberis vulgaris</i> root extract	900 mg/day of berberine	Blood glucose	Do not exceed the recommended daily dose and keep out of the reach of children. Not recommended for use

³⁵ List A of medicinal plants traditionally used in the French Pharmacopoeia

³⁶ List B of medicinal plants traditionally used as is or in preparation form whose potential adverse effects outweigh the expected therapeutic benefit in the French Pharmacopoeia.

³⁷ <https://teleicare.dgccrf.finances.gouv.fr/>

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containing 80% berberine			during pregnancy. Seek medical advice if breastfeeding.
Standardised <i>Berberis aristata</i> root extract	500 mg/day of berberine	-	Reserved for adults and adolescents.
Berberine 97% (<i>Berberis aristata</i>)	516 mg/day of berberine	-	-
Dry <i>Berberis aristata</i> root extract	1125 mg/day of berberine	Maintains normal blood glucose and lipid levels	Not recommended for pregnant women due to the presence of isoquinoline alkaloids. Ask your doctor or pharmacist for advice in the event of concomitant diabetes treatment.
<i>Berberis vulgaris</i> extract	1200 mg/day of berberine	Supports carbohydrate metabolism	-

*The methods of obtaining the extracts and the degrees of purity are not available.

The daily doses of berberine in food supplements recommended by operators range from 250 to 1200 mg (the type of salt is unspecified). These doses are comparable to those tested in the clinical studies analysed above in which pharmacological activity was observed.

Due to the lack of available data on the formulation of food supplements containing berberine, there is uncertainty regarding the dose of exposure to berberine and its potential pharmacological effects. Furthermore, some food supplements contain mixtures of berberine and plant powder, without specifying the total amount of berberine.

Moreover, these food supplements come with claims regarding the “maintenance of normal blood glucose” or “control of blood cholesterol and triglyceride levels”. It should be noted that isoquinoline alkaloids and plants containing them are not included in the list of substances and foodstuffs for which health claims are authorised in Commission Regulation (EU) No 432/2012.

Restrictions of use for these products are sometimes specified during their notification and primarily involve pregnant and breastfeeding women, children and adolescents, as well as the risk of drug interactions.

Furthermore, in North America (United States and Canada), *Hydrastis canadensis* is the plant most commonly used in food supplements containing berberine (Barnes, Bloom, and Nahin 2008). In this context, it should be noted that other substances, such as other isoquinoline alkaloids for *Hydrastis canadensis* and *Tinospora sinensis*, and terpene and furan derivatives for *Tinospora sinensis*, should be taken into account when assessing the risks associated with their consumption.

Levels of berberine and its analogues in the plant parts used in food supplements are given for information in the following table.

Table 8: Levels of berberine and its analogues in the plant parts found in food supplements

Scientific name	Parts used	Level of alkaloids	References
<i>Berberis aquifolium</i> Pursh. syn. <i>Mahonia aquifolium</i> (Pursh) Nutt.	Root	Berberine: 0.5-6% Total alkaloids expressed as berberine > 1% ("Mahonia for homeopathic preparations")	French Pharmacopoeia in force (Bruneton 2016) (WHO 2009)
<i>Berberis aristata</i> DC.	Root	Berberine > 1%	Herbal Medicines Compendium (https://hmc.usp.org)
<i>Berberis vulgaris</i> L.	Root	Berberine (4.5%), magnoflorine (2.1%), berbamine (0.6-1.2%), columbamine (0.3%), jatrorrhizine (0.4%) Total alkaloids expressed as berberine > 2% ("Barberry for homeopathic preparations")	French Pharmacopoeia (WHO 2009)
<i>Coptis japonica</i> (Thunb.) Makino	Root	Berberine 7-9%; palmatine 0.4-0.6%; coptisine 0.4-0.6%	(WHO 1999)

Scientific name	Parts used	Level of alkaloids	References
<i>Coptis teeta</i> Wall. (<i>Coptis chinensis</i> Franch.)	Root	Berberine: 5-7%; palmatine: 1-4%; coptisine: 0.8-2%; berberastine: 1%	(WHO 1999)
<i>Coptis trifolia</i> (L.) Salisb.	Rhizome	Berberine: 4-8%	(WHO 1999)
<i>Coscinium fenestratum</i> (Goetgh.) Colebr.	Root	Berberine: 2-3.5%	(Rojsanga and Gritsanapan 2005)
<i>Hydrastis canadensis</i> L.	Root/rhizome	At least: hydrastine: 2.5%; berberine: 3% Total alkaloids: 2.5-6% including hydrastine (1.5-5%), berberine (0.5-4.5%), canadine (0.5-1%)	European Pharmacopoeia in force (WHO 2007)
<i>Jateorhiza palmata</i> (Lam.) Miers syn. <i>Menispermum palmatum</i>	Root	Contains berberine, palmatine	(Sturm and Stuppner 1998)
<i>Phellodendron amurense</i> Rupr.	Bark	Berberine (1.2-4.75%); magnoflorine (1.1%); palmatine (1.2%); jatrorrhizine (0.5%)	(WHO 2009)
<i>Thalictrum flavum</i> L.	Root	Berberine: around 0.5%	(Ropivia <i>et al.</i> 2010)
<i>Tinospora sinensis</i> (Lour.) Merr.	Root, stem, leaf	Whole plant: palmatine: 0.17%, jatrorrhizine: 0.15%, contains berberine, magnoflorine: 0.05%, other isoquinoline alkaloids	(Bajpai <i>et al.</i> 2016)

3.11 Berberine-containing plants considered in traditional medicine

Several berberine-rich species, in particular plants of the *Berberis* or *Coptis* genera, identified in food supplements in Europe, are commonly used in traditional Chinese and Ayurvedic medicine and included in the Chinese and Indian Pharmacopoeias. They are mainly used in the form of aqueous extracts to treat diarrhoea as well as diabetes, hypertension and hypercholesterolaemia (Guo *et al.* 2012). The standard daily doses described for berberine-containing plants, whether in traditional Chinese medicine (Hempen and Fischer 2007) or by the WHO (WHO 1999, 2009), are around 10 g of plant per day (or the equivalent as plant extract), i.e. 500 to 700 mg per day of berberine and its analogues (Table 8).

Phellodendron amurense Rupr. is one of the 50 fundamental herbs in traditional Chinese medicine, for oral use in the treatment of abdominal pain, diarrhoea, gastroenteritis and urinary tract infections. It has its own WHO monograph (WHO 2009).

Hydrastis canadensis was used by Native North Americans for numerous indications (as a bitter tonic, for gastrointestinal disorders, skin conditions, tumours, tuberculosis, sore throat, eye inflammation) and has its own ESCOP monograph (dyspeptic disorders, as an adjuvant in menorrhagia and dysmenorrhoea, based on tradition) (ESCOP 2013). The American Pharmacopoeia considers that the dried roots and rhizomes of *Hydrastis canadensis* contain at least 2.5% berberine and 2% hydrastine, which is a phthalidisoquinoline alkaloid (Pengelly 2012). According to the European Pharmacopoeia, they contain at least 3% berberine and 2.5% hydrastine. *Hydrastis* (rhizome powder and extracts) is included in the list of the Ministerial Order of 22 February 1990 on exemption from the regulations on poisonous substances intended for human medicine³⁸ (JORF 1990).

An herbal medication (Climaxol®) containing *Hydrastis* (alcoholic extract) is available for the treatment of venous circulation disorders.

3.12 Risks associated with the consumption of foodstuffs containing berberine

Due to numerous pharmacological activities that were identified and in light of the vigilance data, the consumption of foodstuffs (foods in various forms and food supplements) made with berberine or berberine-containing plant extracts may pose risks to human health.

³⁸ Maximum amount of substance provided to the public: 20 g of rhizome powder, 8 g of dry extract.

- **For the general public**

In light of the clinical studies analysed and the adverse effects observed, a risk of hypoglycaemia associated with the consumption of berberine-containing plants or plant preparations was identified. Moreover, based on the clinical data, the risk of hypotension could not be ruled out (Lan *et al.* 2015).

- **For pregnant and breastfeeding women, and for infants**

Experimental studies in animals showed a risk of increased uterine contractions associated with the consumption of berberine-containing plants (McKenna and Plotnikoff 2005). The WHO considers that berberine-containing plants should not be used by pregnant women because of these effects (WHO 2009). Furthermore, in 2015, the ANSM informed the DGCCRF that berberine-containing plants may increase uterine contractions. A warning intended for pregnant women appears in the “Plants” Order.

Berberine may pass into breast milk. This is the reason why its use is not recommended when breastfeeding (Kumar *et al.* 2015). ESCOP affirms that *Hydrastis canadensis* should not be used during pregnancy and breastfeeding (ESCOP 2013). The WHO also considers that *Berberis vulgaris*, *Coptis chinensis* and *Hydrastis canadensis* should not be used during breastfeeding (WHO 2009, 2007, 1999).

Berberine may also cross the placental barrier, as suspicions of toxicity have been suggested for *in utero* exposure (Kumar *et al.* 2015). In cases of prenatal exposure, and in infants during breastfeeding, berberine seems to pose a risk due to the displacement of free bilirubin (Chan 1993). Cases of jaundice in the first week of life and of neurological impairment in infants were associated with the use of *Rhizoma coptidis* during pregnancy. A study showed haemolysis of isolated red blood cells in G6PD³⁹-deficient patients during *in vitro* treatment with a *Rhizoma coptidis* extract (Ho, Goh, and Zhang 2014).

Preparations of berberine-containing plants, especially *Rhizoma coptidis* and *Cortex phellodendri*, were withdrawn from the market and prohibited as imports in Singapore from 1978 to 2012 due to the assumed involvement of berberine in aggravating jaundice, haemolytic anaemia and kernicterus in newborns with potential G6PD deficiency (Linn *et al.* 2012). However, a study undertaken with more than 1000 infants did not show any difference in the incidence of jaundice between the infants exposed to traditional Chinese herbs, including berberine-containing herbs, and the unexposed infants (Ho, Goh, and Zhang 2014).

Moreover, epidemiological data suggested embryotoxicity associated with *Coptis* spp. in more than 14,500 births monitored in Taiwan. In particular, a high prevalence of nervous system malformations was observed (3%), potentially associated with the use of *Rhizoma coptidis* (consumed by 1.5% of pregnant women) and other plants during the 1st trimester of pregnancy (Chuang *et al.* 2006). However, these effects were not formally attributed to berberine.

The data on the reproductive and developmental toxicity of berberine and *Hydrastis canadensis* extracts have shown maternal (Jahnke *et al.* 2006) and embryo-fœtal toxicity and teratogenicity (National Toxicology Program 2002, 2003).

- **For the population with cardiac disorders (excluding drug interactions)**

The WHO also considers that *Berberis*, *Hydrastis* and *Coptis* should not be used by hypertensive individuals with a history of cardiovascular disease (WHO 2009, 2007, 1999).

A *Rhizoma coptidis* extract showed time- and dose-dependent cardiotoxicity in an *in vitro* model⁴⁰ used by the FDA to assess the cardiotoxicity of drugs. Other alkaloids contained in the preparation, such as palmatine, also showed cardiotoxicity with this model (Zhang *et al.* 2018).

³⁹ G6PD: glucose-6-phosphate dehydrogenase

⁴⁰ The real-time cell analysis (RTCA) model

Berberine can lead to cardiotoxicity, with functional bradycardia. A clinical case was observed in a patient consuming berberine to control his blood cholesterol (Cannillo *et al.* 2013).

Because of its ability to inhibit the hERG potassium channel, a target of numerous antiarrhythmic drugs, berberine can also induce cardiotoxicity (Yu *et al.* 2017).

Lastly, there are multiple risks of drug interactions (Feng *et al.* 2018) (see Section 3.8).

4. CONCLUSIONS AND RECOMMENDATIONS OF THE WG ON “PLANTS” AND THE CES ON “HUMAN NUTRITION”

The WG on “Plants” and the CES on “Human Nutrition” reiterate that lists of plants, plant parts, uses and doses authorised in food supplements, as well as restrictions and warnings governing their use, are not harmonised in the European Union. This is true for berberine-containing plants for which use in food supplements is not recommended or authorised in some European countries.

Given the low level of berberine in barberry (*Berberis vulgaris*) fruits and their very low level of consumption in France, the WG and CES consider that this source of exposure to berberine is currently negligible with regard to the consumption of food supplements.

It should be noted that for certain plants, other substances such as other isoquinoline alkaloids (for *Tinospora sinensis* and *Hydrastis canadensis*) and terpene and furan derivatives (for *Tinospora sinensis*) should be taken into account when assessing the risks associated with their consumption.

In light of the available clinical and literature data, the WG on “Plants” and the CES on “Human Nutrition” consider that:

- the minimum dose of berberine of 400 mg/day, used for therapeutic purposes, does not rule out the possibility of lower doses having the same effects;
- adverse effects related to berberine have been observed following the oral administration of berberine from a daily dose of 600 mg in adults.

Moreover, since the available data on the formulation of food supplements containing berberine are insufficient, there is uncertainty regarding the dose of exposure to berberine and its potential effects associated with the use of food supplements. Nonetheless, the daily doses of berberine in food supplements recommended by operators are comparable to those tested in the clinical studies in which pharmacological activity was reported.

Regarding the daily dose of 10 mg of isoquinoline alkaloids (expressed as berberine equivalent) set by the Belgian authorities for food supplements made with plants or plant preparations containing berberine, the WG on “Plants” and the CES on “Human Nutrition” consider that none of the available studies enable these products to be excluded from the scope of medicinal products.

The available toxicological data enabled the CES on “Health reference values” (HRV Committee) to establish and propose an indicative toxicity value (iTV) of 1.7 µg/kg bw/day for berberine. For a 60 kg individual, the maximum daily dose of berberine would be 0.1 mg.

The WG on “Plants” and the CES on “Human Nutrition” consider that the consumption of food supplements made with plants or plant preparations containing berberine can pose risks of gastrointestinal disorders, hypoglycaemia and hypotension.

Toxicological, mechanistic and clinical data on berberine led to the identification of susceptible population groups for which the WG on “Plants” and the CES on “Human Nutrition” consider there is a higher risk associated with the consumption of food supplements and other dietary sources made with berberine or berberine-containing plant extracts. These population groups are:

- pregnant or breastfeeding women;
- diabetic individuals;
- individuals with hepatic disorders;

- individuals with cardiac disorders.

Despite the lack of specific data, the WG on “Plants” and the CES on “Human Nutrition” consider that the risk may also be higher in children and adolescents.

Pharmacokinetic interactions were identified between berberine and the following drugs: cyclosporin, dextromethorphan, digoxin, ketoconazole, losartan, metformin, midazolam, paclitaxel, statins (simvastatin and atorvastatin) and tacrolimus. Moreover, a risk of interaction is possible for other drugs metabolised in particular by CYP2D6 and CYP3A4 or P-gp substrates, and for any drug with a narrow therapeutic range.

Pharmacodynamic interactions are also possible with certain cancer treatments (5-fluorouracile, paclitaxel, camptothecin), macrolides (azithromycin, clarithromycin), statins and hypoglycaemic drugs.

In light of the broad and numerous drug interactions identified, the WG on “Plants” and the CES on “Human Nutrition” warn against consuming food supplements containing berberine in combination with a drug treatment.

Furthermore, the WG on “Plants” and the CES on “Human Nutrition” reiterate that isoquinoline alkaloids and plants containing them are not included in the list of substances and foodstuffs for which health claims are authorised in Commission Regulation (EU) No 432/2012.

5. AGENCY CONCLUSIONS AND RECOMMENDATIONS

In the specific context of the regulations on food supplements, the DGCCRF asked ANSES to identify the conditions most likely to guarantee the safety of use of food supplements made with plants or plant preparations containing berberine.

The Agency endorses the conclusions and recommendations of the Working Group on “Plants” (2016-2019) and the Expert Committee on “Human Nutrition” (2018-2021).

Berberine is an isoquinoline alkaloid found in various plants. It is known to have pharmacological effects. These have been confirmed by the literature for doses greater than or equal to 400 mg/day, and the experts do not rule out the possibility of lower doses having such effects. In particular, the daily dose of 10 mg of isoquinoline alkaloids (expressed as berberine equivalent), set by the Belgian authorities to separate the scope of food supplements from that of medicinal products by function, has no scientific justification. Therefore, ANSES firstly recommends clarifying the regulatory situation for supplements on the market whose consumption leads to doses that clearly generate pharmacological effects.

Furthermore, and from a regulatory standpoint, ANSES notes that in the absence of authorised health claims at European level, the benefits of consuming berberine or plants containing it are not currently recognised.

Moreover, the Agency noted considerable differences between various European countries in terms of the regulatory position relative to food supplements containing berberine (prohibition, limitation to 10 mg, restrictions for certain population groups). Thus, to identify safe conditions of use, the experts assessed the available toxicological data. Due to the poor quality of the available toxicological studies, only an indicative toxicity value (iTV) of 1.7 µg/kg bw/day could be proposed. ANSES reiterates that an iTV is an indicative toxicological benchmark that, while less robust than a toxicity reference value (TRV), can nonetheless be used for temporary risk management purposes, pending the determination of a TRV based on good-quality toxicological studies. This iTV corresponds to a dose of 0.1 mg/day for a 60 kg individual, which is likely exceeded for a large number of food supplements on the market (see Table 7).

Thus, in the absence of new data, their safety of use cannot be guaranteed. ANSES recommends that toxicological studies on berberine, undertaken according to the OECD guidelines, be submitted to the Agency so it may derive a TRV.

Regardless of the availability of new data enabling a more robust health reference value to be derived, ANSES advises pregnant and breastfeeding women, diabetic individuals and individuals with hepatic or cardiac disorders to refrain from consuming food supplements containing berberine, due to the adverse effects they could experience. In the absence of specific data, this recommendation also applies to children and adolescents.

Moreover, ANSES draws the attention of healthcare professionals to the multiple drug interactions occurring with berberine that are likely to compromise the efficacy of certain treatments, especially cancer treatments.

In general, ANSES recommends that consumers:

- avoid the concomitant consumption of several food supplements or of food supplements including numerous ingredients,
- report the consumption of food supplements and concomitant drug treatments to their doctor or pharmacist, due to the risk of interactions.

Lastly, ANSES reminds healthcare professionals and manufacturers of the need to report to its nutriviigilance scheme any adverse effects likely to be associated with the consumption of food supplements about which they become aware.

Dr Roger Genet

KEYWORDS

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ANNEX 1

Presentation of the participants

FOREWORD: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

WORKING GROUP ON “PLANTS” (2016-2019)

Chair

Mr Bernard WENIGER – Retired, University Lecturer (Strasbourg University) – Speciality: pharmacognosy

Members

Ms Sabrina BOUTEFNOUCHET – University Lecturer (Paris-Descartes University) – Speciality: pharmacognosy

Mr Pierre CHAMPY – University Professor (Paris-Sud University) – Speciality: pharmacognosy

Ms Hanh DUFAT – University Lecturer (Paris-Descartes University) – Speciality: pharmacognosy

Mr Mohamed HADDAD – Research Manager (IRD, Toulouse - Institute of Research for Development) – Speciality: pharmacognosy

Mr Thierry HENNEBELLE – University Professor (Lille University) – Speciality: pharmacognosy

Mr Serge MICHALET – University Lecturer (Claude Bernard University, Lyon I) – Speciality: pharmacognosy

Mr Claude MOULIS – Retired, Professor Emeritus – Speciality: pharmacognosy

Ms Céline RIVIERE – University Lecturer (Lille University) – Speciality: pharmacognosy

Ms Catherine VONTHRON-SENECHEAU – University Lecturer (Strasbourg University) – Speciality: pharmacognosy

RAPPORTEURS FROM THE WG ON “PLANTS”

Mr Pierre CHAMPY – University Professor (Paris-Sud University) – Speciality: pharmacognosy

Ms Céline RIVIERE – University Lecturer (Lille II University) – Speciality: pharmacognosy

TOXICOLOGICAL RAPPORTEUR

Ms Anne PLATEL – University Lecturer (Lille University – Institut Pasteur) – Speciality: toxicology

EXPERT COMMITTEE ON “HUMAN NUTRITION” (2018-2021)

Chair

Mr François MARIOTTI – Professor (AgroParisTech) – Specialities: metabolism of proteins, amino acids, nutritional requirements and recommendations, postprandial metabolism, cardiometabolic risk

Members

Mr Frédéric BARREAU – Research Manager (Inserm) – Specialities: chronic inflammatory intestinal diseases, microbiota, host-microbe relationships, barrier function of the intestinal mucosa

Ms Charlotte BEAUDART – Research Manager (University of Liège) – Specialities: epidemiology, public health, meta-analyses, sarcopenia

Ms Catherine BENNETAU-PELISSERO – Professor (Bordeaux Sciences Agro) – Specialities: phyto-oestrogens, isoflavones, endocrine disruptors, bone health, food supplements

Ms Clara BENZI-SCHMID – Federal Food Safety and Veterinary Office (FSVO), Switzerland – Specialities: revision and updating of legal bases of foodstuffs

Ms Marie-Christine BOUTRON-RUAULT – Research Director (CESP Inserm) – Specialities: nutritional epidemiology and cancer, digestive system

Ms Blandine de LAUZON-GUILLAIN – Research Director (INRA, CRESS) – Specialities: epidemiology, infant nutrition, nutrition of pregnant and breastfeeding women, public health

Ms Amandine DIVARET-CHAUVEAU – University Hospital Practitioner (Nancy Regional University Hospital) – Specialities: allergology, epidemiology, complementary feeding, breastfeeding

Ms Christine FEILLET-COUDRAY – Research Director (INRA, Montpellier) – Specialities: metabolism of minerals, oxidative stress

Ms Amandine GAUTIER-STEIN – INRA Research Manager (Inserm “Nutrition, diabetes and brain” unit) – Specialities: energy metabolism, neuroendocrinology, gut-brain axis

Mr Jacques GROBER – University Lecturer (AgroSup Dijon) – Specialities: nutrition, lipids, metabolism of lipoproteins

Mr Jean-François HUNEAU – Professor (AgroParisTech) – Speciality: human nutrition

Ms Emmanuelle KESSE-GUYOT – Research Director (INRA, UMR Inserm U1153/INRA U1125/CNAM/University of Paris 13) – Specialities: epidemiology, nutrition and pathologies, nutrition and public health, food sustainability

Ms Corinne MALPUECH-BRUGERE – University Professor (University of Clermont Auvergne) – Specialities: human nutrition, metabolism of macro- and micro-nutrients

Ms Christine MORAND – Research Director (INRA Clermont-Ferrand) – Specialities: prevention of vascular dysfunctions and related diseases, plant micro-constituents

Ms Beatrice MORIO-LIONDORE – Research Director (INRA Lyon) – Specialities: human nutrition, lipid and energy metabolism

Ms Anne-Sophie ROUSSEAU – University Lecturer (University of Côte d'Azur, UMR/INSERM 1065) – Specialities: nutrition and physical activity, oxidative stress, immunometabolism

Mr Stéphane WALRAND – University Professor-Hospital Practitioner (University of Clermont Auvergne and Gabriel Montpied University Hospital in Clermont-Ferrand) – Specialities: pathophysiology, protein metabolism, vitamin D, amino acids

EXPERT COMMITTEE ON “HEALTH REFERENCE VALUES” (HRV COMMITTEE) (2017-2020)

Chair

Mr Fabrice MICHIELS – Occupational physician/toxicologist at the Inter-company Association for Occupational Health (Corrèze) – Expertise: occupational medicine, toxicology

Vice-Chair

Mr Raymond VINCENT – Retired (formerly Project Officer in the Applications Division (INRS)) – Expertise: chemistry, metrology of pollutants, assessment of occupational risks

Members

Mr Marc Baril – Associate Professor at University of Montreal – Expertise: chemical toxicology, industrial hygiene

Mr Stéphane BINET – Pharmacist-toxicologist in the Research and Studies Department (INRS) – Expertise: general and industrial toxicology

Ms Michèle BISSON – Research Manager at INERIS – Expertise: pharmaceutical toxicology, general toxicology

Ms Anne CHEVALIER – Retired from the French Institute for Public Health Surveillance – Expertise: epidemiology

Ms Fatiha EL-GHISSASSI – Scientist, IARC Monographs Group (IMO). International Agency for Research on Cancer – Expertise: biochemistry specialising in carcinogenesis and genotoxicity

Ms Mounia EL-YAMANI – Unit Manager at *Santé publique France* – Expertise: biochemistry, toxicology

Mr Claude EMOND – Assistant Clinical Professor at University of Montreal – Expertise: toxicology, PBPK modelling, toxicokinetics, nanotoxicology, endocrine disruptors

Mr Rex FITZGERALD – Regulatory toxicology expert at the Swiss Centre for Applied Human Toxicology – Expertise: reproductive toxicology, developmental neurotoxicity, assessment of human risks

Mr Robert GARNIER – Medical toxicologist, Paris Poison Control Centre – Expertise: medical toxicology, occupational medicine

Ms Perrine HOET – Professor at the Catholic University of Louvain. Institute for Experimental and Clinical Research (IREC) – Expertise: medicine, industrial and environmental toxicology

Ms Yuriko IWATSUBO – Doctor-epidemiologist at *Santé publique France* – Expertise: epidemiology of occupational risks

Ms Cécile KAIRO – Health risk assessor at *Santé publique France* – Expertise: doctor of pharmacy specialising in the environment, general toxicology and risk assessment

Ms Laila LAKHAL – INRA Engineer, Toxalim unit – Expertise: toxicology, metabolism, endocrine disruptors

Mr Frédéric LIRUSSI – University Lecturer-Hospital Practitioner at the UFR of Health Sciences & Dijon University Hospital – Expertise: clinical toxicology, analytical toxicology, innate immunity, reprotoxicity

Ms Anne MAITRE – University Professor-Hospital Practitioner at the Laboratory of Occupational and Environmental Toxicology, Grenoble University Hospital; Manager of the “Environment and population health forecasting” team, TIMC Laboratory, Grenoble-Alpes University – Expertise: medicine, toxicology, BMEs, metrology of pollutants, industrial hygiene

Ms Anne PLATEL – Lecturer at the Lille Faculty of Pharmaceutical and Biological Sciences – Genetic Toxicology Laboratory, Institut Pasteur, Lille – Expertise: toxicology, genotoxicity, QSAR

Mr Henri SCHROEDER – Associate Professor at the Faculty of Sciences and Technologies of University of Lorraine – CALBINOTOX Laboratory, EA 7488 – Pharmacist-neurobiologist – Expertise: neurotoxicity, environmental pollutants, animal behaviour, cerebral development, perinatal exposure

Mr Olivier SORG – Head of research group at University of Geneva – Expertise: doctor of science in biochemistry, experimental toxicology, dermatotoxicology

Mr Jérôme THIREAU – CNRS Research Manager – Expertise: doctor of science, animal physiology, cellular biology, cardiotoxicity

Mr Claude VIAU – Retired Full Professor, currently Associate Professor in the Department of Environmental and Occupational Health, School of Public Health, University of Montreal – Expertise: toxicology, biomarkers of exposure, industrial hygiene, metrology of pollutants

Coordination and scientific contributions for the HRV Committee

Ms Aurélie MATHIEU-HUART – Scientific Project Manager – ANSES

ANSES PARTICIPATION FOR THE NUTRITIONAL RISK ASSESSMENT UNIT

Scientific coordination

Mr Youssef EL OUADRHIRI – Scientific Coordinator, Nutritional Risk Assessment Unit – Risk Assessment Department

Scientific contribution

Mr Youssef EL OUADRHIRI – Scientific Coordinator – Risk Assessment Department

Ms Fanny HURET – Scientific Project Leader for Nutrivigilance – Risk Assessment Department

Mr Aymeric DOPTER – Deputy Head of the Nutritional Risk Assessment Unit – Risk Assessment Department

Ms Irène MARGARITIS – Head of the Nutritional Risk Assessment Unit – Seconded University Professor (University of Nice Sophia Antipolis) – Risk Assessment Department

Administrative secretariat

Ms Virginie SADE – Risk Assessment Department