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**Scientific opinion**

DRAFT Scientific Opinion on the safety of plant preparations containing berberine  
EFSA-Q-2022-00803

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DRAFT

# DRAFT Scientific Opinion on the safety of plant preparations containing berberine

EFSA Panel on Nutrition, Novel Foods and Food Allergens

## Abstract

Following a request from the European Commission pursuant to Article 8(2) of Regulation (EC) No 1925/2006, the Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver a scientific opinion on the safety for human consumption of preparations of selected plant species containing berberine, namely *Berberis aquifolium* Pursh (root), *Berberis aristata* DC (root, bark), *Berberis vulgaris* L. (root, bark), *Chelidonium majus* L. (herb), *Coptis japonica* (Thunb.) Makino (rhizome), *Coptis teeta* Wall. (rhizome), *Coptis trifolia* (L.) Salisb. (rhizome), *Cosciniium fenestratum* (Goetgh.) Colebr. (root, stem), *Hydrastis canadensis* L. (rhizome), *Jateorhiza palmata* (Lam.) Miers (root), *Phellodendron amurense* Rupr. (bark), *Thalictrum flavum* L. (root), *Tinospora sinensis* (Lour.) Merr. (root, stem, leaf). Evidence on adverse effects was gathered through systematic literature searches covering berberine as a single compound, other protoberberine alkaloids, and preparations from the plant species. The assessment included in vitro genotoxicity data as well as in vivo animal and human data. Genotoxicity concern for berberine was identified in in vitro assays, which requires confirmation in vivo. There is uncertainty regarding the genotoxicity of other compounds that may be present in the plant preparations, including other protoberberines. Based on evidence of carcinogenic activity of preparations from rhizome/root of *Hydrastis canadensis* L. in rodents, the consumption of such preparations represents a carcinogenic risk for humans. Consumption of preparations of *Chelidonium majus* L. aerial parts has been linked to idiosyncratic herb-induced liver injury in humans. Susceptible individuals cannot be identified, nor can a dose be established below which such reactions would not occur. The toxicity profiles of preparations of the other plant species remain largely unknown due to the lack of adequate toxicity studies. Due to identified safety concerns and insufficient data, no safe intake can currently be established for any of these berberine-containing plant preparations.

## Keywords

Berberine, protoberberine, Berberis, Chelidonium, Coptis, Cosciniium, Hydrastis, Jateorhiza, Phellodendron, Thalictrum, Tinospora, plant preparation, botanical.

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# 175 1 Introduction

## 176 1.1 Background

177 The French authorities have raised concerns regarding a potential risk to consumers linked with the  
178 consumption of plant preparations containing the isoquinoline alkaloid berberine. These concerns  
179 are outlined in an opinion by the French Agency for Food, Environmental and Occupational Health  
180 & Safety (ANSES) on the risks associated with the use of berberine-containing plants as ingredient  
181 in food supplements (ANSES, 2019).

182 The ANSES opinion stresses that the consumption of food supplements made with plants or plant  
183 preparations containing berberine can pose risks including gastrointestinal disorders, hypoglycaemia  
184 and hypotension. As the risk is higher for children and adolescents, pregnant and breastfeeding  
185 women, diabetic individuals and individuals with liver or heart disorders, ANSES advises these  
186 population groups to refrain from consuming berberine-containing food supplements. Moreover, in  
187 light of the numerous drug interactions identified by the experts, ANSES calls for extreme vigilance  
188 by healthcare professionals, since consuming berberine-containing food supplements in combination  
189 with a drug treatment can inhibit its effects or lead to adverse effects.

190 Consequently, the European Commission has initiated the procedure under Article 8(2) of Regulation  
191 (EC) No 1925/2006<sup>1</sup> for plant preparations containing berberine.

## 192 1.2 Terms of reference as provided by the requestor

193 In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002<sup>2</sup>, the European Commission  
194 tasks EFSA to:

195 Review the existing scientific data on the possible link between the intake of preparations of the  
196 following berberine-containing plant parts and an adverse effect on health:

197 *Berberis aquifolium* Pursh (root), *Berberis aristata* DC (root, bark), *Berberis vulgaris* L. (root, bark),  
198 *Chelidonium majus* L. (herb), *Coptis japonica* (Thunb.) Makino (rhizome), *Coptis teeta* Wall.  
199 (rhizome), *Coptis trifolia* (L.) Salisb. (rhizome), *Coscinium fenestratum* (Goetgh.) Colebr. (root,  
200 stem), *Hydrastis canadensis* L. (rhizome), *Jateorhiza palmata* (Lam.) Miers (root), *Phellodendron*  
201 *amurense* Rupr. (bark), *Thalictrum flavum* L. (root), *Tinospora sinensis* (Lour.) Merr. (root, stem,  
202 leaf).

203 Provide advice on a daily intake of the plant preparations containing berberine that does not give  
204 rise to concerns about adverse effects to health for the general population, and as appropriate, for  
205 vulnerable subgroups of the population.

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<sup>1</sup> Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, pp. 26–38.

<sup>2</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

## 206 1.3 Interpretation of the Terms of Reference

207 This scientific assessment will address the potential adverse health effects of the consumption of  
208 preparations of the parts of the plants containing berberine listed in Section 1.2. The term  
209 'preparation' is interpreted as all preparations obtained from '*botanical materials (e.g. whole,*  
210 *fragmented or cut plants, plant parts, algae, fungi and lichens) by various processes (e.g. pressing,*  
211 *squeezing, extraction, fractionation, distillation, concentration, drying up and fermentation)*' (EFSA  
212 Scientific Committee, 2009).

213 Although the mandate puts emphasis on the berberine content of the plant species identified, an  
214 assessment of the possible adverse effects on health of the intake of "preparations of the plants"  
215 (and relevant parts thereof, as specified in the terms of reference (section 1.2) as a whole (i.e.,  
216 beyond their berberine content) was required. Therefore, the assessment will integrate the following  
217 lines of evidence:

- 218 • Evidence of adverse effects of berberine as a single substance;
- 219 • Evidence of adverse effects of other alkaloids of the same family as berberine  
220 (protoberberine alkaloids), given their high structural similarity with berberine and their co-  
221 occurrence in plant species;
- 222 • Evidence of adverse effects of the whole preparations of the respective plant species, and  
223 relevant parts thereof.

224 The following aspects are excluded from the scope of this assessment:

- 225 • Risk-benefit analysis: the assessment does not address possible beneficial health effects of  
226 plant preparations containing berberine;
- 227 • Medicinal products: certain preparations may be classified as medicinal products under  
228 Directive 2001/83/EC<sup>3</sup> in some EU Member States. These are subject to separate legal  
229 provisions and authorisation processes.

230 Upon consultation with the EC, it was clarified that synthetic forms of berberine used in food  
231 supplements or added to foods fall within the scope of Regulation (EU) 2015/2283<sup>4</sup> on novel foods.

## 232 1.4 Context of the assessment

### 233 1.4.1 Evaluations performed by EFSA

234 The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) evaluated  
235 extracts of *Macleaya cordata* (Willd.) R. Br. for use as feed additives (EFSA FEEDAP Panel (EFSA  
236 Panel on Additives and Products or Substances used in Animal Feed) et al., 2024; EFSA FEEDAP  
237 Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) et al., 2023). An  
238 evaluation of the genotoxicity of individual compounds present in the extracts was performed,  
239 including sanguinarine and chelerythrine, two alkaloids which are also present in *Chelidonium majus*  
240 L. herb. The FEEDAP Panel identified a concern for genotoxicity for sanguinarine, based on evidence  
241 of induction of DNA damage upon intraperitoneal (i.p.) exposure in mice, evidence of DNA-

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<sup>3</sup> Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2001, pp. 67–128.

<sup>4</sup> Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, pp. 1–22.

242 intercalating activity and of its potential to produce reactive oxygen species. In vivo investigation of  
243 genotoxicity of sanguinarine at first sites of contact (stomach and duodenum) after oral exposure  
244 was lacking. The same genotoxicity concern was raised for chelerytrine, based on the structural  
245 similarity with sanguinarine.

#### 246 1.4.2 Evaluations performed by the European Medicines Agency

247 An assessment report by the Committee on Herbal Medicinal Products (HMPC) of *Chelidonium majus*  
248 L. (*C. majus*) herb was published in 2011 by the EMA. The committee concluded on a negative risk-  
249 benefit balance based on limited evidence on efficacy and consistent reports of hepatotoxicity. Data  
250 on potential genotoxicity, cytotoxicity and fetotoxicity of sanguinarine were also noted. As of 2008,  
251 the German authorities have applied restrictions to the authorization of *C. majus*-containing  
252 medicinal products (EMA, 2011).

#### 253 1.4.3 Assessments from other EU bodies

254 In 2019, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES)  
255 published an opinion on the safety of use of berberine-containing plants in the composition of food  
256 supplements (ANSES, 2019). ANSES evaluated data from toxicological studies, clinical case reports  
257 and human clinical trials. Data from the French nutriviigilance, pharmacovigilance and toxicovigilance  
258 systems, and foreign vigilance systems were also reviewed. Potential adverse effects included  
259 gastrointestinal symptoms, hypoglycaemia, hypotension, hepatic effects and cardiovascular effects.  
260 Considering a maximum use period of 14 days recommended by manufacturers of food supplements  
261 containing berberine, ANSES chose to establish an acute reference value and selected the increased  
262 relative liver weight observed in a US National Toxicology Program (NTP) 2-week study in male rats  
263 fed *Hydrastis canadensis* L. root powder (3.45% berberine) as the critical effect (NTP, 2010). A  
264 lowest observed adverse effect level (LOAEL) of 155 mg/kg body weight (bw) *Hydrastis canadensis*  
265 L. (*H. canadensis*) root powder was identified, corresponding to 5.35 mg/kg bw per day berberine,  
266 which was converted by allometric scaling to a human-equivalent LOAEL of 1.25 mg/kg bw berberine  
267 per day. By applying an overall uncertainty factor of 750, ANSES set an indicative toxicity value  
268 (iT<sub>V</sub>)<sup>5</sup> for berberine of 1.7 µg/kg bw per day. ANSES discouraged the use of berberine supplements  
269 in pregnant or breastfeeding women, diabetics, and individuals with hepatic or cardiac disorders,  
270 and warned against any concomitant drug treatment due to potential interactions. These  
271 recommendations were extended to children and adolescents.

#### 272 1.4.4 Other evaluations

273 In 2015, the International Agency for Research on Cancer (IARC) classified *H. canadensis* root  
274 powder as possibly carcinogenic to humans (Group 2B), considering evidence in humans as  
275 inadequate, but sufficient in experimental animals (IARC, 2015). The conclusion relied on two 2-  
276 year carcinogenicity studies in mice and rats conducted by the US NTP (NTP, 2010). Regarding  
277 mechanistic data, IARC noted evidence that berberine and its metabolite, berberrubine, can inhibit  
278 DNA topoisomerases.

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<sup>5</sup> According to ANSES, an indicative toxicity value (iT<sub>V</sub>) is a provisional benchmark established when available toxicological data are insufficient to derive a formal toxicological reference value and is intended for use only in the specific context that prompted its derivation. ANSES (Agence Nationale de Sécurité Sanitaire de l'Alimentation de l'Environnement et du Travail). (2019). *Opinion on the safety of use of berberine-containing plants in the composition of food supplements* (2018-SA-0095). <https://www.anses.fr/system/files/NUT2018SA0095EN.pdf>

## 279 2 Data and Methodologies

280 A protocol for the evaluation of the safety in use of plant preparations containing berberine was  
281 developed (EFSA, 2023). The draft of the protocol was released for public consultation from 12 May  
282 to 11 June 2023 and approved by the NDA Panel in its final version on 4 July 2023.

283 Key elements of the protocol are summarised below. Some amendments to the protocol were  
284 applied, which are documented in this section.

### 285 2.1 Problem formulation

286 The overarching risk assessment questions addressed in this assessment are the followings:

- 287 1) Is there a link between dietary exposure to berberine and adverse health effects in humans?
- 288 2) Is there a link between dietary exposure to berberine-containing plant preparations listed in  
289 the mandate and adverse health effects in humans?
- 290 3) What is the maximum level of total chronic dietary exposure (i.e. over a substantial part of  
291 the lifespan) to berberine-containing plant preparations which is unlikely to pose a risk of  
292 adverse health effects to humans? (amendment 1 to the protocol<sup>6</sup>)

#### 293 2.1.1 Definition of the exposure of interest

294 The assessment is restricted to the parts of the plant species that were specified in the mandate  
295 (Table 1Table 1:). Plants of the World Online (POWO) was used as a reference for the identification  
296 of the scientific names of the plant species, their synonyms (Appendix A) and common names (Table  
297 1). The accepted scientific name will be used throughout the opinion.

298 **Table 1:** Plant species and their parts included in the assessment

Scientific name <sup>a</sup>	Plant part in the mandate	Common names <sup>a, b</sup>
<i>Berberis aquifolium</i> Pursh	Root	Oregon grape; holly-leaved barberry
<i>Berberis aristata</i> DC.	Root, bark	Indian barberry
<i>Berberis vulgaris</i> L.	Root, bark	Common barberry
<i>Chelidonium majus</i> L.	Herb	Greater celandine
<i>Coptis japonica</i> (Thunb.) Makino	Rhizome	Japanese goldthread
<i>Coptis teeta</i> Wall.	Rhizome	Indian goldthread
<i>Coptis trifolia</i> (L.) Salisb.	Rhizome	Three-leaf goldthread; savoyane
<i>Coscinonium fenestratum</i> (Goetgh.) Colebr.	Root, stem	Tree turmeric; false calumba
<i>Hydrastis canadensis</i> L.	Rhizome	Goldenseal; orange root; eye-balm
<i>Jateorhiza palmata</i> (Lam.) Miers	Root	Calumba
<i>Phellodendron amurense</i> Rupr.	Bark	Amur cork tree
<i>Thalictrum flavum</i> L.	Root	Common meadow-rue
<i>Tinospora sinensis</i> (Lour.) Merr.	Root, stem, leaf	Chinese tinospora

299 (a): Source: Plants of the World Online (<https://powo.science.kew.org>).

300 (b): Some common plant names are used for multiple plant species, therefore some of the common names listed may refer to different  
301 plant species depending on context or region.

<sup>6</sup> Amendment 1 to the protocol: Revision of the formulation of the 3<sup>rd</sup> question to align with the wording used in the terms of reference.

302 The scope of the present assessment focuses on berberine, which was specified in the mandate as  
 303 the substance of concern. Other protoberberine alkaloids present in preparations of these plants are  
 304 also considered, given their high structural similarity with berberine and their co-occurrence (Section  
 305 1.3). These include berberastine, berberrubine, columbamine, coptisine, corysamine,  
 306 demethyleneberberine, epiberberine, fissisaine, groenlandicine, jatrorrhizine, palmatine, stephaine,  
 307 thalidastine, thalifendine.<sup>7</sup>

308 Other constituents present in the plant preparations have not been evaluated systematically.  
 309 Information on such constituents is included in the opinion where relevant for hazard identification,  
 310 in particular regarding genotoxicity.

### 311 2.1.2 Formulation of the assessment sub-questions

312 The overarching risk assessment questions were further specified into assessment sub-questions  
 313 (sQs) and the methods to address each sQ were selected, as outlined in Table 2. The sQs identified  
 314 in the protocol have been amended to include other relevant protoberberines (EFSA, 2023).

315 The following potential adverse health effects were identified in the protocol: acute toxicity;  
 316 genotoxicity; liver, lung, other organ chronic toxicity; carcinogenicity; developmental toxicity;  
 317 immunotoxicity; cardiac effects; diarrhoea, constipation, abdominal pain, nausea; headache,  
 318 hypotension, dizziness; hypoglycaemia; body weight loss indicative of toxicity; interactions with  
 319 medicinal products.

320 **Table 2:** Assessment sub-questions

Number	Sub-questions	Method to answer sub-questions
1	What is the absorption, distribution, metabolism and excretion (ADME) of berberine and the other relevant protoberberines <sup>a</sup> ?	Narrative review Call for data
2	2a) Is there a causal relationship between dietary exposure to berberine or the other relevant protoberberines, and the identified potential adverse health effect(s)? 2b) Is there a causal relationship between the dietary exposure to preparations of the selected plant species (and relevant parts thereof) <sup>b</sup> and the identified potential adverse health effect(s)?	Systematic searches and reviews (Amendment 2 of the protocol <sup>8</sup> ) Call for data
3	What is the potential mode(s) of action for the relationships found?	Narrative review, Call for data
4	What is the dose-response relationship between dietary exposure to berberine, the other relevant protoberberines, preparations of the selected plant species and risk of the identified potential adverse health effects?	Systematic searches and reviews (Amendment 2 of the protocol) Call for data

<sup>7</sup> Protoberberines initially identified in the protocol which were not identified in the plant species included in the mandate, on the basis of the analytical data collected, were not further considered.

<sup>8</sup> Amendment 2 of the protocol: description of the method corrected for accuracy; the full systematic review process, including RoB appraisal, was applied to animal toxicity studies; human studies were retrieved through systematic searches and screening against predefined criteria, no RoB appraisal was applied; see text.

Number	Sub-questions	Method to answer sub-questions
	5a) Content data: what is the content of berberine and other relevant protoberberines in the selected plant species (and relevant parts thereof)? (Amendment 3 of the protocol <sup>9</sup> )	Systematic searches and narrative review (Amendment 4 of the protocol <sup>10</sup> ) Call for data
	5b) Content data: <ul style="list-style-type: none"> <li>What is the content of berberine in foods, including food supplements?</li> </ul>	Call for data, Intel GNPD
5	5c) Consumption data: <ul style="list-style-type: none"> <li>What is the consumption of foods, including food supplements, containing berberine?</li> <li>What are the use levels of berberine containing food supplements recommended by manufacturers?</li> </ul>	EFSA food consumption database Call for data, Intel GNPD
	5d) Exposure: what is the distribution of chronic dietary exposure to berberine from all dietary sources by population group in EU countries?	Dietary exposure estimations conducted by EFSA by combining data from sub-questions 5b and 5c

321 Abbreviations: ADME, absorption, distribution, metabolism and excretion; EFSA, European Food Safety Authority; EU, European Union;  
322 GNPD, global new products database.

323 <sup>a</sup> Berberastine, berberrubine, columbamine, coptisine, corysamine, demethyleneberberine, epiberberine, fisisaine, groenlandicine,  
324 jatrorrhizine, palmatine, stephaine, thalidastine, thalifendine.

325 <sup>b</sup> *Berberis aquifolium* Pursh (root), *Berberis aristata* DC (root, bark), *Berberis vulgaris* L. (root, bark), *Chelidonium majus* L. (herb), *Coptis*  
326 *japonica* (Thunb.) Makino (rhizome), *Coptis teeta* Wall. (rhizome), *Coptis trifolia* (L.) Salisb. (rhizome), *Coscinium fenestratum* (Goetgh.)  
327 Colebr. (root, stem), *Hydrastis canadensis* L. (rhizome), *Jateorhiza palmata* (Lam.) Miers (root), *Phellodendron amurense* Rupr. (bark),  
328 *Thalictrum flavum* L. (root), *Tinospora sinensis* (Lour.) Merr. (root, stem, leaf).

## 329 2.2 Data

### 330 2.2.1 Systematic searches and data collection

331 Evidence retrieval and study selection according to criteria laid down in the protocol (EFSA, 2023)  
332 were conducted by the University of Padova as contractor<sup>11</sup> in collaboration with the Istituto  
333 Zooprofilattico Sperimentale delle Venezie. A detailed description of the work performed is published  
334 in the final report of this outsourcing project (Gregori D, 2026).

335 The search strategy to address sQ2 and 5a was created by EFSA information specialists and run by  
336 a contractor (Gregori D, 2026). PubMed and Embase were searched until the 16<sup>th</sup> of October 2023,  
337 while the Cochrane database was searched until the 9<sup>th</sup> of January 2024. Grey literature (i.e.  
338 literature not indexed in the selected literature databases) was not searched. Retrieved articles were  
339 screened in duplicate in Distiller SR® at title and abstract level, also with the use of the artificial  
340 intelligence tool of Distiller SR®, and at full text level for inclusion/exclusion criteria according to  
341 the criteria defined in the protocol (EFSA, 2023). Conflicts were solved by consensus between two  
342 reviewers. Relevant reviews were hand-searched for additional pertinent studies.

<sup>9</sup> Amendment 3 of the protocol: question reformulated for clarity.

<sup>10</sup> Amendment 4 of the protocol: description of the methods corrected for accuracy; content data of berberine and other relevant protoberberines in the selected plant species were collected through systematic searches complemented by narrative reviews. See text.

<sup>11</sup> Call for tender OC/EFSA/MESE/2022/03 – SC11.

343 Expert opinions, editorials, letters to the editors not reporting on original data, meetings abstracts  
344 and posters were excluded. Only publications with a full text in English were included in the  
345 assessment. No restriction on the publication year was applied.

346 The numbers of articles screened at different steps of the review and the final number of articles in  
347 this opinion, as described in Appendix B, reflect the considerations of the Panel and, therefore,  
348 deviate from the numbers given by the contractor in its technical report.

#### 349 2.2.1.1 Eligibility criteria for sQ2 and outcome of the search

350 Eligible exposures were oral preparations of purified berberine (any purity), purified protoberberine  
351 alkaloids (any purity) among those listed in section 2.1.1, and any preparation from the plant species  
352 (and plant parts thereof) included in the mandate (Amendment 5<sup>12</sup> of the protocol), at any  
353 concentrations.

354 *Human studies:* Case reports and case series, prospective cohort studies, nested case-control  
355 studies, case-cohort studies were eligible. Studies were eligible if they involved individuals of any  
356 age, who were healthy or suffering from a disease.

357 *Animal studies:* any in vivo studies on mammalian animals were eligible. Eligible designs included  
358 single and multiple dose toxicity studies of any duration and studies designed to assess  
359 beneficial/pharmacological effects or mechanisms, which reported on safety-related endpoints.  
360 Studies in animal disease models, including obese animals, were excluded following a protocol  
361 amendment (Amendment 6 of the protocol<sup>13</sup>) as pathological conditions can alter normal physiology  
362 and findings of such studies may not be readily comparable to those obtained in studies in healthy  
363 animals which form the basis of hazard identification.

364 *In vitro studies:* in vitro studies in bacteria (*Salmonella typhimurium*, *Escherichia coli*) and  
365 mammalian cells addressing genotoxicity endpoints were eligible.

366 *In silico studies:* in silico studies (e.g. quantitative structure-activity relationship (QSAR) modelling)  
367 addressing genotoxicity endpoints were eligible.

368 In addition to the searches conducted by the contractor, EFSA performed an ad hoc search for  
369 randomised controlled trials (RCTs) investigating supplementation with berberine or berberine-  
370 containing plant preparations and reporting on adverse events in the intervention and control groups  
371 (number, event type). The searches were carried out in PubMed and the Cochrane database for  
372 RCTs in March 2024 (berberine<sup>14</sup>) and December 2025 (berberine-containing plant preparations<sup>15</sup>).

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<sup>12</sup> Amendment 5 to the protocol: Collection of data on preparations of berberine-containing plants other than those included in the mandate was also foreseen, as supportive evidence (Annex A, EFSA (European Food Safety Authority). (2023). Protocol for the Scientific Opinion on the evaluation of the safety in use of plant preparations containing berberine. *EFSA Supporting Publications*, 20(9), 8246E. <https://doi.org/10.2903/sp.efsa.2023.EN-8246> ). In view of the data available, the Panel decided not to consider these data further.

<sup>13</sup> Amendment 6 of the protocol: No restriction regarding animal disease models was initially foreseen in the protocol.

<sup>14</sup> Search string in Pubmed: berberine[All Fields] OR berberine[Text Word] OR berberine[Mesh] and applying the database filter "Randomized Controlled Trial" and "Clinical trial"  
Search string in the Cochrane database: berberine

<sup>15</sup> Search string in Pubmed: "Berberis aquifolium" OR "Oregon-grape" OR "holly-leaved barberry" OR "Berberis aristata" OR "Indian barberry" OR "tree turmeric" OR "berberis vulgaris" OR "barberry" OR "common barberry" OR "Chelidonium majus" OR "greater celandine" OR "nipplewort" OR "coptis japonica" OR "Japanese goldthread" OR "coptis teeta" OR "goldthread" OR "Indian goldthread" OR "coptis trifolia" OR "three leaf goldthread" OR "savoyane" OR "coscinium fenestratum" OR "yellow vine" OR "false calumba" OR "colombo weed" OR "hydrastis canadensis" OR "goldenseal" OR "jateorhiza palmata" OR "calumba" OR "phellodendron amurense" OR "amur cork tree"

373 Studies which used a combination of berberine or berberine containing plants with another  
374 treatment, lacked an appropriate control, did not report on the adverse events for all groups, or  
375 were not in English were excluded. Flow diagrams of the study selection process are provided in  
376 Appendix B. Thirty-five publications on in vivo and in vitro genotoxicity studies on berberine or  
377 relevant plant preparations were eligible. Fifty-one publications on animal studies on general toxicity  
378 and 14 on interactions with medicinal products for berberine, other protoberberines or relevant plant  
379 preparations were also eligible. Publications on human studies included 27 case reports and case  
380 series for berberine or relevant plant preparations, 28 RCTs on berberine supplementation and 11  
381 clinical studies addressing interactions with medicinal products.

#### 382 2.2.1.2 Eligibility criteria for sQ5 and outcome of the search

383 Analytical studies providing the content of berberine in food supplements and plants preparations  
384 were eligible, if they provided information on the analytical methods used.

385 For sQ5, 1787 publications were screened at title and abstract level, 305 at full text level and 253  
386 were initially included by the contractor. As the Panel decided to depict in the opinion the content  
387 of berberine and other protoberberines in eligible plant parts rather than in plant preparations as  
388 originally anticipated (protocol amendment 7), only those publications were used in the assessment  
389 which allowed the calculation of the content in the plant parts of interest. At the step of data  
390 extraction, studies investigating plant species or parts of the plants not included in the mandate  
391 were further excluded (data cleaning).

392 Additional publications were added through snowballing of publications on the characterisation of  
393 the plant species performed by a botanical expert from the WG and EFSA staff. Following the  
394 application of these further restrictions and searches, a total of 60 studies were included for this  
395 question.

#### 396 2.2.2 Narrative reviews

397 Relevant information was collected from existing evaluations, reviews and other publications  
398 retrieved through ad hoc searches in bibliographic databases and references cited therein regarding:

- 399 • the botanical characterisation of the plant species included in the mandate, their common  
400 names, their geographical distribution and additional data on their content of berberine and  
401 other protoberberines in addition to the systematic search conducted by the contractor;
- 402 • the absorption, distribution, metabolism and excretion (ADME) of berberine and the other  
403 relevant protoberberines;
- 404 • data on adverse effects of other compounds that may be contained in the plant preparations;
- 405 • potential mode(s) of action.

#### 406 2.2.3 Call for data

407 A call for data was launched by EFSA on 5 July 2023 and open until 12 January 2024 to offer  
408 interested parties (e.g. governments, food business operators, national food authorities, research

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OR "thalictrum flavum" OR "common meadow-rue" OR "monk's rhubarb" OR "tinospora sinensis" OR "Chinese tinospora" and applying the database filter "Randomized Controlled Trial" and "Clinical trial"

Search string in the Cochrane database: "Berberis aquifolium" OR "Oregon-grape" OR "holly-leaved barberry" OR "Berberis aristata" OR "Indian barberry" OR "tree turmeric" OR "berberis vulgaris" OR "barberry" OR "common barberry" OR "Chelidonium majus" OR "greater celandine" OR "nipplewort" OR "coptis japonica" OR "Japanese goldthread" OR "coptis teeta" OR "goldthread" OR "Indian goldthread" OR "coptis trifolia" OR "three leaf goldthread" OR "savoyane" OR "coscinium fenestratum" OR "yellow vine" OR "false calumba" OR "colombo weed" OR "hydrastis canadensis" OR "goldenseal" OR "jateorhiza palmata" OR "calumba" OR "phellodendron amurense" OR "amur cork tree" OR "thalictrum flavum" OR "common meadow-rue" OR "monk's rhubarb" OR "tinospora sinensis" OR "Chinese tinospora".

409 institutions, academia) and other stakeholders the opportunity to submit documented information  
410 (published and unpublished) relevant to the evaluation of the safety of preparations containing  
411 berberine and other protoberberine alkaloids listed in Section 1.3.<sup>16</sup> Third parties were also given  
412 the possibility to submit data through the annual call for continuous collection of chemical  
413 contaminants occurrence data in food and feed.<sup>17</sup>

414 The purpose of the call was to retrieve:

- 415 1) Analytical data on the content of berberine and other protoberberine alkaloids in preparations  
416 of plants used in food supplements;
- 417 2) Use levels recommended by manufacturers for food supplements containing berberine and  
418 other protoberberines;
- 419 3) Biological and toxicological data to support the assessment of a causal relationship between  
420 dietary exposure to berberine as single substance and/or in plant preparations and the  
421 identified potential adverse effects, including data on absorption, digestion, absorption and  
422 metabolism (ADME) for berberine and within the food matrix.

423 Details on the interested parties, the type of documents received and how these data were  
424 considered in the assessment are provided in Section 7. Data on the content of berberine and other  
425 protoberberine alkaloids of extracts of *Berberis aristata* DC. (*B. aristata*) root or bark and food  
426 supplements containing extracts of *B. aristata* root or bark and the daily doses recommended by the  
427 manufacturers, were submitted by the Società italiana di scienze applicate alle piante officinali e ai  
428 prodotti per la salute (SISTE). Data on the content of berberine and other protoberberine alkaloids  
429 in *B. aristata* bark extracts and *Berberis vulgaris* L. bark extracts were submitted by the European  
430 Federation of Associations of Health Product Manufacturers (EHPM).

431 Additional data on berberine content of food supplements were provided by Bundesamt für  
432 Verbraucherschutz und Lebensmittelsicherheit (BVL) through the EFSA Annual call for continuous  
433 collection of chemical contaminants occurrence data in food and feed. However, no information was  
434 provided on the source of berberine nor on the recommended proposed uses by the manufacturer.  
435 Therefore, these data were not considered for the exposure assessment.

#### 436 2.2.4 EFSA Food consumption database

437 The use of the food consumption data from the EFSA Comprehensive European Food Consumption  
438 Database (hereinafter referred to as Comprehensive Database) (EFSA, 2011) to estimate the  
439 exposure to berberine from food supplements (sQ5) was considered. However, the consumption of  
440 food supplements might not be properly captured by the surveys included in the EFSA  
441 Comprehensive Database due to different reasons, such as the survey design, use of short-term  
442 instruments (24 h recalls and food records) or the number of subjects covered. Consequently, data  
443 could not be used in view of the limited coverage of botanical food supplements consumption in the  
444 database.

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<sup>16</sup> <https://www.efsa.europa.eu/en/call/call-data-scientific-opinion-evaluation-safety-use-plant-preparations-containing-berberine>

<sup>17</sup> <https://www.efsa.europa.eu/en/call/annual-call-continuous-collection-chemical-contaminants-occurrence-data-food-and-feed-0>. The data submitted through the annual call followed the requirements of EFSA Guidance on Standard Sample Description EFSA (European Food Safety Authority). (2013). Standard Sample Description ver. 2.0. *EFSA Journal*, 11(10), 3424. <https://doi.org/10.2903/j.efsa.2013.3424>.

## 445 2.2.5 Mintel Global New Products Database

446 In addition to the call for data, the Mintel's Global New Products Database (GNPD)<sup>18</sup> was used to  
447 collect information on foods, which contain preparations of the plant species included in the  
448 mandate. The search covered food products, including food supplements, which reported berberine,  
449 the other protoberberine alkaloids identified or the name of any of the plant species (i.e. scientific  
450 name and most used common names in English as identified in Table 1) on their label, i.e. on the  
451 product description or in the list of ingredients<sup>19</sup>. The search covered all products launched on the  
452 EU market in the previous 10 years, from October 2015 to October 2025<sup>20</sup>.

453 For all identified products, the following data were collected: type of product; plant name and  
454 plant(s) part(s) used; content of berberine and protoberberine alkaloids (mg/serving<sup>21</sup>) and the  
455 proposed uses (i.e. number of servings/day) recommended by manufacturers.

## 456 2.3 Methodologies

### 457 2.3.1 Characterisation of the plant species in the mandate

458 Quantitative data on the content of berberine and other protoberberine are provided in the  
459 characterisation subsection of the respective plant species in Section 3, and in Annex A. The data  
460 collection was restricted to the plant parts of interest (Table 1).

461 Analytical data were converted to a common unit (g/100 g dry weight (DW)) and expressed as  
462 amount in the plant part used as source material to allow comparison across studies. Additional  
463 details related to conversions are reported in Annex A.

### 464 2.3.2 Hazard identification and characterisation

465 The following guidance documents were used: EFSA's guidance on transparency in the scientific  
466 aspects of risk assessment (EFSA, 2009), EFSA's guidance on the application of the systematic  
467 review methodology in food and feed safety assessments (EFSA, 2010), EFSA's principles and  
468 processes for dealing with data and evidence in scientific assessments (EFSA, 2015), EFSA Scientific  
469 Committee guidance on statistical significance and biological relevance (EFSA Scientific Committee,  
470 2011b), EFSA Scientific Committee guidance on the assessment of the biological relevance of data  
471 in scientific assessments (EFSA Scientific Committee et al., 2017c), EFSA Scientific Committee  
472 guidance on the use of the weight of evidence (WoE) approach in scientific assessments (EFSA  
473 Scientific Committee et al., 2017b), EFSA Scientific Committee guidance on uncertainty analysis in

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<sup>18</sup> The Mintel GNPD contains information on over 4 million food and food products including food supplements, beverages, of which more than 1,1 million are or have been available on the European food market. Twenty-five out of the twenty-seven EU Member States and Norway are present in the database. The database provides the compulsory ingredient information reported on product labels and the nutrition declaration when available. Available online: <http://www.mintel.com/globalnew-products-database>.

<sup>19</sup> Full Text Search matches "Berberis aquifolium" OR "Oregon-grape" OR "holly-leaved barberry" OR "B. aristata" OR "Indian barberry" OR "tree turmeric" OR "berberis vulgaris" OR "barberry" OR "common barberry" OR "Chelidonium majus" OR "greater celandine" OR "nipplewort" OR "coptis japonica" OR "Japanese goldthread" OR "coptis teeta" OR "goldthread" OR "Indian goldthread" OR "coptis trifolia" OR "three leaf goldthread" OR "savoyane" OR "coscinium fenestratum" OR "yellow vine" OR "false calumba" OR "colombo weed" OR "hydrastis canadensis" OR "goldenseal" OR "jateorhiza palmata" OR "calumba" OR "phellodendron amurense" OR "amur cork tree" OR "thalictrum flavum" OR "common meadow-rue" OR "monk's rhubarb" OR "tinospora sinensis" OR "Chinese tinospora" with word variants and EU Countries matches EU Countries with word variants null.

<sup>20</sup> Since the number of products launched in the past five years was small, the time limit for the search was extended to include all products launched in the EU in the previous 10 years from the date of access (October 2025).

<sup>21</sup> The Mintel GNPD provides data on the amount of berberine and/or plant preparation per serving. Servings are defined by the food manufacturer and vary across products (e.g. 1 serving = 1 pill or 3 pills, depending on the products).

474 scientific assessments (EFSA Scientific Committee, 2018), risk assessment of substances which are  
475 both genotoxic and carcinogenic (EFSA, 2005), genotoxicity testing strategies (EFSA Scientific  
476 Committee, 2011a; EFSA Scientific Committee et al., 2017a), genotoxicity assessment of chemical  
477 mixtures (EFSA Scientific Committee, 2019), harmonised approach for reporting reliability and  
478 relevance of genotoxicity studies (EFSA et al., 2023), and uncertainty analysis in scientific  
479 assessments (EFSA Scientific Committee, 2018). Additionally, the risk assessment took into account  
480 the EFSA Scientific Committee guidance on safety assessment of botanicals and botanical  
481 preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009).

### 482 2.3.2.1 Genotoxicity assessment

#### 483 *Assessment of eligible studies*

484 In line with EFSA's guidance on the genotoxicity assessment of chemical mixtures (EFSA Scientific  
485 Committee, 2019), the genotoxicity of individually identified components in the plant preparation  
486 and the plant preparation itself were considered. If one or more components of a plant preparation  
487 are shown to be genotoxic in vivo, the whole plant preparation is considered to raise concern for  
488 genotoxicity.

489 All eligible studies were extracted in structured tables (Appendix E). The studies were grouped based  
490 on genetic endpoints and test systems. The study results were assessed as positive, negative,  
491 equivocal or inconclusive. The term 'equivocal result' usually refers to a situation in which not all the  
492 requirements for a clear positive result have been met (e.g. positive trend was observed, but the  
493 dose-response relationship was not statistically significant). Results are considered inconclusive  
494 where no clear finding is achieved due to some limitation of the test or procedure (EFSA Scientific  
495 Committee, 2011a).

496 Details on the approach to appraise the reliability and the relevance of genotoxicity studies are  
497 described in **Errore. L'origine riferimento non è stata trovata.** The reliability of the study is  
498 scored using numerical values based on the scoring system of (Klimisch et al., 1997), where 1  
499 corresponds to 'Reliable without restrictions', 2 corresponds to 'Reliable with restrictions', 3  
500 corresponds to 'Not reliable' and 4 corresponds to 'Not assignable'. The relevance of the test system  
501 and the relevance of the study results are categorised into 'high', 'limited' or 'low' relevance.

502 Studies investigating modes of action should be considered as supporting elements to the lines of  
503 evidence for the genotoxicity endpoints.

#### 504 *Quantitative Structure-Activity Relationship (QSAR) and read-across methods*

505 Five lines of evidence were used to evaluate the potential genotoxicity of berberine, protoberberines  
506 and other alkaloids using in silico QSAR and read-across methods:

- 507 1) Quantification of structural similarity, using VEGA similarity algorithm as described by (Floris  
508 et al., 2014);
- 509 2) Identification of relevant structures in the skeleton of the substances, including structural  
510 alerts (SA) specific for mutagenicity, using the ToxRead software (version 0.25) (Gini et al.,  
511 2014), and SAs specific for micronuclei (MN) induction, using the SARpy model (version 1)  
512 (Ferrari et al., 2013);

- 513 3) Prediction of mutagenicity in Ames test based on CAESAR model (version 2.1.13) (Ferrari &  
514 Gini, 2010), ISS model (version 1.0.3) (Benigni R, 2008), SARpy model (version 1) (Ferrari  
515 et al., 2013), kNN model (version 0.9) (Benfenati et al., 2015), and VEGA Consensus model  
516 (Mombelli et al., 2022); the training and test sets used in the model contained 5764 and  
517 12240 substances.
- 518 4) Prediction of MN induction in the in vitro MN assay, based on SARpy model, and the in vivo  
519 MN assay, based on SARpy and kNN models (Baderna et al., 2020); the training and test  
520 sets used in the in vitro and in vivo models contained 293 and 87 substances and 1209 and  
521 19 substances, respectively.

522 The models were applied to the following compounds:

- 523 • All protoberberines detected in the plant species included in the mandate: berberastine,  
524 berberrubine, columbamine, coptisine, corysamine, demethyleneberberine, epiberberine,  
525 fisisaine, groenlandicine, jatrorrhizine, palmatine, stephamine, thalidastine, thalifendine
- 526 • Other relevant alkaloids:
  - 527 ○ canadine and (-)- $\beta$ -hydrastine, present in preparations of the rhizome/root of *H.*  
528 *canadensis*
  - 529 ○ sanguinarine and chelerythrine, present in preparations of the herb of *C. majus*

530 All models were run using the VEGA hub, version 1.2.4.<sup>22</sup> Further information on the methods and  
531 results is provided in Annex C.

### 532 *Weighing and integration of the evidence*

533 A qualitative, expert-guided approach was used to weigh the evidence and draw conclusions  
534 regarding the relationship between the exposure to berberine, other protoberberine alkaloids,  
535 preparations of the plant species, and the genotoxicity endpoints considered, in line with the EFSA  
536 guidance on genotoxicity assessment (EFSA et al., 2023). Genotoxicity studies evaluated as of high  
537 or limited relevance, as well as mechanistic studies on single constituents, were included in the WoE.  
538 The key components of the WoE approach include the reliability and relevance of the available  
539 studies, the consistency of findings across studies, and the biological plausibility of the observed  
540 effects based on mechanistic data. With respect to biological plausibility, studies investigating modes  
541 of action and the QSAR predictions were considered as supporting evidence to the main lines of  
542 evidence addressing genotoxicity endpoints.

### 543 2.3.2.2 General toxicity assessment

#### 544 *Assessment of eligible studies*

545 The internal validity (risk of bias, RoB) of all eligible animal studies identified through the literature  
546 search was critically appraised using the Office of Health Assessment and Translation (OHAT) RoB  
547 tool developed by the US NTP (OHAT/NTP, 2015). The RoB criteria and rating instructions provided  
548 therein were tailored to the specific research questions and the specific dataset retrieved. The OHAT  
549 RoB tool proposes five response options for each RoB question: definitely low RoB (++), probably  
550 low RoB (+), not reported (NR), probably high RoB (-), definitely high RoB (--). Studies were  
551 allocated to RoB tiers reflecting their overall internal validity, i.e. at low (tier 1), moderate (tier 2)

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<sup>22</sup> Available online [www.vegahub.eu](http://www.vegahub.eu).

552 or high (tier 3) RoB, according to the approach proposed by OHAT. The questions, the instructions  
553 for assessors and the algorithm used to integrate the answers to the individual questions into an  
554 overall RoB tier, as well as the results of the RoB appraisal, are given in Appendix D.

555 The RoB appraisal was performed independently in duplicate by experts of the EFSA Working Group  
556 (WG) on other substances (multiple dose level toxicological studies) or EFSA staff (single dose  
557 studies). Discrepancies regarding RoB judgments were discussed among the assessors. An  
558 agreement was reached in all cases.

559 No systematic RoB appraisal was applied to human studies, as they were limited to case reports,  
560 case series, and RCTs in which the endpoints of interest (adverse effects) were reported as ancillary  
561 data in the publications.

### 562 *Weighing and integration of the evidence*

563 An expert-guided approach was used to weigh the evidence (WoE) and draw conclusions regarding  
564 the relationship between the exposure to berberine, other protoberberine alkaloids, and  
565 preparations of the plant species and the endpoints considered. The key components of the WoE  
566 approach include the reliability (including RoB) and relevance of the available studies, the  
567 consistency of findings across studies, including consideration of consistency across streams of  
568 evidence (humans vs animals), and biological plausibility based on mechanistic data (EFSA Scientific  
569 Committee et al., 2017b).

570 The evidence on berberine and other protoberberine alkaloids was integrated narratively by taking  
571 into account similarities and differences in chemical structure and toxicokinetic behaviour and the  
572 consistency of findings across studies. The overall evidence on berberine, other protoberberine  
573 alkaloids, and preparations of berberine-containing plant species was subsequently integrated by  
574 evaluating the consistency of findings across exposure types, including considerations of critical  
575 doses and dose–response relationships where available, and associated uncertainties and data gaps.

### 576 2.3.3 Exposure assessment

577 A total of 1,253 analytical results on the content of berberine and other protoberberine alkaloids in  
578 extracts of *B. aristata* root and bark and in samples of commercial food supplements containing  
579 preparations of *B. aristata* root and bark were submitted to EFSA. Results referring to plant extracts  
580 (n=821) were excluded from the exposure assessment as they do not necessarily reflect the  
581 concentrations in the final food supplement as consumed. Analytical data on the content of berberine  
582 and other protoberberine alkaloids in food supplements and other data submitted by interested  
583 parties were cleaned according to the procedure and methodology described in Annex D.

584 An evaluation of the distribution of chronic dietary exposure to berberine from all dietary sources  
585 was not possible due to the limited data available regarding foods containing berberine plant  
586 preparations in Mintel GNPD (section 2.2.5) and individual eating occasions regarding the  
587 consumption of such products and berberine-containing food supplements in the EFSA  
588 Comprehensive Database (section 2.2.4). Instead, theoretical daily exposures of consumers of  
589 berberine-containing food supplements were calculated based on the occurrence data and  
590 recommended use levels which were received from stakeholders through the call for data and  
591 collected from the Mintel GNPD. To calculate the theoretical daily exposure to berberine, the mean

592 concentration of berberine in the product (mg/serving) was multiplied by its use level recommended  
593 by the manufacturers (number of servings per day).

## 594 3 Assessment

### 595 3.1 Berberine and protoberberine alkaloids

#### 596 3.1.1 Chemistry

597 Berberine ( $C_{20}H_{18}NO_4^+$ , CAS no: 2086–83-1) (Figure 3), also known as umbellatine, is a quaternary  
598 ammonium ion and is an isoquinoline alkaloid of the protoberberine subgroup (Dewick, 2009). It  
599 has a bright yellow colour. The protoberberine alkaloid family consists of compounds sharing the  
600 same protoberberine molecular skeleton, characterised by a tetracyclic ring system which is based  
601 on the dibenzo[*a,g*]quinolizidine system (Figure 3). These tetracyclic alkaloids are derived from  
602 benzyloquinolines and coupling with the isoquinoline N-methyl group, which becomes the  
603 “berberine bridge” carbon (C-8) (Da-Cunha et al., 2005).

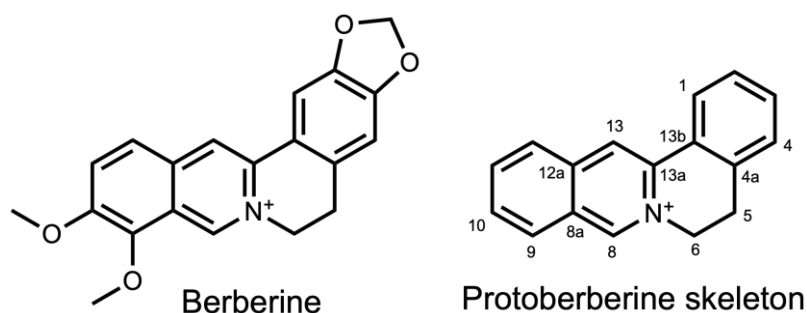
604 Berberine is naturally present in plants and can also be obtained by chemical synthesis (Tajiri et al.,  
605 2021). In food supplements, berberine is found in plant preparations or concentrated extracts  
606 thereof, of variable composition. Berberine cation readily form salts, with berberine chloride<sup>23</sup> and  
607 berberine sulphate being the most common forms in food supplements. Berberine chloride is soluble  
608 in organic solvents, and sparingly soluble in water (Battu et al., 2010). Berberine sulphate is more  
609 soluble than the chloride salt under physiological conditions (Miyazaki et al., 1981). Other salts, e.g.  
610 with organic acids, may have different physicochemical properties (Cui et al., 2018; Xu et al., 2022).  
611 The form and plant source of berberine used in studies published in literature is not always reported.  
612 In this opinion, this information is included in the study descriptions whenever available.

613 Berberine and protoberberines constitute a structurally related class of alkaloids frequently co-  
614 occurring in plant species. Variations in protoberberine profiles among different plant taxa are  
615 attributed to genetic, enzymatic, and environmental influences that govern the regulation of  
616 biosynthetic pathways, leading to species-specific alkaloid compositions (see, for instance, Wu et al.  
617 (2023)).

618 Among the other protoberberine alkaloids identified in the protocol (EFSA, 2023), the following have  
619 been detected in the plant species and plant parts under evaluation: berberrubine, berberastine,  
620 columbamine, coptisine, demethyleneberberine, groenlandicine, jatrorrhizine, corysamine,  
621 fisisaine, stephamine, thalidastine, thalifendine. The protoberberines profile of the respective plant  
622 species is provided in the plant-specific characterisation sub-sections of Section 3 and in Annex A.

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<sup>23</sup> Berberine hydrochloride is sometimes used as a synonym (PubChem CID 12456, <https://pubchem.ncbi.nlm.nih.gov/compound/12456>, accessed on 12 December 2025).



624 **Figure 1.** Berberine and protoberberine skeleton.

625

### 626 3.1.2 Absorption, distribution, metabolism and excretion (ADME)

#### 627 3.1.2.1 Absorption and metabolism of berberine in the gastrointestinal tract

628 Berberine intestinal absorption is limited by its physicochemical properties (hydrophilic nature with  
 629 an experimental logP in the range of  $-1.2$  to  $-1.5$ , yet poor solubility in water (1-3 g/L in water))  
 630 (Battu et al., 2010; Spinozzi et al., 2014), and by P-glycoprotein (P-gp)-mediated efflux (Cui et al.,  
 631 2015; Zhang et al., 2019). Some data indicate that the gut microbiota may reduce berberine to  
 632 dihydroberberine through nitroreductase activity. Dihydroberberine is lipophilic and was found to  
 633 have a 5-fold higher intestinal absorption rate than berberine and to quickly revert to berberine via  
 634 oxidation after entering the intestinal epithelium in rats (Liu et al., 2010). When incubating berberine  
 635 with human intestinal bacteria cultures in vitro (100  $\mu\text{g}/\text{mL}$ ), around 4% of the berberine dose was  
 636 converted to dihydroberberine after 24 hours. Therefore, the gut microbiota may contribute to  
 637 berberine absorption.

638 Berberine undergoes extensive first-pass metabolism in the enterocytes. In a pharmacokinetic  
 639 experiment in rats receiving an intragastric dose of 100 mg/kg bw, 56% of the dose remained  
 640 unabsorbed, while 43.8% was calculated to be metabolised in the enterocytes and 0.2% to enter  
 641 the portal vein (Liu et al., 2010). Oxidative demethylation to form berberrubine, followed by  
 642 glucuronidation, was identified as the primary intestinal metabolic pathway, while  
 643 demethyleneberberine, its glucuronide conjugate, and jatrorrhizine were also detected (Figure 2).

644 The gut microbiota was also found to convert berberine into oxyberberine under anaerobic  
 645 conditions (Li et al., 2020). In vivo, 1.49% and 0.05% of an oral berberine dose were excreted as  
 646 oxyberberine in the faeces of normal and pseudo-germ-free rats, respectively.

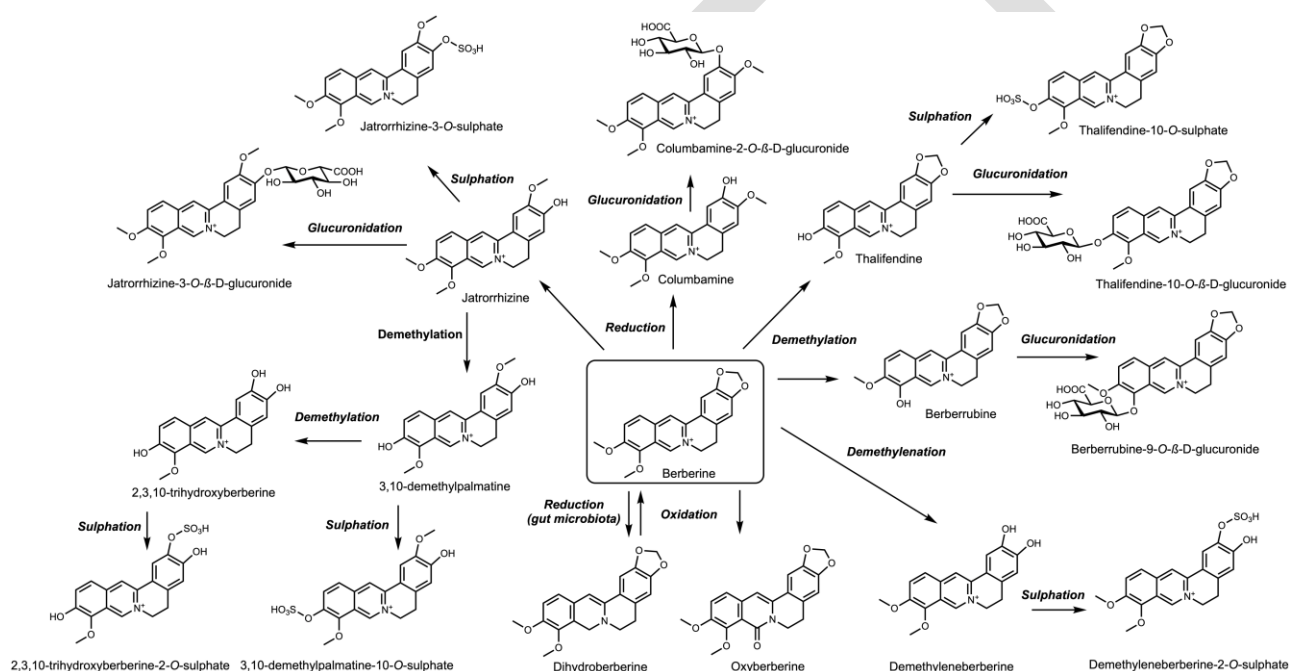
#### 647 3.1.2.2 Hepatic metabolism of berberine

648 Active transport mechanisms are involved in hepatic uptake of berberine (Li et al., 2020). In the  
 649 liver of rats and humans, the demethylation product berberrubine is the main phase-I metabolite of  
 650 berberine (Chen et al., 2021; Huang et al., 2023). Other metabolites include thalifendine and  
 651 demethyleneberberine, and the reduction products jatrorrhizine and columbamine (Hua et al., 2007;  
 652 Moon et al., 2021; Petrangolini et al., 2021; Spinozzi et al., 2014) (Figure 2). Cytochrome P450  
 653 (CYP) 2D6 is the predominant enzyme involved in the demethylation and demethylation of  
 654 berberine, followed by CYP1A2 and CYP3A4 (Li et al., 2011). Phase-I products are rapidly conjugated

655 with glucuronic acid by UDP-glucuronosyltransferases (UGTs) and with sulfate by sulfotransferases  
656 (SULTs) (Spinozzi et al., 2014).

657 Active excretion of unmodified berberine or its metabolites into bile and enterohepatic circulation  
658 was demonstrated in rats (Liu et al., 2010; Tsai & Tsai, 2004). In rats, the main metabolites  
659 measured in the bile are berberrubine, thalifendine, demethyleneberberine, and jatrorrhizine, along  
660 with their conjugated derivatives (Feng et al., 2020; Ma et al., 2013). Rat experiments (Feng et al.,  
661 2020; Ma et al., 2013) indicate that berberine and its metabolites can undergo enterohepatic  
662 circulation but the proportion of orally administered berberine excreted through the bile is small,  
663 with the largest fraction being recovered in faeces. Comparison of berberine toxicokinetics in  
664 conventional and pseudo germ-free rats further showed that the gut microbiota can affect the  
665 enterohepatic circulation of berberine metabolites and the chemical profiles of metabolites detected  
666 in the circulation (Zuo et al., 2006).

667



668

669 **Figure 2.** Main berberine metabolites and metabolic pathways. In rats, oxidative demethylation to form  
670 berberrubine, followed by glucuronidation, were identified as the main metabolic pathways, both in the  
671 gastrointestinal tract and in the liver (Feng et al., 2020, 2022). Other phase-I metabolites include thalifendine  
672 and demethyleneberberine, and the reduction products jatrorrhizine and columbamine (Feng et al, 2020). The  
673 gut microbiota can contribute to the formation of metabolites such as dihydroberberine and oxyberberine.  
674 Phase-I products are rapidly conjugated to glucuronide and sulphate forms in the liver (Feng et al., 2020; Ma  
675 et al., 2013), with phase-II metabolites being the predominant forms detected in circulation (Feng et al., 2020,  
676 2022). Berberine metabolism is qualitatively similar between rats and humans (Pan et al., 2002; Qiu et al.,  
677 2008; Spinozzi et al., 2014), although the quantitative contribution of the individual pathways may differ.

### 678 3.1.2.3 Systemic circulation and distribution of berberine and its metabolites

679 Low plasma concentrations of berberine were found after oral administration of berberine in rats  
680 (Choudhury et al., 2022; Sahibzada et al., 2021), rabbits (Choudhury et al., 2022; Sahibzada et al.,  
681 2021) and dogs (Feng et al., 2018), indicating low systemic availability of the parent compound.  
682 Similarly, in healthy human adults, a mean  $C_{max}$  in plasma below 0.4 ng/mL was reported after single

683 dose oral administration of 400 mg to 500 mg berberine. Peak concentrations were reached 6-8  
684 hours after administration (Hua et al., 2007; Moon et al., 2021; Petrangolini et al., 2021; Spinozzi  
685 et al., 2014).

686 Notably, protein-bound berberine may represent a significant fraction of berberine in the circulation.  
687 In whole blood, the  $AUC_{0-\infty}$  of protein-bound berberine was found to be approximately 4.6 times  
688 higher than that of unbound berberine after single dose oral administration of 400 mg/kg bw in rats  
689 (Huang et al., 2023). This indicates that analytical methods measuring unbound berberine in plasma  
690 underestimate total berberine concentrations in the circulation. Also, a fraction of berberine was  
691 found to be converted to oxyberberine in blood (Chen et al., 2021; Huang et al., 2023). After  
692 administering a berberine dose of 400 mg/kg bw to rats, the  $AUC_{0-\infty}$  ( $\pm$  SD) of total berberine and  
693 oxyberberine were  $7.533 \pm 0.808$  and  $3.947 \pm 0.412$   $\mu\text{g/mL}$  per hour, respectively (Huang et al.,  
694 2023).

695 In rats, phase-II metabolites are the main metabolites detected in blood circulation (Feng et al.,  
696 2020, 2022). After an oral dose of 48.2 mg/kg berberine, the  $AUC_{0-48\text{h}}$  values of berberrubine-9-*O*-  
697  $\beta$ -D-glucuronide, demethyleneberberine-2-*O*- $\beta$ -D-glucuronide, thalfendine-10-*O*- $\beta$ -D-glucuronide,  
698 and jatrorrhizine-3-*O*- $\beta$ -D-glucuronide were found to be 165-, 65-, 23-, and 10-fold higher than that  
699 of berberine, respectively (Feng et al., 2020). In the same study, berberrubine was the main phase-  
700 I metabolite present in plasma, with an  $AUC_{0-48\text{h}}$  value 3.7 higher than that of berberine, whereas  
701 the systemic exposure to demethyleneberberine was substantially lower than the parent compound.  
702 These data indicate that berberine phase-II metabolites represent the main forms of systemic  
703 exposure after oral consumption of berberine.

704 In healthy humans, (Spinozzi et al., 2014) identified berberrubine as the main phase-I metabolite  
705 present in the circulation, reaching a maximum plasma concentration 10 times higher than that of  
706 berberine. The authors noted that the higher lipophilicity of berberrubine due to tautomerisation to  
707 an electroneutral quinoid form could contribute to a higher intestinal absorption compared to  
708 berberine. After repeated administration of berberine (15 mg/kg bw per day for 3 months), berberine  
709 reached a maximum steady-state concentration in plasma of 1.3 ng/mL in 12 hypercholesterolaemic  
710 individuals, while the mean concentrations of its primary metabolites berberrubine,  
711 demethyleneberberine and jatrorrhizine were 2.2 ng/mL, 1.8 ng/mL and 0.6 ng/mL, respectively.  
712 Phase-II metabolites were not quantified in that study.

713 After single dose oral administration of 200 mg/kg bw to rats, berberine was rapidly distributed to  
714 the liver, followed by the kidneys. The  $AUC_{0-t}$  in liver of berberine and its metabolites was 10 and  
715 30 times higher than in plasma, respectively. Lower concentrations than detected in plasma were  
716 detected in lungs, muscle, brain, heart, pancreas and adipose tissue (Tan et al., 2013a; Tan et al.,  
717 2013b).

718 No data was retrieved regarding the ability of berberine or its metabolites to cross the placental  
719 barrier.

#### 720 3.1.2.4 Excretion

721 Faeces are the primary route of excretion for berberine and its first-pass metabolites. In rats,  
722 berberrubine and intact berberine were the most abundant forms recovered in faeces following oral

723 administration of berberine, accounting for nearly 95% of the total amount excreted via this route,  
724 whereas smaller amounts of demethyleneberberine and jatrorrhizine were also detected (Feng et  
725 al., 2020).

726 In urine, berberrubine-9-*O*- $\beta$ -D-glucuronide was the most abundant metabolite identified after oral  
727 administration of berberine to rats (approximately 66% of total urinary metabolites), followed by  
728 berberrubine (19%), demethyleneberberine (6%), and other glucuronidated and sulphated  
729 metabolites (Feng et al., 2020). In healthy humans, Pan et al. (2002) isolated three berberine-  
730 derived metabolites in urine following oral administration of berberine chloride (900 mg/day for  
731 three days) to five individuals. These metabolites were identified as sulphate conjugates, specifically  
732 demethyleneberberine-2-*O*-sulphate (the major metabolite), jatrorrhizine-3-*O*-sulphate, and  
733 thalifendine-10-*O*-sulphate. In a subsequent study, Qiu et al. (2008) confirmed the presence of  
734 demethyleneberberine and jatrorrhizine sulphate conjugates in the urine of humans receiving the  
735 same dose of berberine chloride, and additionally identified the sulphated form of  
736 demethylpalmatine, as well as the glucuronidated forms of berberrubine, columbamine,  
737 jatrorrhizine, and thalifendine.

738 No data was retrieved regarding the transfer of berberine or its metabolites into human milk.

#### 739 3.1.2.5 Factors affecting ADME

740 In a pharmacokinetic study of oral administration of berberine, jatrorrhizine and palmatine, (Alolga  
741 et al., 2015) observed higher serum AUCs of the three compounds in individuals from African origins  
742 ( $n = 3$ ) vs Asian origins ( $n = 3$ ). The sample size was insufficient for adequate statistical  
743 comparisons. In another study involving 40 medication-free Chinese individuals, oral administration  
744 of berberine resulted in higher plasma concentrations in hyperlipidaemic individuals ( $n = 30$ ; 7  
745 ng/mL) compared to individuals with blood lipids and glucose concentrations in the normal range ( $n$   
746 = 10; 4 ng/mL,  $p < 0.05$ ) (Wang et al., 2017b). Authors mentioned that variations in the gut  
747 microbiota composition could contribute to these differences (Alolga et al., 2015; Wang et al.,  
748 2017b), including through the conversion of berberine into dihydroberberine (Section 3.1.2.1).

749 Regarding potential matrix effects, compared to pure berberine, higher absorption, intestinal  
750 disposal and hepatic exposure were found in mice when an equivalent dose of berberine (200-600  
751 mg/kg bw) was administered as *Coptidis* rhizome extract (Li et al., 2018; Ma et al., 2013; Zhao et  
752 al., 2021), but not as *Coptidis* rhizome-*Glycyrrhizae* radix and rhizome extracts (Li et al., 2018). At  
753 lower doses (60 mg/kg bw), differences were smaller compared to higher doses (Li et al., 2018).  
754 Other substances present in *Coptidis* rhizome extracts might affect the solubility and absorption of  
755 berberine (Ma et al., 2013; Zhao et al., 2021).

#### 756 3.1.2.6 Other protoberberine alkaloids present in berberine-containing plant preparations

757 Using a Caco-2 cell model and intestinal perfusion in rats, the intestinal apparent permeability  
758 coefficient and absorption rate of the protoberberines coptisine, palmatine, jatrorrhizine and  
759 columbamine were found to be lower than that of berberine. P-gp-mediated efflux affected the  
760 absorption of these alkaloids (Cui et al., 2015; Zhang et al., 2019). Pharmacokinetic studies in rats  
761 confirmed the lower bioavailability of coptisine (Yan et al., 2017), palmatine (Song et al., 2021), and  
762 jatrorrhizine (Shi et al., 2012) compared to berberine, while epiberberine bioavailability was reported  
763 to be higher (Chen et al., 2018).

764 Coptisine, palmatine, jatrorrhizine and columbamine are metabolised through similar pathways as  
765 berberine, in particular through demethylation and glucuronidation reactions (Shi et al., 2012; Su et  
766 al., 2015; Wang et al., 2017a). In human liver microsomes, Liu et al. (2015) identified CYP2D6 as  
767 the predominant enzyme involved in the metabolism of coptisine, while palmatine was mainly  
768 metabolised by CYP1A2. In an in vitro model, coptisine showed the strongest inhibition of berberine  
769 metabolism, while palmatine and jatrorrhizine had little inhibitory effect. In another experiment  
770 using human hepatocyte suspensions and recombinant human cytochrome P450 enzymes, (Vrba et  
771 al., 2015) found that CYP2D6 was the dominant enzyme mediating O-demethylation of palmatine,  
772 with CYP1A2 playing a secondary role.

773 Palmatine and jatrorrhizine share some common degradation products with berberine. For instance,  
774 jatrorrhizine, and its isomer columbamine, have been identified as metabolites of both berberine  
775 and palmatine, and demethyleneberberine as a metabolite of berberine, palmatine and jatrorrhizine  
776 (Su et al., 2015; Wang et al., 2017a).

777 Feng et al. (2022) demonstrated the interconnected metabolic pathways among protoberberine  
778 alkaloids present in berberine-containing plant preparations by investigating the toxicokinetics of  
779 berberine, jatrorrhizine, palmatine, epiberberine, and coptisine following oral administration of a  
780 preparation of *Coptis chinensis* rhizome to rats. Berberrubine was identified both as a demethylation  
781 metabolite of berberine and as a reduction metabolite of coptisine, while demethyleneberberine  
782 arose from demethylation of berberine and demethylation of jatrorrhizine. In addition, palmatine  
783 and jatrorrhizine could undergo mutual interconversion in vivo.

#### 784 3.1.2.7 Concluding remarks

785 Berberine and other protoberberine alkaloids commonly co-occur in plant-derived preparations, with  
786 their specific profiles and relative abundances varying according to the plant species. Because of  
787 their structural similarities, some commonalities in absorption mechanisms and metabolic pathways  
788 are to be expected between berberine and co-occurring protoberberines in plant preparations. Some  
789 experimental data indicate common absorption mechanisms and degradation reactions in the  
790 intestines. Some in vitro data indicate potential competition between berberine and coptisine at the  
791 level of hepatic metabolism by CYP2D6. Berberine, palmatine and jatrorrhizine share some common  
792 degradation products.

793 Low intestinal absorption, partly due to active efflux mediated by P-gp transporters, with extensive  
794 intestinal and hepatic first-pass metabolism, explain the low plasma concentrations of berberine  
795 upon oral ingestion. With respect to tissue distribution, berberine and its metabolites are  
796 predominantly found in the gastrointestinal tract, liver and kidneys. The demethylation product  
797 berberrubine has been identified as the predominant phase I metabolite present in the circulation  
798 upon oral administration of berberine in rats and humans. Other phase I metabolites include  
799 thalifendine, demethyleneberberine, jatrorrhizine and columbamine. In rats and humans, these  
800 metabolites are mainly metabolised via glucuronidation and sulphation in the intestine and the liver.  
801 The gut microbiota is also involved in the metabolism of berberine, contributing to the formation of  
802 metabolites such as dihydroberberine and oxyberberine, which have a higher intestinal absorption  
803 rate than berberine. However, the magnitude of the effect of the gut microbiota on the overall  
804 bioavailability of berberine is likely modest. To date, more than 20 berberine-related metabolites  
805 have been identified, whose contributions to effects of berberine in vivo are not well characterised.

806 Once absorbed, berberine and its metabolites are predominantly excreted via faeces and, to a lesser  
807 extent, urine.

808 After oral consumption of berberine-plant preparations, co-occurring alkaloids may interact at the  
809 level of intestinal transport and first-pass metabolism, thereby influencing the qualitative and  
810 quantitative profile of protoberberines metabolites present in the gastrointestinal tract, liver and  
811 systemic circulation.

### 812 3.1.3 Genotoxicity

#### 813 3.1.3.1 In vitro studies

##### 814 *Mutagenicity studies*

815 Sun et al. (2025) conducted a hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene  
816 mutation test in mouse lymphoma L5178Y cells, in accordance with OECD TG 476 and in compliance  
817 with GLP. Cells were exposed to berberine chloride for 3 h, at concentrations up to 511 µM without  
818 S9-mix and up to 484.1 µM with S9-mix. A concentration-dependent, statistically significant increase  
819 in HPRT mutation frequencies (exceeding the upper limit of historical negative control of the testing  
820 facility) was observed in test conditions without S9-mix, with cytotoxicity levels not exceeding 80%.  
821 High variability in mutation frequency was noted at the highest concentration tested; however, since  
822 relative survival remained within acceptable limits (approximately 20%) and positive results were  
823 observed at multiple concentrations, the Panel considers that the data indicate that berberine is  
824 inherently capable of inducing gene mutations. With S9-mix, berberine chloride did not induce any  
825 statistically significant increases in HPRT mutation frequencies compared to the negative control  
826 [dimethyl sulfoxide (DMSO)]. The Panel considers this study as reliable without restrictions (Klimisch  
827 score 1) and with high relevance of both the test system and the study results.

828 Sun et al. (2025) also conducted a targeted bacterial reverse mutation assay. *S. typhimurium* strain  
829 TA98 was treated with berberine chloride (purity 99%) at concentrations up to 1,095 µg/plate,  
830 without S9-mix. A dose-dependent increase (approximately 2.5-fold) in the number of revertant  
831 colonies was observed, reaching the highest level at 547.5 µg/plate. As this was a targeted study,  
832 key elements of the Ames test OECD TG 471, such as concurrent negative and positive controls,  
833 were not included. This, however, does not invalidate the experimental outcome. The study is  
834 considered reliable with restrictions (Klimisch score 2) and of limited relevance regarding the study  
835 results. Importantly, the positive results in TA98 are biologically relevant, as they are consistent with  
836 other reports and contribute to the overall weight of evidence for mutagenicity.

837 In another bacterial reverse mutation study (NTP, 2010), berberine chloride was tested at  
838 concentrations of up to 100 µg/plate (without S9-mix) and up to 1,000 µg/plate (with S9-mix), using  
839 *S. typhimurium* strains TA97, TA98, TA100 and TA1535. The selection of the highest concentrations  
840 was influenced by observed cytotoxicity. There were no dose-related increases in the number of  
841 revertant colonies, and all increases were below 2-fold as compared to the negative control. Positive  
842 controls were functional. The Panel notes the lack of information regarding the purity of the test  
843 substance, and that not all *S. typhimurium* and *E. coli* strains requested by the OECD TG 471 were  
844 tested. Due to these critical limitations, the study is considered reliable with restrictions (Klimisch  
845 score 2), and the relevance of the study results is limited.

846 The Panel notes differences in the concentration ranges tested in the TA98 strain between the (NTP,  
847 2010) study (up to 100 µg/plate) and the Sun et al. (2025) study (up to 1,095 µg/plate), which may  
848 be attributable to variations in toxicity associated with the purity of the test compounds.

849 In a bacterial reverse mutation study (Nozaka et al., 1990), using the pre-incubation method, *S.*  
850 *typhimurium* strains TA100 and TA98 were exposed to unspecified concentrations of berberine  
851 hydrochloride (purity confirmed by internal analysis), with and without metabolic activation system  
852 (S9-mix). The results indicated no mutagenicity of berberine hydrochloride with S9-mix and 'weak'  
853 mutagenicity in the TA98 strain without S9-mix. Due to several critical deviations from OECD TG  
854 471 (only two bacterial strains tested, absence of concentration information, unclear reporting of  
855 the results), the Panel considers the results inconclusive, the study as not reliable (Klimisch score  
856 3) and the relevance of the study results low.

857 Pasqual et al. (1993) conducted an SOS chromotest on berberine chloride. *E. coli* strain PQ37 was  
858 exposed to concentrations of up to 10,000 ng/test for 2h. Berberine chloride did not induce  $\beta$ -  
859 galactosidase synthesis, a marker of the SOS DNA repair response, either in the presence or absence  
860 of S9-mix. The study authors concluded that it is not genotoxic under non-growth conditions. In the  
861 same study, point- and frameshift mutations, as well as mitochondrial mutations and crossing-over,  
862 were investigated in proficient and repair-deficient *S. cerevisiae* strains. When yeast cells were  
863 treated during non-growth stages, results were negative. However, significant increases in  
864 frameshift mutations and mitotic recombination were observed in both DNA-repair proficient and  
865 deficient strains during mitotic growth stage. A mutant blocked in the DNA strand-break repair  
866 pathway (rad52-1) was found to be the most sensitive to the cytotoxic effect of berberine. Although  
867 the study is methodologically sound and mechanistically relevant, the assays were not conducted  
868 under GLP conditions and are not OECD-validated. Therefore, the Panel considers the study as  
869 reliable with restrictions (Klimisch score 2) and the relevance of the test system and study results  
870 as limited.

#### 871 *Chromosomal damage studies*

872 Liu et al. (2009) conducted a micronucleus assay using human osteosarcoma cell lines (U2OS). Cells  
873 were treated with berberine at concentration levels of up to 50 µg/mL for 48 h. A significant increase  
874 in micronuclei frequency was observed at all concentrations tested. Although a decrease in  
875 micronucleus frequency was observed at the highest concentration (cytotoxicity >60%), the  
876 positive, dose-related increase at lower concentrations indicate that berberine chloride can induce  
877 chromosomal damage in vitro. Nevertheless, the use of a non-standard cell line (U2OS) and other  
878 deviations from the OECD TG 487 (Appendix C) limit the relevance of the findings. The Panel  
879 considers the study to be reliable with restrictions (Klimisch score 2) and the relevance of the test  
880 system and results to be limited.

881 Sun et al. (2025) reported results from an in vitro micronucleus assay internally conducted using  
882 two cell-lines, L5178Y tk +/- mouse lymphoma and human TK6 cells. Cells were exposed to  
883 berberine chloride at concentrations up to 376 (L5178Y) and 200 µM (TK6) for 4 h, followed by a  
884 20 h recovery period. Micronuclei were assessed by flow cytometry (BD FACSCanto II), with analysis  
885 of at least 10,000 healthy nuclei per sample. The assays were performed without metabolic  
886 activation (S9-mix), and no positive control was included. In TK6 cells, statistically significant  
887 increases in micronuclei frequency were observed at the two highest concentrations (100 and 200

888  $\mu\text{M}$ ), with cytotoxicity remaining below 60%, indicating in vitro clastogenic and/or aneugenic  
889 potential. In L5178Y cells, a comparable pattern was seen; however, excessive cytotoxicity (greater  
890 than 60%) prevented any definitive interpretation of the results. Due to the limited information  
891 reported (Klimisch score 2), the Panel considers the relevance of the study results to be limited for  
892 TK6 cells and low for L5178Y cells, considering also the equivocal results observed in this assay.

### 893 *DNA damage studies*

894 DNA damage studies are summarised collectively because they provide supporting evidence in the  
895 genotoxicity assessment and many studies shared similar experimental designs and methodological  
896 limitations.

897 Studies assessing DNA damage induced by berberine have primarily used the alkaline comet assay  
898 in various cell lines, most frequently human or murine cancer-derived or immortalised cell lines (Gao  
899 et al., 2019; Secerli et al., 2023; Sun et al., 2019; Xia et al., 2021).

900 Comet assay studies, conducted under controlled experimental conditions (Gao et al., 2019; Jagetia  
901 Rao, 2014; Mantena et al., 2006; Secerli et al., 2023; Sun et al., 2019; Szeto et al., 2002; Xia et al.,  
902 2021), provided consistent evidence (Gao et al., 2019; Jagetia Rao, 2014; Mantena et al., 2006; Xia  
903 et al., 2021) that berberine can induce DNA strand breaks and related lesions, including single- and  
904 double-strand breaks, abasic sites, and DNA repair intermediates, in both murine and human cell  
905 lines. One study (Hou et al., 2017) comparing ovarian cancer-derived cell lines with non-  
906 transformed, immortalised epithelial cells (FTE-187) reported lower levels of DNA damage in the  
907 latter; however, most available data are derived from cancer-derived cell lines, often in the context  
908 of investigating berberine's anti-tumour properties.

909 Some of these studies reported the concomitant induction of oxidative stress-related markers,  
910 including increased ROS production and changes in mitochondrial function, following berberine  
911 exposure (Gu et al., 2015; Hou et al., 2017). Direct assessment of oxidative DNA base damage  
912 yielded inconsistent results. An increase in 8-hydroxy-2'-deoxyguanosine levels was reported in one  
913 study (Hou et al., 2017), whereas no increase in oxidised DNA bases was detected using an  
914 Fpg/Endo III-modified comet assay in another study (Jantová et al., 2006).

915 Double-strand breaks (DSBs) induction by berberine was investigated in several studies using  $\gamma$ -  
916 H2AX as a marker. Multiple studies have consistently demonstrated that berberine induces H2AX  
917 phosphorylation, as detected by the  $\gamma$ -H2AX foci assay (Inoue et al., 2021). This assay is highly  
918 sensitive for detecting DSBs, although its specificity may be limited. Other DNA damage types, such  
919 as replication stress or oxidative damage, as well as apoptosis-induced fragmentation, can also  
920 trigger  $\gamma$ -H2AX foci, leading to potential false positives. Studies based solely on Western blot analysis  
921 without foci visualisation (e.g. immunofluorescence microscopy) were excluded, as the method lacks  
922 the resolution needed to reliably detect DNA double-strand breaks at the cellular level. The strongest  
923 evidence for DSB induction by berberine comes from two studies (Gu et al., 2015; Hou et al., 2017;  
924 Hou et al., 2022; Inoue et al., 2021; Sun et al., 2019; Xia et al., 2021). (Inoue et al., 2021) confirmed  
925 the presence of DSBs in cancer cell lines using pulsed field gel electrophoresis (PFGE), a reliable  
926 method for detecting DSBs, under conditions where apoptosis was pharmacologically inhibited. In a  
927 time-course experiment [up to 24h exposure with 1  $\mu\text{M}$  camptothecin (CPT) and 20  $\mu\text{M}$  berberine],  
928 the accumulation of DSBs, expressed as the fold increase in broken DNA relative to the negative

929 control, was approximately 2-fold for berberine and 5-fold for CPT. This indicates that berberine  
930 induces a comparatively mild level of DSBs relative to the classical topoisomerase II inhibitor CPT.  
931 It is of note that accumulation of DSBs occurred around DNA replication sites and was suppressed  
932 by aphidicolin, suggesting that DSBs primarily occur at replication sites. Similarly, Hou et al. (2017)  
933 demonstrated the concurrent induction of  $\gamma$ -H2AX and RAD51 foci in ovarian cancer cells, supporting  
934 both DSB induction and the activation of homologous recombination repair pathways.

935 In the remaining studies on berberine Inoue et al. (2021), high cytotoxicity (>70-80%) or concurrent  
936 apoptosis was observed, which may confound the results. Studies in which cytotoxicity or apoptosis  
937 levels exceeded acceptable thresholds, or where DNA damage was assessed using parameters not  
938 aligned with OECD TG 489 recommendations (e.g. olive tail moment/tail moment or tail length used  
939 as standalone measures without supporting quality criteria), are considered of inadequate reliability  
940 (Klimisch score 3) and therefore considered of low relevance.

941 Studies with other eligible protoberberine alkaloids are limited. Available data include two comet  
942 assays with coptisine (Kim et al., 2020) and palmatine (Chen et al., 2013), as well as an assessment  
943 of DSB using PFGE with coptisine chloride and palmatine (Inoue et al., 2021). In the study by Kim  
944 et al. (2020) coptisine induced an increase in DNA damage as measured by tail length, although the  
945 interpretation is limited by the occurrence of apoptosis at the only dose tested, therefore the results  
946 are inconclusive. Chen et al. (2013) reported a moderate induction of DNA breaks in human  
947 hepatoma HepG2 cells by palmatine relative to berberine, but the data were not shown. Inoue et  
948 al. (2021) reported that coptisine chloride, similarly to berberine, induced a mild accumulation of  
949 DSBs in normal and cancer cell lines, while palmatine did not. Additionally, *in silico* predictions of  
950 genotoxic potential for various food-relevant plant metabolites identified coptisine as potentially  
951 mutagenic (Gluck et al., 2018).

#### 952 3.1.3.2 *In vivo* studies

953 Bone-marrow from B6C3F1 mice was used in an *in vivo* MN assay (NTP, 2010) where animals (both  
954 sexes) were administered intraperitoneally berberine chloride over three consecutive days at 24h  
955 intervals. Preliminary range-finding studies were performed to determine the bone marrow toxicity  
956 and to guide dose selection. For the main experiment, berberine chloride was administered at doses  
957 ranging from 0 (phosphate-buffered saline as negative control) to 658 mg/kg bw. Cyclophosphamide  
958 was used as positive control. Formation of micronuclei was assessed in 2,000 polychromatic  
959 erythrocytes (PCEs) in each of five animals per group. In addition, the percentage of PCEs relative  
960 to the total erythrocyte population in the bone marrow was scored for each dose group as a measure  
961 of toxicity. No increases in the frequency of micronucleated PCEs were seen. No dose-related change  
962 in the percentage of PCEs in the bone marrow were observed, suggesting that exposure to berberine  
963 chloride did not induce clastogenic and/or aneugenic effects and toxicity under the tested conditions.  
964 The Panel considers that the lack of toxicity in the bone marrow might suggest limited or no exposure  
965 to berberine chloride when administered via *i.p.*, even at higher doses. Therefore, the study is  
966 considered reliable with restrictions (Klimisch score 2) while the results are considered inconclusive.  
967 Furthermore, as per OECD TG 474, *i.p.* administration is generally not recommended since it is not  
968 an intended route of human exposure, and no justification is given for its use. The relevance of the  
969 results is therefore considered low.

970 In the two in vivo comet assays (Xu et al., 2014a; Xu et al., 2014b), DNA damage was assessed in  
971 heart and bone marrow cells of male mice administered berberine by gavage at doses up to 120  
972 mg/kg bw per day. In both tested systems, berberine was reported to induce significant and dose-  
973 dependent increase of DNA damage in all dose-groups compared to the controls, as observed in  
974 %DNA in tail, tail length and tail moment. However, no rationale was provided for selecting non-  
975 standard target organs (e.g. heart), and the information on methodology – critical for evaluating  
976 study reliability according to OECD TG 489 – was limited and lacked key information, including cell  
977 isolation procedures, cytotoxicity data and detailed descriptions of the assay and scoring system.  
978 Furthermore, histopathological observations were not reported. Consequently, the Panel considers  
979 these two studies as not reliable (Klimisch score 3), and the relevance of study results as low.

### 980 3.1.3.3 Proposed modes of action

981 The primary mechanisms involve DNA topoisomerases and DNA intercalation.

#### 982 *DNA topoisomerases*

983 Berberine, as well as the related protoberberine alkaloids palmatine and coptisine, has been shown  
984 to interfere with DNA topoisomerases. These alkaloids inhibit Topo I in a catalytic manner in a cell-  
985 free system (Chen et al., 2013), and also in cellular models. Notably, (Inoue et al., 2021), showed  
986 that berberine and coptisine induce mild increases in DSBs, particularly during replication, in a Topo  
987 I-dependent manner involving the MUS81–EME1 endonuclease complex in different cell lines,  
988 suggesting that DSBs arise at stalled replication forks.

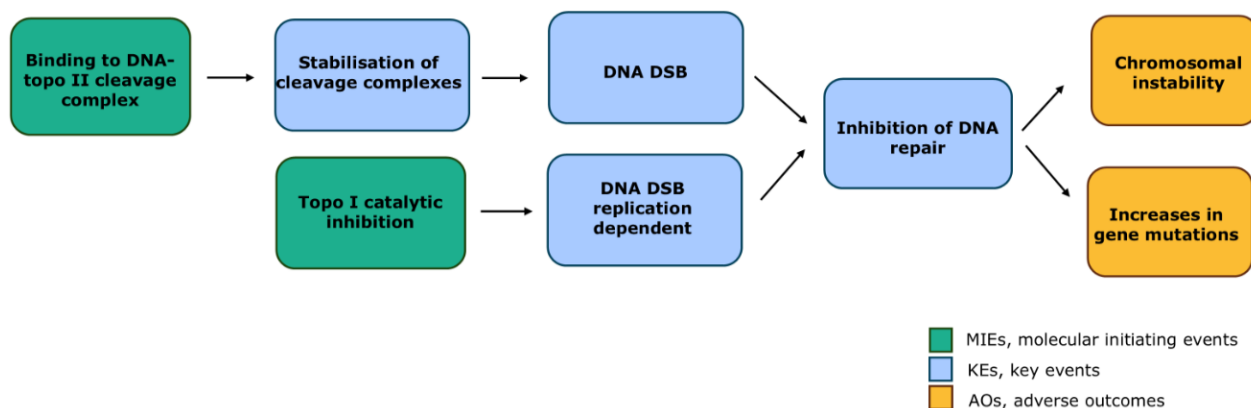
989 A dual mode of action has been identified for berberine on Topo II, acting both as a catalytic inhibitor  
990 and as a Topo II poison through stabilization of the Topo II–DNA cleavage complex. In HepG2 cells,  
991 induction of  $\gamma$ -H2AX has been reported in association with Topo II inhibition following targeted gene  
992 silencing Chen et al. (2013), supporting the occurrence of DSB formation in a cellular context.  
993 Consistently, Sun et al. (2025) confirmed catalytic inhibition of Topo II in vitro and stabilisation of  
994 the Topo II–DNA cleavage complex using an in vivo complex of enzyme (ICE) assay in mouse  
995 lymphoma L5178Y cells.

#### 996 *DNA intercalation*

997 Berberine has been consistently reported to intercalate into DNA, a process favoured by its planar  
998 tetracyclic structure (Chatterjee et al., 2015; Chen et al., 2004; Mazzini et al., 2003), and to bind to  
999 alternative structures, such as G-quadruplexes (Naasani et al., 1999; Parkinson et al., 2002). These  
1000 interactions may destabilise DNA structure and promote replication slippage and base  
1001 insertion/deletion events, resulting in frameshift mutations and contributing to chromosomal  
1002 damage through DNA break formation.

1003 Additional mechanisms that may further contribute to genomic instability are the induction of  
1004 oxidative DNA damage (Hou et al., 2017), modulation of DNA repair pathways (e.g. downregulation  
1005 of XRCC1 (Gao et al., 2019) and RAD51 (Hou et al., 2017), and effects on histone acetylation and  
1006 methylation (Mishra et al., 2020; Wang et al., 2016). These processes may exacerbate DNA damage  
1007 persistence and chromosomal instability but are considered supportive rather than primary  
1008 mechanisms.

1009 The Panel notes that interaction with topoisomerases and DNA provides biological plausibility for the  
 1010 chromosomal damage and mutagenic effects observed in vitro (Figure 3). Although limited,  
 1011 mechanistic data reported for structurally related protoberberine alkaloids support a similar mode  
 1012 of action.



1013  
 1014 **Figure 3.** Diagram of the adverse outcome pathway (AOP) describing the genotoxic MoA of Topo II poisons.  
 1015 *Source:* adapted from (Sasaki et al., 2020) to the potential MoA of berberine.

#### 1016 3.1.3.4 QSAR predictions

1017 In silico modelling of structural similarity and genotoxicity predictions was applied to berberine,  
 1018 berberastine, berberrubine, columbamine, coptisine, corysamine, demethyleneberberine,  
 1019 epiberberine, fississaine, groenlandicine, jatrorrhizine, palmatine, stephamine, thalidastine and  
 1020 thalifendine. The results are provided in Annex C. Key findings are summarised below.

1021 Protoberberines share the same skeleton, consisting of four rings, three of which are aromatic, with  
 1022 a positive charge on the nitrogen. Oxygen atoms are attached to the rings, as either ether or hydroxy  
 1023 groups. High structural similarity between berberine and the protoberberines included in the analysis  
 1024 was predicted by the VEGA algorithm IstSimilarity (structural similarity score >0.94).

1025 All protoberberines were predicted to be mutagenic by the VEGA consensus model. The consensus  
 1026 score was of 0.5 or higher, indicative of reliable prediction, for all protoberberines except for  
 1027 berberastine, with a consensus score of 0.425, close to this threshold. A common structural alert for  
 1028 mutagenicity was identified in berberine and all other protoberberines (including berberastine),  
 1029 which corresponds to heterocyclic polycyclic aromatic hydrocarbons with three or more fused rings  
 1030 (SA19).

1031 Regarding micronucleus induction, the VEGA in vitro MN model provided similar predictions  
 1032 protoberberines included in the analysis, indicating genotoxicity. However, because of low model  
 1033 reliability, no conclusion can be reached for this endpoint based on the QSAR predictions. Regarding  
 1034 VEGA in vivo MN model, no prediction could be produced for stephamine because of conflicting  
 1035 results from the two submodels. Identical predictions were obtained for the other protoberberines  
 1036 included in the analysis, which indicated non-genotoxicity with moderate model reliability.

1037 The Panel notes that for the modelling of in vitro Ames mutagenicity, the training and test datasets  
1038 used were extensive (section 2.3.2.1), providing robust model predictions. By contrast, for the  
1039 models of in vitro and in vivo micronucleus assays, the limited and less robust datasets used may  
1040 limit the applicability of the models, resulting in predictions of lower certainty.

#### 1041 3.1.3.5 Discussion

##### 1042 *Mutagenic effects*

1043 The results from the available bacterial reverse mutation assays indicated that berberine is not  
1044 mutagenic in the majority of *S. typhimurium* strains tested. However, there is a pattern of weak  
1045 positive results for the TA98 strain across several studies with berberine alone (without S9-mix)  
1046 (Nozaka et al., 1990; Sun et al., 2025) or with plant extracts containing berberine (with and without  
1047 S9-mix) (Akiyama et al., 2019; Sun et al., 2025) (section 3.1.3.1). In contrast, the (NTP, 2010)  
1048 reported negative results for berberine chloride in the TA98 strain. However, these results may be  
1049 attributed to suboptimal dosing, constrained by observed toxicity. Notably, the NTP study did not  
1050 report the compound's purity, or the vehicle used, raising the possibility that impurities or vehicle-  
1051 related effects could have influenced the toxicity profile and masked mutagenic responses. Taken  
1052 together, the available evidence supports the conclusion that berberine exhibits weak mutagenic  
1053 activity in bacteria, primarily inducing frame-shift mutations. This conclusion is further supported by  
1054 findings in Pasqual et al. (1993), where berberine induced frame-shift mutations, but not point  
1055 mutations, in both DNA-repair proficient and deficient strains during mitotic growth stages, were  
1056 seen, reinforcing berberine mutational specificity.

1057 Berberine chloride did not exhibit mutagenic activity in the SOS chromotest using *Escherichia coli*  
1058 strain PQ37, both with and without S9, and no genotoxicity was detected in *Saccharomyces*  
1059 *cerevisiae* strains exposed during non-growth stages (Pasqual et al., 1993). These findings suggest  
1060 that berberine does not trigger the bacterial SOS DNA damage response and that active DNA  
1061 replication and associated DNA damage response pathways are necessary for its genotoxic effects  
1062 to manifest. The authors proposed that the genotoxic effects of berberine in the yeast test system  
1063 were likely due to its intercalation into yeast DNA, leading to disruption of topoisomerase enzyme  
1064 activities.

1065 A recent study by Sun et al. (2025) provided evidence that berberine is mutagenic in an in vitro  
1066 HPRT mutation assay using L5178Y mouse cells. This assay detected a range of mutations including  
1067 base-pair substitutions, frame-shift mutations, splice site mutations, large deletions, duplications or  
1068 chromosomal damage, in mammalian cells. The mutagenic effect of berberine was observed only in  
1069 the absence of metabolic activation (S9). Topoisomerase inhibitors induced mutagenic responses in  
1070 the Ames TA98 strain (Nakanomyo et al., 1986); additionally, a literature survey by Sun et al. (2025)  
1071 reported that common topoisomerase II inhibitors induced mutations in the HPRT gene in  
1072 mammalian cells, in the absence of S9, indicating a direct mutagenic potential. This activity is  
1073 attributed to their ability to interfere with DNA topoisomerase-driven processes, inducing DSBs. The  
1074 mutagenicity of berberine in the TA98 strain and in mammalian cells without metabolic activation  
1075 may reflect a similar mechanism, suggesting that berberine can directly cause DNA damage through  
1076 topoisomerase inhibition. In addition, the abolishment of the mutagenic response in the presence  
1077 of metabolic activation suggests that berberine may undergo modification by liver enzymes, which  
1078 may reduce its ability to inhibit topoisomerases or produce metabolites that are less genotoxic.

1079 QSAR analysis identified a common structural alert for mutagenicity present in berberine, and all the  
1080 protoberberines triggered by their heterocyclic polycyclic aromatic structure, with three or more  
1081 fused rings.

1082 In vivo mutation data in mammalian cells are lacking.

1083 Overall, the available data support the conclusion that berberine presents mutagenic activity in  
1084 mammalian cells in vitro. Supporting evidence, though of limited reliability and relevance, is provided  
1085 by bacterial and yeast mutation assays, which suggest that berberine induces frameshift mutations.  
1086 This pattern is consistent with the genotoxic effects commonly associated with topoisomerase  
1087 inhibitors (reviewed in Pommier et al. (2010).

#### 1088 *Chromosomal-damaging effects*

1089 Two in vitro studies demonstrated berberine's ability to induce micronuclei in the human U2OS  
1090 cancer cell line (Liu et al., 2009) and in the human lymphoblastoid TK6 cell line (Sun et al., 2025).  
1091 However, both studies presented several deviations from the OECD TG guidelines making the study  
1092 findings of limited relevance. Evidence of induction of recombinogenic events (gene conversion and  
1093 crossing-over) in yeast (Pasqual et al., 1993) further supports the potential for berberine to cause  
1094 genomic instability, as suggested by the observed micronuclei induction in mammalian cells. Only  
1095 one in vivo genotoxicity study in mice model has been identified. In this study, berberine chloride  
1096 showed negative results (as concluded by the study authors) in the in vivo MN assay conducted in  
1097 bone marrow PCEs, with no dose-related changes in the percentage of PCEs of male mice exposed  
1098 intraperitoneally (NTP, 2010). However, in addition to the non-physiological route of administration  
1099 (i.p.), the absence of bone marrow toxicity may reflect insufficient exposure, as even high doses  
1100 (up to 658 mg/kg bw) may be ineffective if the compound does not adequately reach or persist in  
1101 the target tissue. Although i.p. administration generally maximises systemic availability, berberine  
1102 undergoes rapid metabolism (see section 3.1.2), so systemic concentration may have remained too  
1103 low to affect the bone marrow. Moreover, i.p. dosing bypasses the gastrointestinal tract, limiting  
1104 local exposure of gut epithelial cells. Therefore, the lack of genotoxic effects observed in this study  
1105 does not preclude potential effects in other organs with higher or localized exposure, such as the  
1106 liver or GI tract. The Panel considers that this study is inconclusive.

1107 Although evidence of chromosomal damage is limited in in vitro assays and inconclusive in one in  
1108 vivo MN assay, berberine's ability to inhibit topoisomerases, thus inducing DSBs, provides biological  
1109 plausibility for mutagenic and clastogenic and/or aneugenic effects.

#### 1110 *DNA damaging effects*

1111 Several studies have reported positive results using the in vitro comet assay to investigate berberine-  
1112 induced DNA damage (Chen et al., 2013; Gao et al., 2019; Gu et al., 2015; Hou et al., 2017; Jagetia  
1113 Rao, 2014; Jantová et al., 2006; Kim et al., 2020; Lin et al., 2006; Szeto et al., 2002). This assay  
1114 primarily detects SSBs but can also identify DSBs. Additionally, it is capable of detecting alkali-labile  
1115 sites, which often result from depurination or depyrimidination, as well as oxidative DNA damage  
1116 and DNA repair intermediates. The induction of oxidative DNA damage by berberine was confirmed  
1117 by measuring 8-oxo-dG level in a cancer-derived cell line (Hou et al., 2017). There is no validated  
1118 testing guideline for the in vitro comet assay. In evaluating the reliability and relevance of data sets,  
1119 several experimental conditions were carefully assessed, with a particular focus on cytotoxicity and

1120 apoptosis. Overall, the consistent findings in various cell lines, including human cells, supports the  
1121 conclusion that berberine is capable of inducing DNA damage in in vitro cell systems, in line with its  
1122 capacity to generate ROS.

1123 Positive results have also been reported in studies employing the  $\gamma$ -H2AX foci assay, which is  
1124 considered a sensitive indicator of DNA damage and, in particular, of DSBs (Hou et al., 2017; Inoue  
1125 et al., 2021; Wang et al., 2012; Zhao et al., 2023; Zhu et al., 2014). In the absence of an OECD  
1126 test guideline, interpretation of this assay involves important caveats regarding the specificity of the  
1127 DNA damage detected; however, these aspects were carefully taken into account in the evaluation  
1128 process (see section 3.1.1). The available evidence consistently shows that berberine induces DNA  
1129 damage-associated foci, likely reflecting DSB formation, in mammalian cells. Several studies  
1130 strengthened these observations by combining  $\gamma$ -H2AX analysis with complementary endpoints of  
1131 DNA damage, such as markers of homologous recombination repair (e.g. RAD51) (Hou et al., 2022)  
1132 or alternative methods capable of detecting DSBs (Inoue et al., 2021). These combined approaches  
1133 support the interpretation that berberine induces both SSBs and DSBs, with DSBs arising primarily  
1134 through replication-associated mechanisms.

1135 DNA damage, as detected by the comet assay, was reported in heart and bone marrow cells of mice  
1136 treated with berberine via gavage (Xu et al., 2014a; Xu et al., 2014b). However, these studies had  
1137 significant experimental limitations, which undermined the reliability of the findings.

1138 Overall, while SSBs appear to be the primary type of DNA damage induced by berberine, several  
1139 studies also indicated the induction of DSBs. SSBs are typically repaired quickly, posing a relatively  
1140 minor threat to DNA integrity; however, berberine can interfere with their repair, thereby increasing  
1141 the likelihood of DSB formation. Additionally, the inhibition of DNA topoisomerases by berberine is  
1142 another potential source of DSBs. DSBs are among the most lethal DNA lesions and tend to persist  
1143 longer, posing a significant challenge to genomic stability.

1144 Regarding other protoberberine alkaloids, the available data for coptisine and palmatine, though  
1145 limited, suggest a MoA similar to that of berberine.

#### 1146 *Role of metabolism in the genotoxicity of berberine*

1147 Berberine undergoes metabolic conversion in vivo, producing metabolites such as berberrubine and  
1148 dihydroberberine (see section 3.1.2). In vitro mutagenicity studies in bacterial and mammalian cells  
1149 show positive results with the parent compound only in the absence of metabolic activation (Sun et  
1150 al., 2025), suggesting that berberine has direct mutagenic potential.

1151 Data on metabolites provide additional mechanistic insights. Dihydroberberine, the reduced form of  
1152 berberine is reported to be negative in bacterial and mammalian genotoxicity assays, as well as in  
1153 in vivo MN tests (Lewis & Falk, 2022). These results, however, are presented only narratively,  
1154 without supporting data. Although dihydroberberine is known to be rapidly oxidised back to  
1155 berberine in vivo, this conversion is unlikely to occur efficiently in standard in vitro genotoxicity test  
1156 systems, where the enzymatic machinery required for re-oxidation is absent or limited. In addition,  
1157 as dihydroberberine is a metabolite of berberine that is converted back to berberine in the body, its  
1158 evaluation should also consider berberine's genotoxicity to account for risks from in vivo conversion.  
1159 Consequently, the negative findings for dihydroberberine reflect the lack of intrinsic genotoxic  
1160 potential of the reduced metabolite itself, rather than providing evidence against the genotoxic

1161 potential of berberine. In contrast, berberrubine, the main plasma metabolite of berberine, has been  
 1162 shown to specifically inhibit Topo II. Kim et al. (1998) showed in vitro that berberrubine stabilises  
 1163 the Topo II cleavage complex, similar to etoposide, but at distinct cleavage sites, suggesting a  
 1164 different binding mode or sequence preference. This catalytic inhibition correlates, in a  
 1165 concentration-dependent manner, with DNA intercalation, suggesting a mixed MoA as also described  
 1166 for berberine. These findings are consistent with a clastogenic MoA. In this study berberrubine was  
 1167 found to be a markedly more potent Topo II inhibitor than berberine.

1168 *Weighing the evidence*

1169 Table 3 summarises the key studies considered for the WoE evaluation, after excluding those  
 1170 considered of low relevance. As a result of this exclusion, the WoE evaluation was limited to in vitro  
 1171 studies. The table presents a consolidated view of the genotoxicity endpoints, including gene  
 1172 mutation, chromosomal instability, and DNA damage, alongside the relevance of the results for each  
 1173 endpoint, the consistency of supporting studies and the biological plausibility based on mechanistic  
 1174 insights. A qualitative narrative description is used for consistency and biological plausibility.

1175 **Table 3:** Integration of the evidence on berberine genotoxicity within a WoE approach

Endpoint Category	Assay type	Outcome	Relevance of the study results	Consistency	Biological plausibility
<b>Gene mutation</b>	Frame-shift mutation in bacteria and yeast	Positive	Limited	Two concordant studies in different test systems	Mutation specificity relevant to inhibition of DNA topoisomerases
	HPRT mutation in a mouse cell line	Positive	High	One study	Confirmation of mutagenic activity in mammalian cells
	Point mutation in yeast	Negative	Limited	One study	Negative result potentially confirming mutation specificity
<b>Chromosomal damage</b>	MN formation in human cell lines	Positive	Limited	Two concordant studies	Chromosomal effect mechanistically supported by DNA topoisomerases inhibition
	Crossing-over in yeast	Positive	Limited	One study	Mechanistically plausible by inhibition of recombination
<b>DNA damage</b>	DNA breaks and repair intermediates in human/murine cell lines	Positive	Limited	Five concordant studies	In line with DNA breaks and ROS-induced DNA damage
	DNA DSBs in human cell lines	Positive	Limited	Five concordant studies	Strong mechanistic alignment with DSBs induced by inhibitors of DNA topoisomerases

1176 Abbreviations: DSBs, double-strand breaks; HPRT, hypoxanthine-guanine phosphoribosyl transferase; MN, micronucleus.

1177 The WoE evaluation of genotoxicity for berberine is primarily based on a set of in vitro studies of  
 1178 variable methodological robustness, biological relevance, and mechanistic support. Across the  
 1179 genotoxicity endpoints assessed, there is a consistent pattern of in vitro positive findings for DNA  
 1180 damage and gene mutation. These findings are mechanistically coherent with the well-documented  
 1181 MoA of berberine involving topoisomerase inhibition and ROS generation. However, the limited

1182 number of high-reliability studies and the lack of conclusive in vivo evidence restrict the overall  
1183 certainty of genotoxicity.

### 1184 3.1.4 Acute toxicity

#### 1185 3.1.4.1 Berberine

1186 Nine publications which reported on single dose administration of berberine in mammalian species  
1187 were retrieved from the systematic search of the literature. Of these, five publications (Bokka et al.,  
1188 2023; Ma et al., 2016; Peng et al., 2007; Peng et al., 2004; Wu et al., 2019) did not investigate the  
1189 acute toxicity of berberine and are not further considered. The remaining four studies evaluated the  
1190 acute toxicity of berberine in mice (Kheir et al., 2010; Subaiea et al., 2017; Yi et al., 2013; Zhang  
1191 et al., 2022b). The LD50 estimates derived from these studies cover a range from 713 to >2000  
1192 mg/kg bw berberine in mice (Table 4).

#### 1193 3.1.4.2 Other protoberberines

1194 Three oral acute toxicity studies in mice were retrieved, which evaluated coptisine, epiberberine,  
1195 palmatine (Yi et al., 2013), columbamine (Wang et al., 2016) and jatrorrhizine (Wu et al., 2014).  
1196 None of them claimed to have complied with OECD requirements for acute toxicity studies. The LD50  
1197 estimates derived from these studies are reported in Table 5.

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1198 **Table 4:** LD50 for berberine in mice

Publication	Species	Sex (n/group)	Test material <sup>c</sup>	Exposure route	Doses (mg/kg bw per day) <sup>d</sup>	LD50 (mg/kg bw per day)	Comment
<b>Kheir et al. (2010)</b>	ICR Mice	F (n = 5 <sup>a</sup> ) M (n = 5 <sup>a</sup> )	BBR Cl	Gavage	0, 5200, 10400, 20800, 41600, 83200 <sup>(e)</sup>	Oral route: ND i.v. route: 9.04 i.p. route: 57.61	Mortality at 41600 mg/kg: 20% in M and 40% in F Mortality at 83200 mg/kg: 25% in M and 25% in F Maximum tolerated oral dose estimated at 20800 mg/kg bw.
<b>Subaiea et al. (2017)</b>	Swiss Albino Mice	F (n = 6)	BBR	Gavage	0, 2000	>2000	No mortality
<b>Zhang et al. (2022b)</b>	ICR Mice	F (UaDP : n = 9 <sup>b</sup> ; mKM : n = 6)	BBR Cl	NR	UaDP: 0, 790, 2500, 5000 mKM: 0, 703, 1406, 2812, 5628, 11250	UaDP: 2954.93 (95% CI 2160.05, 3749.8) mKM: 2825.5 (95% CI 1613, 4038)	
<b>Yi et al. (2013)</b>	KM mice	F (n = 5) M (n = 5)	BBR	Gavage	0, 409, 512, 640, 800, 1000	713.6 (M and F combined)	Sex-specific differences not reported

1199 Abbreviations: BBR, berberine (unspecified form); bw, body weight; CI, confidence interval; Cl, chloride; F, females; ICR, Institute of Cancer Research; LD50, median lethal dose; KM, Kunming  
1200 mice; M, males; mKM, modified Karber method; ND, not determined; NR, not reported; UaDP, up-and-down procedure; i.v., intravenous; i.p., intraperitoneal; n, number.

1201 <sup>a</sup> 1 M and 1 F in the lowest dose group and 4 M and 4 F in the highest dose group.

1202 <sup>b</sup> Total number of mice tested until stop criteria were met.

1203 <sup>c</sup> BBR relates to berberine in an unspecified form.

1204 <sup>d</sup> Dose of test material.

1205 <sup>e</sup> Accumulated dosages through repeated administration once every hour.

1206

1207 **Table 5:** LD50 for protoberberines in mice

Publication	Species	Sex, n/ group	Test material <sup>a</sup>	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>	LD50 (mg/kg bw)	Comment
<b>Yi et al. (2013)</b>	KM mice	F (n = 5) M (n = 5)	Coptisine	Gavage	0, 579, 694, 833, 1,000, 1,200	852.12 (M and F combined)	Sex-specific differences not reported
		F (n = 5) M (n = 5)	Epiberberine	Gavage	670, 931, 1295, 1800, 2500	1360 (M and F combined)	
		F (n = 5) M (n = 5)	Palmatine	Gavage	670, 931, 1295, 1800, 2500	1533.68 (M and F combined)	

<b>Wu et al. (2014)</b>	KM mice	F (n = 5) M (n = 5)	Jatrorrhizine	Gavage	0, 1,000, 2,000, 4,000, 8,000, 16,000	5500 (M and F combined)	Sex-specific differences not reported
<b>Wang et al. (2016)</b>	KM mice	F (n = 5) M (n = 5)	Columbamine	Gavage	400, 600, 900, 1,350, 2,025, 3,037.5, 4,556.3, 6,834.4	1524.6 (95% CI: 1185-1960.22) (M and F combined)	Sex-specific differences not reported

Abbreviations: bw, body weight; CI, confidence interval; d, day; F, females; LD50, median lethal dose; KM, Kunming mice; M, males; not reported; n, number.

<sup>a</sup> Dose of the test material

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1217 [3.1.5 General toxicity](#)

1218 [3.1.5.1 Berberine](#)

1219 [Subchronic toxicity study](#)

1220 One 90-day study investigating toxicity of berberine in rats (Yi et al., 2013) was retrieved  
1221 through the systematic search of the literature (Table 6).

1222 **Table 6:** General characteristics of the subchronic toxicity study of berberine in rats

Publication	RoB Tier	Species	Sex (n/group)	Duration (days)	Test material <sup>a</sup>	Mode of administration	Doses (mg/kg bw per day) <sup>c</sup>
Yi et al. (2013)	2	SD rats	M (n=5) F (n=5)	90	BBR (extracted from <i>Coptis</i> species; purity NR)	Unclear <sup>b</sup>	0, 156

1223 Abbreviations: BBR, berberine (unspecified form); F, females, RoB, Risk of Bias; SD, Sprague Dawley, M, males; n, number;  
1224 NR, not reported; bw, body weight.

1225 <sup>a</sup> BBR relates to berberine in an unspecified form.

1226 <sup>b</sup> The authors reported that 'treatment group received a dose of 156 mg/kg at daily diet' and 'the individual dose was adjusted  
1227 for the body weight to maintain the target dose level for all rats'.

1228 <sup>c</sup> Dose of test material.

1229

1230 Sprague-Dawley (SD) rats received berberine at a dose of 156 mg/kg bw per day or no  
1231 treatment (control) (n=10 per group; n=5 per sex).

1232 No mortality or toxicity symptoms were observed in rats exposed to berberine at a dose of  
1233 156 mg/kg bw per day or in the control. No differences in body weight and relative organ  
1234 weights (heart, liver, spleen, lung, kidney, stomach, brain) were found between the  
1235 treatment and control group. No treatment-related effects were detected based on gross  
1236 necropsy, histopathology, urinalysis and clinical biochemistry. Standard haematological  
1237 parameters were not reported. The Panel notes that in this study no effects on the endpoints  
1238 investigated were detected in rats at 156 mg/kg bw per day of berberine for 90 days.  
1239 However, main methodological limitations of the study included the unknown purity of the  
1240 test material, the limited number of animals per group and the limited number of organs  
1241 and endpoints investigated. The study is at moderate risk of bias (tier 2) (Appendix D).

1242 [Other studies in mammalian animals](#)

1243 Through a systematic literature search, 21 publications were retrieved, reporting on 18  
1244 studies in mammalian animals that were not primarily designed to assess toxicity but  
1245 investigated some safety-related endpoints (Table 7). Of these, 12 studies reported in 15  
1246 publications were designed to investigate beneficial/pharmacological effects of berberine  
1247 upon oral administration (Adefegha et al., 2021; Akhzari et al., 2024; Akhzari et al., 2019;  
1248 Alagal et al., 2023; Ghavipanje et al., 2021a, 2021b, 2022a, 2022b; Gholampour & Keikha,  
1249 2018; Hasanein et al., 2017; Hassanein et al., 2019; Hu et al., 2012; Jia et al., 2020; Maurya  
1250 et al., 2016; Zhang et al., 2012). The remaining six studies were designed to investigate  
1251 underlying mechanisms (Chatuphonprasert et al., 2012; Gu et al., 2012; Guo et al., 2011;  
1252 Heidarian et al., 2014; Moghaddam et al., 2013; Tian et al., 2019).

1253 Study characteristics and relevant findings are provided in Table 7. Fourteen studies were  
1254 conducted in rats (dose range: 10-380 mg/kg bw per day, duration range: 7-126 days),  
1255 three in mice (dose range: 10-300 mg/kg bw per day, duration range: 5-14 days), and one  
1256 in goats (dose range: 25-100 mg/kg bw per day, duration: 42 days).

1257 Maurya et al. (2016) reported elevated serum concentrations of thyroid hormones (thyroid-  
1258 stimulating hormone (TSH) and thyroxine (T4)), aspartate aminotransferase (AST), alkaline  
1259 phosphatase (ALP), and alanine aminotransferase (ALT)), triglycerides and reduced serum  
1260 cholesterol (high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-  
1261 density lipoprotein (VLDL)) concentrations in berberine-treated female rats (6 per group)  
1262 receiving 100 mg/kg bw per day berberine chloride (unknown purity) for 35 days compared  
1263 to control animals treated with saline. The mode of oral administration was not reported.  
1264 The Panel notes that no elevations of liver enzymes were found in other studies investigating  
1265 these parameters at similar or higher doses (Akhzari et al., 2024; Akhzari et al., 2019; Yi et  
1266 al., 2013). The Panel also notes that this is the only animal study which investigated effect  
1267 of berberine on thyroid hormones concentrations. The Panel considers that limitations in the  
1268 conduct and reporting of the study (tier 3) do not allow drawing conclusions from this study.

1269 The other studies did not raise safety concerns with respect to the endpoints investigated  
1270 (Table 7).

1271

1272 Overall, the Panel notes that most studies (12 studies) were assessed as having a high risk  
1273 of bias (tier 3), while the remaining studies (7 studies) were at moderate risk of bias (tier  
1274 2), and that methods and findings were often not adequately reported. Due to their  
1275 methodological limitations and the limited range of safety-related endpoints investigated,  
1276 the Panel considers that the studies provide only limited support for hazard identification  
1277 and cannot be used to identify a reference point.

1278

1279

1280 **Table 7:** Overview of pharmacological and mechanistic studies reporting on safety-related parameters, by species, dose and duration

Publication	RoB Tier	Species	Sex, n/group	Duration (days)	Test material <sup>a</sup>	Mode of administration	Doses (mg/kg bw per day) <sup>b</sup>	Safety-related findings
<b>Rats</b>								
<b>Gholampour and Keikha (2018)</b>	<b>3</b>	Wistar	M, n=7	14	BBR	Gavage	0, 10	No mortality No histopathological abnormalities in liver tissues No effect on AST, ALT, ALP, LDH in liver tissue homogenates; no effect on serum Bil, TP, Alb, Chol, TG, Glu; no effect on CrCl, fractional excretion of Na+
<b>Jia et al. (2020)</b>	<b>2</b>	Wistar	M, n=7	7	BBR Cl	Gavage	0, 25, 50	No mortality No histopathological abnormalities in colon tissues
<b>Hassanein et al. (2019)</b>	<b>3</b>	Albino	M, n=8	10	BBR	Gavage	0, 50	No mortality No histopathological abnormalities in kidneys tissues, no effect on serum urea, uric acid and Alb
<b>Hasanein et al. (2017)</b>	<b>2</b>	Wistar	M, n=7	56	BBR	Gavage	0, 50	No mortality, no effect on AST, ALP, ALT or serum Alb No histopathological abnormalities in liver
<b>Heidarian et al. (2014)</b>	<b>3</b>	Wistar	M, n=8	60	BBR Cl	Gavage	0, 90	No mortality No histopathological abnormalities in liver
<b>Adefegha et al. (2021)</b>	<b>2</b>	Wistar	M, n=7	14	BBR Cl	Gavage	0, 50, 100	No mortality No histopathological abnormalities in penile tissues (corpora cavernosum, corpora spongiosum and urethra) At mid dose: ↓serum testosterone, ↓luteinising hormone
<b>Maurya et al. (2016)</b>	<b>3</b>	Wistar	F, n=6	35	BBR Cl	Gavage	0, 100	No mortality ↑T4, ↑TSH, ↑AST, ↑ALT, ↑ALP, ↑TG, ↓LDL, ↓HDL, ↓Chol, No effects on haematological parameters
<b>Akhzari et al. (2019)</b>	<b>3</b>	SD	M, n=8	60	BBR Cl	Gavage	0, 100	No mortality No histopathological abnormalities in liver, no effect on AST or Alb
<b>Akhzari et al. (2024)</b>	<b>3</b>	SD	M, n=8	60	BBR Cl	Gavage	0, 100	No mortality No histopathological abnormalities in liver, no effect on ALT, AST
<b>Moghaddam et al. (2013)</b>	<b>3</b>	Wistar	M, n=6	84	BBR Cl	Gavage	0, 100	No mortality Reduced bw No effect on serum Glu

Publication	RoB Tier	Species	Sex, n/group	Duration (days)	Test material <sup>a</sup>	Mode of administration	Doses (mg/kg bw per day) <sup>b</sup>	Safety-related findings
<b>Rats</b>								
Zhang et al. (2012)	3	Wistar	M, n=10	126	BBR Cl	Gavage	0, 100	No mortality No effect on serum Glu, insulin
Alagal et al. (2023)	3	Wistar	M, n=8	21	BBR	NR	0, 200	No mortality No effect on kidney morphology, no effect on serum urea, creatinine, Alb and CrCl
Gu et al. (2012)	2	Wistar	M, n=9	154	BBR Cl	Gavage	0, 200	No mortality No histopathological abnormalities in liver No effect on serum Glu, TG, insulin
Hu et al. (2012)	2	SD	F, n=8	14	BBR Cl	Gavage	0, 380	No mortality ↓ serum TG
<b>Mice</b>								
Tian et al. (2019)	3	C57BL/6 mice	M, n= 6	5	BBR Cl	Dough pills	0, 100	No mortality or signs of toxicity No histopathological abnormalities in liver, no effect on serum AST, ALT
Chatuphonprasert et al. (2012)	3	DDY mice	M, n=7	14	BBR Cl	Gavage	0, 200	No mortality No effect on serum Glu
Guo et al. (2011)	3	C57BL/6 mice	M, n=6	14	BBR Cl	Gavage	10, 30, 100, 300	No mortality No histopathological abnormalities in liver, no effect on serum ALT
<b>Goats</b>								
Ghaviapanje et al. (2021a, 2021b, 2022a, 2022b)	2	Saanen Goats	F, n=6	42	BBR Cl	Gelatine capsules	0, 25, 50, 100	No effect on serum AST, ALT and ALP, serum Glu; ↑serum Alb

1281 Abbreviations: Alb, albumin concentration; ALP, alkaline phosphatase concentration; ALT, alanine aminotransferase concentration; AST, aspartate amino transferase concentration; BBR, berberine  
1282 (unspecified form); Bil, bilirubin; bw, body weight; Chol, cholesterol; Cl, chloride; CrCl, creatinine clearance; DDY, Deutschland, Denken, and Yoken; F, females; Glu, glucose; HDL, High-Density  
1283 Lipoprotein-cholesterol; LDL, Low-Density Lipoprotein-cholesterol; LDH, lactate dehydrogenase; M, males; n, number; Na, sodium; NR, not reported; RoB, Risk of Bias; SD, Sprague-Dawley; TG,  
1284 triglycerides; TP, total protein; TSH, Thyroid-Stimulating Hormone; T4, thyroxine.

1285 <sup>a</sup> BBR relates to berberine in an unspecified form.

1286 <sup>b</sup> Dose of the test material.

1287 3.1.5.2 Other protoberberines

1288 Subchronic toxicity studies

1289 Four sub-chronic toxicity studies in rats were retrieved through the systematic search of the  
 1290 literature, which evaluated coptisine, epiberberine and palmatine (Yi et al., 2013), berberrubine  
 1291 (Wang et al., 2020), jatrorrhizine (Wu et al., 2014) and columbamine (Wang et al., 2016) (Table 8).

1292 **Table 8:** General characteristics of the subchronic toxicity studies of protoberberine in rats

Author	RoB Tier	Species	Sex n/ group	Duration (days)	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>b</sup>
Wang et al. (2020)	2	SD rats	M, n=8	42	synthetic berberrubine (purity>98%)	Gavage	0, 25, 50, 100
Wu et al. (2014)	2	SD rats	F, M, n=5	90	jatrorrhizine (extracted from <i>Coptidis</i> rhizome, purity NR)	Feed	0, 70
Yi et al. (2013)	2	SD rats	F, M n=10	90	coptisine, epiberberine, palmatine (extracted from <i>Coptis</i> species; purity NR)	Unclear <sup>b</sup>	0, 156
Wang et al. (2016)	2	SD rats	F, M n =10	90	columbamine (extracted from <i>Coptidis</i> rhizome, purity NR)	Gavage	0, 154

1293 Abbreviations: BBR: berberine; bw: body weight; F, females; M, males; n, number; RoB, Risk of Bias; SD, Sprague Dawley; NR, not  
 1294 reported.

1295 <sup>b</sup> The authors report that "treatment group received a dose of 156 mg/kg at daily diet" and "the individual dose was adjusted for the  
 1296 body weight to maintain the target dose level for all rats".

1297 <sup>c</sup> Dose of the test material.

1298  
 1299 Wang et al. (2020) investigated the hepatotoxic effects of synthetic berberrubine (purity >98%) in  
 1300 male rats administered 0 (control vehicle, 0.7% CMC-Na solution), 25, 50, 100 mg/kg bw per day  
 1301 by gavage for 6 weeks. Serum ALT and AST concentrations were elevated in all dose groups after  
 1302 one week of treatment (statistically significant for AST), gradually decreasing between week 2 and  
 1303 4, and were comparable to controls by the end of the study. Liver histopathology performed in three  
 1304 animals per dose group showed inflammatory cell infiltration and a 'slight' oedema in the highest  
 1305 dose group (100 mg/kg bw per day). Results from liver histopathology were not reported for the  
 1306 lower dose groups. The Panel notes that histopathological findings in the high dose group may be  
 1307 indicative of liver toxicity. However, only three animals per group were examined for liver  
 1308 histopathology and the reporting of study findings was incomplete. Aside from hepatotoxicity, no  
 1309 other toxic effects were investigated. This study is at moderate risk of bias (tier 2).

1310 A 90-day study in rats evaluated the toxicity of coptisine, palmatine, and epiberberine at a dose of  
 1311 156 mg/kg bw per day (Yi et al., 2013). For all three alkaloids, no macroscopic toxicities or gross  
 1312 abnormalities were observed in any treatment group, and histopathological findings were  
 1313 comparable between treated and control animals. Differences in body weight were not statistically  
 1314 significant in any of the alkaloid groups of both sexes compared to the control. Relative weights of  
 1315 all organs investigated in the epiberberine and palmatine groups were not statistically significantly

1316 different compared to the control. For coptisine, kidney weight was statistically significantly higher  
1317 than the control. Gross necropsy and microscopic evaluation did not identify treatment-related  
1318 lesions. Total bilirubin concentrations were statistically significantly higher in the palmatine male  
1319 group and lower in the epiberberine male group compared to the control. No significant changes  
1320 were reported in other parameters (urinalysis and serum biochemistry). Standard haematological  
1321 examination was not performed. The Panel notes the isolated findings regarding kidney weight and  
1322 total bilirubin concentrations after administration of coptisine, palmatine, and epiberberine,  
1323 respectively. The methodological limitations of the study, i.e. limited number of animals per group,  
1324 single dose, and its poor reporting preclude an evaluation of the toxicological relevance of these  
1325 findings. The Panel also notes that the purity of the test material is unknown. The study is at  
1326 moderate risk of bias (tier 2).

1327 One 90-day study investigated the effect of a single dose of 70 mg/kg bw per day of jatrorrhizine  
1328 (unknown purity) or distilled water (control) in male and female rats (Wu et al., 2014). The mode  
1329 of administration was not described. No histopathological findings were reported for the selected  
1330 organs (heart, liver, spleen, lung and kidney), but data were not shown. Differences in body and  
1331 relative organ weights (heart, liver, spleen, lung and kidney), and clinical biochemistry (serum and  
1332 urine) between intervention and control groups were not statistically significant. Standard  
1333 haematological examination was not reported/performed. The Panel notes that this study does not  
1334 raise a safety concern in rats regarding an adverse effect of jatrorrhizine at a dose of 70 mg/kg bw  
1335 per day for 90 days on the endpoints investigated. However, main methodological limitations of the  
1336 study included the unknown purity of the test material, the limited number of animals per group,  
1337 the limited number of organs and endpoints investigated, and incomplete reporting. The study is at  
1338 moderate risk of bias (tier 2).

1339 One 90-day study investigated the effect of a single dose of 154 mg/kg bw per day columbamine  
1340 (purity >98.0%) or 0.9% saline (control) in male and female rats (Wang et al., 2016). The mode of  
1341 administration was not described. No significant changes were identified in body and relative weight  
1342 of heart, liver, spleen, lung, kidney, stomach and brain. Changes in serum clinical biochemistry were  
1343 not significant. Standard haematological examination and histopathological findings were not  
1344 reported. The Panel notes that this study does not raise safety concerns in rats with respect to an  
1345 adverse effect of columbamine at a dose of 154 mg/kg bw per day for 90 days on the endpoints  
1346 investigated. However, main methodological limitations included the unknown purity of the test  
1347 material, the limited number of organs and endpoints investigated, and incomplete reporting. The  
1348 study is at moderate risk of bias (tier 2).

### 1349 3.1.6 Developmental toxicity

#### 1350 3.1.6.1 Berberine

1351 One publication reporting on four different developmental toxicity studies in mice and rats  
1352 commissioned by the US NTP<sup>24</sup> was retrieved from the systematic review of the literature (Jahnke  
1353 et al., 2006) (Table 9).

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<sup>24</sup> NTP. Berberine chloride dihydrate (5956-60-5). Chemical Effects in Biological Systems (CEBS). Research Triangle Park, NC (USA): National Toxicology Program (NTP). Accessed 2025-12-08. DOI: <https://doi.org/10.22427/NTP-DATA-DTXSID5030820>.

1354 **Table 9:** General characteristics of developmental toxicity studies of berberine in rats and mice

Publication	Tier	Species	Sex n/group	Duration (days)	Test material <sup>a</sup>	Mode of administration	Doses (mg/kg bw per day) <sup>b</sup>
<b>Jahnke et al. (2006)</b>	<b>2</b>	SD rats	F, n=25 F, n=25	GD 6-20 GD 6-19	BBR Cl dihydrate (unspecified source; purity: 87.7% expressed as berberine Cl)	Feed Gavage	0, 282, 531, 1313 <sup>c</sup> 0, 1000 <sup>d</sup>
		Swiss Albino (CD-1) mice	F, n=25 F, n=25	GD 6-17 GD 6-16		Feed Gavage	0, 569, 841, 1155 <sup>e</sup> 0, 1000 <sup>d</sup>

1355 Abbreviations: BBR, berberine; bw, body weight; CD, Charles River; Cl, chloride; F, females; GD, gestational day; n, number; SD, Sprague  
1356 Dawley.

1357 <sup>a</sup> form as reported in the publication.

1358 <sup>b</sup> dose of test material.

1359 <sup>c</sup> equivalent to 223, 420, 1040 mg/kg bw per day berberine chloride.

1360 <sup>d</sup> equivalent to 792 mg/kg bw per day berberine chloride.

1361 <sup>e</sup> equivalent to 450, 666, 1013 mg/kg bw per day berberine chloride.

1362

1363 In two feeding studies, SD rats and Swiss Albino (CD-1) mice were exposed to multiple doses of  
1364 berberine chloride dihydrate (purity 87.7% expressed as berberine chloride). Due to scattering of  
1365 feed in the high dose groups, a gavage study was subsequently conducted in both species, at an  
1366 equivalent dose (1000 mg/kg bw per day) vs control vehicle (0.5% methylcellulose solution).

1367 In the feeding study, rat dams received 0, 282, 531, 1313 mg/kg bw per day berberine chloride  
1368 dihydrate from gestational day (GD) 6 to GD 20. Regarding maternal toxicity outcomes, a dose-  
1369 dependent decrease in body weight gain during gestation was observed, with statistically significant  
1370 differences at doses of 531 and 1313 mg/kg bw per day. Body weight gain corrected for uterine  
1371 weight decreased by 9% at 531 mg/kg bw per day and by 52% at 1313 mg/kg bw per day. Unusual  
1372 variability in maternal feed intake was observed in the highest dose group, which the authors  
1373 attributed to palatability issues. Overall, the relative feed consumption during the treatment period  
1374 was higher in the high-dose group than in the other groups. Relative liver weight in the group  
1375 administered 1313 mg/kg bw per day was 5% lower than control. In the gavage study, a significant  
1376 reduction in maternal body weight gain corrected for uterine weight and a significant reduction in  
1377 relative liver weight were also observed; feed intake was lower in the treatment group vs controls.  
1378 Regarding foetal outcomes, average foetal body weight per litter was significantly reduced at 1313  
1379 mg/kg bw per day in both sexes (-6%) in the feeding study, while no differences were observed  
1380 when the dose of 1000 mg/kg bw was administered by gavage. There were no between-group  
1381 differences in external, visceral, or skeletal malformations in either study. The authors identified a  
1382 maternal no observed adverse effect level (NOAEL) of 282 mg/kg bw per day for berberine chloride  
1383 dihydrate (223 mg/kg bw per day berberine chloride) (feeding study), and a developmental NOAEL  
1384 of 1000 mg/kg bw per day for berberine chloride dihydrate (792 mg/kg bw per day berberine  
1385 chloride) (gavage study). The Panel concurs with the derivation of these NOAELs. The study is at  
1386 moderate risk of bias (tier 2).

1387 In the feeding study in mice, dams received 0, 569, 841, 1155 mg/kg bw per day berberine from  
1388 GD 6 to GD 17. Feeding scattering was observed in the high dose group. No between-group  
1389 differences in maternal weight gain were detected. Regarding maternal toxicity outcomes, a linear  
1390 trend for increased relative liver weights was detected, but no significant between-group differences  
1391 were found. In the gavage study, four animals were removed from the treatment group because of

1392 misdirected dose, and one from the control group due to a preexisting condition. Among the  
1393 remaining animals, maternal mortality/moribundity was observed in 7/21 of the treated mice (reason  
1394 unexplained; histopathology was not performed). Increased water consumption was observed  
1395 during the treatment period in the feeding study (dose-dependent, with statistically significant  
1396 between-group differences at 841 and 1155 mg/kg bw per day) and the gavage study. Regarding  
1397 foetal outcomes, no between-group differences in foetal weight were found in the feeding study,  
1398 while a statistically lower average foetal body weight/litter (5% in males, 6% in females) was  
1399 observed in the gavage study, which was considered biologically relevant by the study authors  
1400 (variation of 1.5–2.7% in historical controls). A non-statistically significant dose-related increase in  
1401 the number of malformations was observed. The incidence of cleft palate exhibited a dose-  
1402 dependent increase (not statistically significant), with 0 case/291 fetuses (0/23 litters) in the control  
1403 group, 1 case/294 fetuses (1/22 litters) in the low-dose group, 2 cases/287 fetuses (1/23 litters)  
1404 in the mid-dose group, and 6 cases/261 fetuses (2/21 litters) in the high-dose group. Based on  
1405 comparisons with historical control data, the number of cleft palates per litter were considered non-  
1406 treatment-related in the low dose, an equivocal effect in the mid dose and higher than expected in  
1407 the highest dose group. There was no effect on the incidence of cleft palate in the gavage study.  
1408 The authors identified a maternal NOAEL of 569 mg/kg bw per day berberine chloride dihydrate  
1409 (450 mg/kg bw per day berberine chloride) (feeding study) and a developmental NOAEL of 841  
1410 mg/kg bw per day (666 mg/kg bw per day berberine chloride) (feeding study). The Panel concurs  
1411 with the derivation of these NOAELs. For the gavage study, the Panel notes that 11 out of the 25  
1412 animals were not available for examination due to misdirected dose (n=4) or death or moribundity  
1413 for an unexplained reason (n=7). The Panel also notes that the study started at GD 6, thus findings  
1414 in the early pregnancy state could not be assessed. The study is at moderate risk of bias (tier 2).

#### 1415 3.1.6.2 Other protoberberines

1416 No eligible studies were retrieved.

#### 1417 3.1.7 Human randomised controlled trials

1418 Twenty-eight RCTs in adult individuals evaluating berberine supplementation and assessing the  
1419 occurrence of adverse effects were identified. Twenty-six were parallel-group, placebo-controlled  
1420 trials including 3628 participants (1818 allocated to berberine and 1810 to placebo), and two were  
1421 crossover trials including a total of 20 participants. Overall, 3648 unique participants were included  
1422 (Appendix FAppendix F).

1423 Studies were conducted in Bangladesh (1 trial), China (19 trials), India (1 trial), Iran (2 trials), Italy  
1424 (2 trial), Mexico (2 trials), and USA (1 trial). Most trials recruited participants based on specific  
1425 disease conditions (i.e. cancer (3 trials), colorectal adenoma (2 trials), cardiovascular disease (CVD)  
1426 (2 trials), infectious diarrhoea (2 trials), diarrhoea-predominant irritable bowel syndrome (1 trial),  
1427 ulcerative colitis (1 trial), non-alcoholic fatty liver disease (NAFLD) (1 trial), polycystic ovary  
1428 syndrome (PCOS) (1 trial), schizophrenia (3 trials), type 2 diabetes mellitus (T2DM) (3 trials),  
1429 COVID-19 (1 trial), human immunodeficiency virus (HIV) infection (1 trial) and risk factors for CVD  
1430 or T2DM (7 trials), while 2 trials recruited apparently healthy individuals. Two publications included  
1431 more than one population (Appendix F). The study size ranged between 5 and 1108 participants.

1432 Berberine formulations were described as berberine, berberine hydrochloride or berberine sulphate,  
1433 with the source material reported in 3 trials (two used berberine extracted from *Berberis aristata*

1434 root and one trial isolate from *Coptidis* rhizome). The purity was either not reported (23 trials) or  
1435  $\geq 97\%$  (5 trials). The daily doses of berberine ranged between 376 mg and 2000 mg, median 1000  
1436 mg. The study duration ranged between 1 day and 2 years, median 10 weeks.

1437 Study characteristics and results are provided in Appendix F. Relevant findings are summarised  
1438 below.

1439 Gastrointestinal symptoms, including constipation, diarrhoea, nausea, abdominal pain/discomfort,  
1440 were more frequently reported in participants receiving the berberine supplementation compared to  
1441 the placebo (An et al., 2014; Chen et al., 2015; Chen et al., 2020; Derosa et al., 2013; Kong et al.,  
1442 2004; Omidvar Tehrani et al., 2023; Panigrahi & Mohanty, 2023; Pu et al., 2023; Rabbani et al.,  
1443 1987; Ruiz-Herrera et al., 2023; Xu et al., 2020; Yan et al., 2015; Zeng et al., 2003; Zhang et al.,  
1444 2022a; Zhang et al., 2020; Zhang et al., 2008). The daily dose of berberine ranged between 400  
1445 mg and 2000 mg in these trials.

1446 In one RCT in individuals with ulcerative colitis, who were randomised to a daily dose of 900 mg  
1447 berberine hydrochloride (purity  $>98\%$ , isolated from *Coptidis* rhizome; n=12) or placebo (n=4) for  
1448 three months, one participant in the treatment group was found to have a clinically meaningful  
1449 elevation of serum AST and ALT at month three, which was resolved at 1-month post-treatment.  
1450 There was no case in the placebo group. The authors judged the event as probably related to the  
1451 consumption of the supplement (Xu et al., 2020). In another RCT in individuals with colorectal  
1452 adenomas randomly assigned to receive a daily dose of 600 mg berberine hydrochloride (purity  
1453  $\geq 97\%$ , source unspecified; n=446) or placebo (n=478) for two years, one participant in the  
1454 treatment group had raised serum ALT (3  $\times$  the normal level). ALT values went back to normal after  
1455 2 weeks. The article does not report at which follow up visit this was observed and whether the  
1456 participant stopped the supplementation. No case was reported in the placebo group (Chen et al.,  
1457 2020).

1458 In another RCT, patients undergoing coronary artery bypass grafting were randomised to receive  
1459 1200 mg/day berberine (formulation NR; n=100) or a placebo (n=100) for 7 days before surgery.  
1460 In the berberine group, mild rash and mild constipation were reported in two and four patients,  
1461 respectively. Three episodes of major postoperative bleeding occurred in the berberine group,  
1462 compared to none in the placebo group. According to the authors, these events were not attributed  
1463 to the berberine treatment (Zhang et al., 2022a). The Panel notes that berberine has been found to  
1464 exhibit antiplatelet effects in vitro and in animal models (Wang et al., 2021). Recent experimental  
1465 data also suggest anticoagulant activity (Wang et al., 2018). Such effects were observed at high  
1466 doses and clinical relevance in humans is unknown. Postoperative bleeding occurs in 2-5% of cases  
1467 after coronary artery bypass grafting (Dimberg et al., 2018; Heimisdottir et al., 2022; Mehta et al.,  
1468 2009).

1469 The Panel notes two cases of elevated transaminases associated with berberine supplementation.  
1470 In one case the relationship with the supplement was considered probable by the study authors,  
1471 while in the second case it cannot be evaluated. The Panel notes that transient gastrointestinal  
1472 symptoms of constipation, diarrhoea, nausea, abdominal pain/discomfort were more frequently  
1473 reported in participants receiving the berberine supplementation (daily dose between 400 mg and  
1474 1500 mg) compared to the placebo. A dose below which gastrointestinal (GI) symptoms do not  
1475 occur cannot be established.

### 1476 3.1.8 Human case reports

1477 Three cases of adverse events suspected to be related to the consumption of berberine-containing  
1478 food supplements were retrieved from the literature (reported in four publications: (Cannillo et al.,  
1479 2013; Déléaval et al., 2022; Florek et al., 2018; Labadie et al., 2018) (Appendix G).

1480 A 56-year-old woman was admitted to the hospital for syncope and episodes of prolonged dizziness  
1481 in the past month. Her medical history was unremarkable. She had been taking hemp oil as a food  
1482 supplement (containing cannabidiol and cannabigerol; 6 times the recommended dose) for 4 months  
1483 and had started a berberine-containing supplement (formulation NR; 250 mg/day) in the last 6  
1484 weeks. Hypotension, together with long QT syndrome and sinus bradycardia at electrocardiogram  
1485 (ECG) were noted, which resolved within five days after cessation of both supplements (Déléaval et  
1486 al., 2022).

1487 A 53-year-old overweight man practicing regular physical exercise who started taking "berberine"  
1488 (formulation and dose NR) 6 days earlier was referred to the hospital for fatigue and dyspnoea upon  
1489 exertion. The patient did not take any medication, and his medical history was unremarkable. An  
1490 ECG showed sinus bradycardia, first-degree atrioventricular (AV) block, and competitive junctional  
1491 rhythm. An ergometric stress test was performed 24h after stopping "berberine", which confirmed  
1492 cardiac rhythm alterations and loss of AV synchronization during stress. The symptoms resolved  
1493 after "berberine" consumption was discontinued for 10 days and the ergometric stress test was  
1494 normal, although sinus bradycardia and first-degree AV block persisted in the ECG at rest and the  
1495 Holter ECG was compatible with a latent hypervagotonic state (Cannillo et al., 2013).

1496 A case of symmetrical drug-related intertriginous and flexural exanthema (SDRIFE) in a 54-year-old  
1497 man was attributed to the consumption of a berberine supplement (berberine HCl extracted from *B.*  
1498 *aristata* (unknown purity) at a dose of 1200 mg/day) that started two months prior to the event.  
1499 There was evidence of perivascular oedema with dermal lymphocytic and eosinophilic infiltration  
1500 upon biopsy. The patient had been treated for hypertension with aliskiren for 7 years. Contact with  
1501 known allergens was excluded. The rash resolved within two weeks after discontinuation of the  
1502 supplement. The authors proposed that the triggering agent could be berberine or tartrazine used  
1503 as a food colour in the supplement (Florek et al., 2018; Labadie et al., 2018). The Panel notes that  
1504 rash is one of the potential adverse effects of aliskiren and an interaction with the drug might also  
1505 be possible.

1506 The Panel notes two cases of sinus bradycardia and one case of SDRIFE associated with the  
1507 consumption of berberine supplements. No standardised causality assessment was performed. No  
1508 other cases of cardiac side effects or SDRIFE were found in the literature. In the three cases, there  
1509 is high uncertainty regarding the identification of the causing factor, due to the poor description of  
1510 the supplements or concurrent ingestion of other substances or ingredients. The Panel considers  
1511 that these case reports do not allow to conclude on hazards associated with intake of berberine-  
1512 containing food supplements.

### 1513 3.1.9 Interactions with medicinal products

1514 A total of 16 publications were retrieved which investigated the possible interactions between  
1515 berberine and medicinal products in humans (four clinical studies and two case reports) or animals  
1516 (12 studies).

### 1517 3.1.9.1 Human clinical studies

1518 In a crossover study (Guo et al., 2012), the interaction between berberine and CYP enzymes was  
1519 investigated in 17 healthy male Chinese volunteers. After 14-day supplementation with berberine  
1520 (300 mg three times daily; form and purity NR) or a placebo, midazolam, omeprazole,  
1521 dextromethorphan, losartan, and caffeine were administered as probe drugs to evaluate the  
1522 activities of CYP3A4, 2C19, 2D6, 2C9, and CYP1A2, respectively. Statistically significant increases in  
1523 midazolam systemic exposure<sup>25</sup> (AUC<sub>0-12</sub> +37%), 0-8 h dextromethorphan/dextrorphan urinary ratio  
1524 (9-fold), and losartan/E-3174 plasma ratio (2-fold) (decrease in CYP3A4, CYP2D6 and CYP2C9  
1525 activity) were found upon berberine administration compared to placebo. Pharmacokinetic  
1526 parameters for omeprazole or caffeine were not affected. The Panel notes that no information about  
1527 the choice of timing of drug administration and sample collection were provided and the impact of  
1528 pharmacogenetics (ethnicity, gender) was not assessed.

1529 In renal transplant patients from China (n=52 each group), co-administration of berberine chloride  
1530 (200 mg, three times daily; purity NR) with ciclosporine A (CsA, 2-6 mg/kg bw per day) increased  
1531 steady-state CsA blood concentrations (331.9±139.8 µg/L vs 256.6±119.5 µg/L, p<0.05) and  
1532 altered its pharmacokinetics (AUC<sub>0-12</sub> +34.5%, t<sub>max</sub> +1.7h, t<sub>1/2</sub> +2.7h, all p<0.05) (Wu et al., 2005).  
1533 In 6 further patients (3 female, 3 male, pre-treated with CsA), co-administration of 300 mg dose of  
1534 berberine (three times daily; form and purity NR) with a CsA dose of 3 mg/kg bw (twice daily) for  
1535 12 days increased CsA AUC<sub>0-12</sub> by approximately 19% (p<0.05), while t<sub>1/2</sub> and t<sub>max</sub> were unaffected  
1536 (Wu et al., 2005). In 2 studies with 12 healthy male volunteers (6 in each study), the effects of  
1537 repeated berberine administration (300 mg, twice daily for 10 days) on CsA systemic exposure were  
1538 assessed at a higher CsA dose (6 mg/kg bw) and a lower CsA dose (3 mg/kg bw) (Xin et al., 2006).  
1539 While no influence on CsA systemic exposure was observed in the higher-dose group, a significant  
1540 increase in the mean CsA AUC was found in the lower-dose group. As several methodological  
1541 changes were made to the study design between the two groups, no definitive overall conclusion  
1542 can be drawn. The authors of these studies proposed that the interaction between berberine and  
1543 CsA may involve CYP3A4, the main enzyme involved in CsA metabolism, and/or p-glycoprotein, its  
1544 main transporter, but further investigation is needed to elucidate the mechanisms.

1545 In an open-label, parallel RCT in healthy male and female adults (Li et al., 2019), no evidence of  
1546 pharmacokinetic interactions was found upon co-administration of berberine (300 mg three times a  
1547 day; form and purity NR) and simvastatin (40 mg/day) or fenofibrate (200 mg/day) (each group  
1548 n=12) over a period of 7 days. The Panel notes that simvastatin is primarily metabolised by CYP3A4  
1549 and CYP2C6 (Talreja & Cassagnol, 2025), while CYP is not involved in the metabolism of fenofibrate  
1550 (Caldwell, 2008).

### 1551 3.1.9.2 Human case reports

1552 In a 16-year-old patient with idiopathic nephrotic syndrome, co-administration of berberine (200  
1553 mg, three times daily; form and purity NR) with tacrolimus (6.5 mg, twice daily) led to a rapid and  
1554 significant increase in serum tacrolimus concentrations (from 8 to 22 ng/mL), exceeding the  
1555 therapeutic range (Hou et al., 2013). This was accompanied by a rise in serum creatinine  
1556 concentrations (from 62 to 109 µmol/L). Dose reduction of tacrolimus to 3 mg/day normalized both

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<sup>25</sup> In the context of interactions with medicinal products, the term 'systemic exposure' refers to the concentration of active substances and relevant metabolites in the systemic circulation over time, typically quantified by pharmacokinetic parameters.

1557 parameters. Genetic phenotyping indicated that CYP3A4 was the primary enzyme responsible for  
1558 tacrolimus metabolism in this patient. The authors proposed that CYP3A4 inhibition by berberine  
1559 could have led to increased tacrolimus systemic exposure.

1560 A 68-year-old man, treated with rivaroxaban for atrial fibrillation for several years, presented with  
1561 jaundice and vomiting three weeks after commencing berberine 500 mg twice daily (form and purity  
1562 not reported) (Bonnichsen et al., 2022). Clinical investigations confirmed acute liver injury (ALI),  
1563 with highly elevated transaminases (AST 4789 U/L, ALT 5858 U/L). The international normalised  
1564 ratio (INR) was greater than 12.0 indicating impaired coagulation function. Rivaroxaban trough  
1565 concentration was found to be at 284 ng/mL (reference range: 20–150 ng/mL), despite his last dose  
1566 being 4 days prior. Other common aetiologies of ALI, including viral, autoimmune, genetic, and  
1567 paracetamol, were excluded. Following supportive care, the patient's liver biochemistry and  
1568 coagulation parameters normalised within 6 months. The authors proposed that berberine may have  
1569 increased rivaroxaban systemic exposure by inhibiting P-gp and CYP3A4, thus reducing rivaroxaban  
1570 metabolism.

### 1571 3.1.9.3 Animal studies

1572 In animal models, berberine was found to induce CYP3A4, decreasing the systemic exposure to  
1573 lovastatin in rats (Cui et al., 2016), while also inhibiting CYP2C9/CYP3A4, increasing the systemic  
1574 exposure of gliclazide in rabbits (Bokka et al., 2023), and amlodipine, losartan and midazolam in  
1575 rats (Li et al., 2016c; Liu et al., 2018). Two studies found no effect on CYP3A-mediated metabolism  
1576 in rats when using simvastatin (Liu et al., 2015) or carbamazepine (Qiu et al., 2009) as probe drugs.

1577 Transporter-mediated effects of berberine have been reported, with some studies reporting an  
1578 inhibition of intestinal P-glycoprotein (P-gp) leading to increased systemic exposure of drugs such  
1579 as CsA, ketoconazole, rhodamine 123, and digoxin (Qiu et al., 2009; Zhou et al., 2012). Inconsistent  
1580 effects of berberine on other transporters, such as OCT1 and OCT2/MATE1, were found, with reports  
1581 of both inhibition and no effect on metformin pharmacokinetics, depending on the testing conditions  
1582 (Lyu et al., 2019; Shi et al., 2019).

1583 Other studies in rats reported decreased systemic exposure to ciprofloxacin after pre-treatment with  
1584 berberine (Hwang et al., 2012), whereas co-administration of berberine increased systemic exposure  
1585 to fenofibric acid and irbesartan (Li et al., 2016b; Zhou et al., 2021).

### 1586 3.1.9.4 Conclusions on interactions with medicinal products

1587 The Panel notes that available data on potential interactions between berberine and medicinal  
1588 products are limited due to the heterogeneous designs of the available studies and the lack of studies  
1589 addressing pharmacogenetics (ethnicity, gender). One interaction study indicates that berberine  
1590 may inhibit CYP2D6, CYP2C9, and CYP3A4 activities. Two case reports also point to a potential  
1591 inhibition of CYP3A4. Animal data regarding berberine interaction with CYP enzymes and drug  
1592 transporters, such as P-gp, are limited and inconsistent. The Panel notes that there are substantial  
1593 uncertainties in extrapolating animal data to humans due to potential species differences in drug  
1594 metabolic pathways. The Panel considers that, despite their limitations, available data indicate  
1595 possible inhibition of CYP3A4 and possibly CYP2D6 and CYP2C9 by berberine.

### 1596 3.1.10 Concluding remarks

1597 In mammals, although berberine has been the most widely studied compound in terms of ADME,  
1598 available experimental data on other protoberberines suggest overlapping absorption mechanisms,  
1599 metabolism in the GI tract, and metabolic and elimination pathways. Therefore, interactions between  
1600 berberine and co-occurring protoberberines are complex, involving both external exposure (e.g. co-  
1601 ingestion through plant preparations) and systemic exposure (e.g. bioconversions and interactions  
1602 within the body). The Panel considers that a holistic risk assessment approach considering berberine  
1603 and other co-occurring protoberberines is necessary.

1604 Consistent in vitro findings, supported by mechanistic data, raise a concern regarding the genotoxic  
1605 potential of berberine particularly with respect to chromosomal damage and mutagenicity. Data on  
1606 the genotoxic potential of berberine in vivo are inconclusive. The genotoxicity of other  
1607 protoberberines has been poorly studied. In silico modelling structural similarity and genotoxicity  
1608 predictions was applied to berberine and other protoberberine alkaloids. Regarding mutagenicity,  
1609 QSAR predictions identified common structural alerts across the protoberberine family. Mutagenicity  
1610 was predicted by the VEGA models for Ames test for all protoberberines included in the analysis,  
1611 with good model reliability. The Panel notes that these results support grouping of berberine and  
1612 protoberberines together on the grounds of chemical similarity and common mutagenicity alerts,  
1613 that could also form the basis for application of read-across (EFSA Scientific Committee et al., 2025).  
1614 Regarding chromosomal damage, the uncertainty was high for the in vitro MN predictions obtained  
1615 from the VEGA models and conclusions could not be reached for this endpoint. Regarding the in  
1616 vivo MN predictions, the VEGA models did not raise concerns for genotoxicity, however, with  
1617 moderate model reliability. Overall, the Panel considers that there is a genotoxic concern regarding  
1618 the consumption of berberine and other protoberberines.

1619 Maternal and developmental toxicity of berberine was investigated in two multiple dose studies, in  
1620 rats and mice. In rats, decreased maternal body and liver weight (NOAEL = 223 mg/kg bw per day  
1621 berberine chloride) and decreased foetal body weights (NOAEL = 792 mg/kg bw per day berberine  
1622 chloride) were reported. In mice, increased water intake was observed in the dams (NOAEL = 450  
1623 mg/kg bw per day berberine chloride) and an increase in the percentage of fetuses with  
1624 malformations (cleft palate) was found (NOAEL = 666 mg/kg bw per day berberine chloride). The  
1625 Panel, however, notes that early pregnancy state was not addressed by both studies.

1626 No effects on the investigated endpoints were detected in rats at doses of 156 mg/kg bw per day  
1627 of berberine, 70 mg/kg bw per day of jatrorrhizine, or 154 mg/kg bw per day of columbamine for  
1628 90 days. For berberrubine, the Panel notes histopathological findings that may be indicative of liver  
1629 toxicity at a dose of 100 mg/kg bw per day administered for 42 days. Following administration of  
1630 156 mg/kg bw per day for 90 days, increased kidney weight was observed after coptisine  
1631 administration, increased total bilirubin after palmatine administration and decreased total bilirubin  
1632 after epiberberine administration. The studies limitations and poor reporting preclude the evaluation  
1633 of the toxicological relevance of these findings. Overall, the Panel notes that the available 90-day  
1634 toxicity studies on the respective protoberberines had several methodological and reporting  
1635 limitations and were considered at moderate to high risk of bias. Therefore, the Panel considers that  
1636 these studies are insufficient for hazard identification and characterisation.

1637 No safety concerns were identified in the other mammalian studies on berberine, which were  
 1638 primarily designed to study beneficial/pharmacological effects or underlying mechanisms. The Panel,  
 1639 however, notes that these studies provided limited information for hazard identification and  
 1640 characterisation given the restricted number of safety-related endpoints investigated.

1641 Three case reports reporting adverse effects in individuals consuming berberine supplements have  
 1642 been found in the literature, however a causal link with berberine intake cannot be ascertained. In  
 1643 available clinical trials, gastrointestinal symptoms were the most frequent adverse events associated  
 1644 with berberine supplementation. Two trial participants receiving berberine supplementation were  
 1645 found to have elevated transaminases. There is no published case report of clinically apparent liver  
 1646 injury associated with the consumption of berberine supplements. The Panel considers that the  
 1647 evidence is not sufficient to establish or refute that the increase in transaminase concentrations  
 1648 observed in these two individuals is linked to berberine consumption. The Panel notes that clinical  
 1649 trials investigated a limited number of safety related parameters and cannot be used to establish  
 1650 safe doses of berberine supplementation.

1651 Available data indicate that berberine has the potential to inhibit CYP3A4 and possibly CYP2D6 and  
 1652 CYP2C9 and thus has potential to interact with various medicinal products.

### 1653 3.2 *Berberis aquifolium* (root)

#### 1654 3.2.1 Characterisation of the plant

1655 *Berberis aquifolium* Pursh (*B. aquifolium*) is an evergreen perennial shrub that belongs to the  
 1656 Berberidaceae family native to North America. Its common names in English are Oregon grape and  
 1657 holly-leaved barberry, the list of synonyms is provided in Appendix A. Berberine and palmatine are  
 1658 present in *B. aquifolium* root with contents <1% and <0.1% w/w DW, respectively. Available  
 1659 analytical data regarding protoberberine alkaloids and other alkaloids are reported in Table 10.  
 1660 Details of the analytical studies are provided in Annex A.

1661 **Table 10:** Alkaloids content in *B. aquifolium* root

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	0.0008–0.705	(Tuzimski et al., 2023; Uzaşçı & Erim, 2014)
<b>Palmatine</b>	0.030–0.063	(Tuzimski et al., 2023)
<b>Other alkaloids</b>		
<b>Chelerythrine</b>	ND	(Tuzimski et al., 2023)
<b>Chelidonine</b>	ND	(Tuzimski et al., 2023)
<b>Magnoflorine</b>	ND	(Tuzimski et al., 2023)
<b>Protopine</b>	ND	(Tuzimski et al., 2023)
<b>Sanguinarine</b>	ND	(Tuzimski et al., 2023)

1662 Abbreviations: DW, dry weight; ND, not detected; w, weight.

#### 1663 3.2.2 Genotoxicity

1664 No eligible genotoxicity studies were retrieved.

1665 **3.2.3 General toxicity**

1666 No eligible toxicity studies in mammalian species were retrieved.

1667 **3.3 *Berberis aristata* (root, bark)**

1668 **3.3.1 Characterisation of the plant**

1669 *B. aristata* is a woody plant, with a yellow to brown bark covered with three-branched thorns, that  
 1670 belongs to the Berberidaceae family. It is native to temperate and tropical Asia. Its common names  
 1671 in English are Indian barberry or tree turmeric. The list of synonyms is provided in Appendix A.

1672 In the root of *B. aristata*, berberine, palmatine and jatrorrhizine are the predominant protoberberine  
 1673 alkaloids, whereas columbamine was quantified at <0.01% w/w dry weight. Other alkaloids  
 1674 comprise magnoflorine, with isocorydine, glaucine, berbamine and tetrahydroberberine (canadine)  
 1675 present in lower amounts. In the bark of *B. aristata*, small amount of berberine (<0.01% w/w DW)  
 1676 was reported, while several other protoberberine alkaloids – including berberastine, berberrubine,  
 1677 demethyleneberberine, corysamine, fissisaine, stephabine, thalidastine and thalifendine – were also  
 1678 detected. Available analytical data regarding protoberberine alkaloids and other alkaloids are  
 1679 reported in Table 11. Details of the analytical studies are provided in Annex A.

1680 **Table 11:** Alkaloids content in root and bark of *B. aristata*

Alkaloid	Content range (% w/w DW)	Plant part	Reference
<b>Protoberberine alkaloids</b>			
<b>Berberine</b>	0.02–12.18	Root	(Basera et al., 2021; Singh et al., 2015; Uzaşçi & Erim, 2014)
	0.003	Bark	(Khan et al., 2020)
<b>Jatrorrhizine</b>	0.005–1.44	Root	(Basera et al., 2021; Singh et al., 2015)
	NQ	Bark	Unpublished data (Biagi, 2023; SISTE, 2023)
<b>Columbamine</b>	0.001–0.003	Root	(Basera et al., 2021)
<b>Palmatine</b>	0.006–7.89	Root	(Basera et al., 2021; Singh et al., 2015)
	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Berberastine</b>	NQ	Bark	Unpublished data (Biagi, 2023)
	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Berberrubine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
	NQ	Bark	(Rigillo et al., 2024); Unpublished data (Biagi, 2023)
<b>Demethyleneberberine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Corysamine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Fissisaine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Stephabine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Thalidastine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Thalifendine</b>	NQ	Bark	Unpublished data (SISTE, 2023)
<b>Other alkaloids</b>			
<b>Magnoflorine</b>	0.006–1.64	Root	(Basera et al., 2021) (Singh et al., 2015)

Alkaloid	Content range (% w/w DW)	Plant part	Reference
Isocorydine	0.025–0.030	Root	(Singh et al., 2015)
Glaucine	ND–0.006	Root	(Singh et al., 2015)
Tetrahydroberberine	ND–0.0003	Root	(Singh et al., 2015)
Berberamine	0.00004–0.004	Root	(Basera et al., 2021)

1681 Abbreviations: DW, dry weight; ND, not detected; NQ, detected but not quantified in the plant material; w, weight.

### 1682 3.3.2 Genotoxicity

1683 One bacterial reverse mutation assay on *B. aristata* (Sood et al., 2019) was retrieved from the  
 1684 literature. *S. typhimurium* strain MTCC 1251 (not listed as recommended strain in OECD TG 471)  
 1685 was exposed to an aqueous extract of the root bark of *B. aristata* (berberine content not reported)  
 1686 at unknown concentration(s). The authors reported that the plant extract did not cause any  
 1687 mutagenic effect. However, in the absence of information regarding the concentrations tested, and  
 1688 of a negative control, the Panel considers the results as inconclusive. The study was scored as not  
 1689 reliable (Klimisch score 3), and the relevance of study results as low.

### 1690 3.3.3 Acute toxicity

1691 Two publications, which reported on single dose administration of preparations of roots or bark of  
 1692 *B. aristata* in mammalian species, were retrieved from the systematic search of the literature  
 1693 (Section 2.3). One publication (Akhtar et al., 2008) did not investigate acute toxicity and is not  
 1694 further considered. Another study evaluated the acute toxicity of ethanolic and aqueous extracts of  
 1695 *B. aristata* in mice (Joshi et al., 2011). The LD50 of berberine derived from this study is reported in  
 1696 Table 12.

1697 **Table 12:** LD50 for *B. aristata* in mice

Publication	Species	Sex n/group	Test material	Exposure route	Doses (mg/kg bw per day) <sup>a</sup>	LD50 mg/kg bw	Comment
Joshi et al. (2011)	Swiss albino mice	NR	<i>B. aristata</i> , stem bark ethanolic and aqueous extract	Gavage	2000, 5000	>5000	No mortality

1698 Abbreviations: NR, not reported; bw, body weight; LD50, median lethal dose; n, number.  
 1699 <sup>a</sup> Dose of test material.

### 1700 3.3.4 General toxicity

1701 No eligible toxicity studies in mammalian species were retrieved.

## 1702 3.4 *Berberis vulgaris* (root, bark)

### 1703 3.4.1 Characterisation of the plant

1704 *Berberis vulgaris* L. (*B. vulgaris*) is a deciduous shrub that belongs to the Berberidaceae family,  
 1705 native to central and southern Europe, southern England, northwest Africa and western Asia. Its  
 1706 common name in English is common barberry. The list of synonyms is provided in Appendix A.

1707 Berberine is the most abundant protoberberine alkaloid in *B. vulgaris* root and bark, whereas  
 1708 jatrorrhizine and palmatine are present in lower amounts. Thalifendine, epiberberine and  
 1709 demethyleneberberine were also detected in *B. vulgaris* root, whereas berberrubine has also been  
 1710 detected in the bark. One study also identified the other alkaloids berbamine and oxyacanthine in  
 1711 *B. vulgaris* root. Available analytical data regarding protoberberine alkaloids and other alkaloids are  
 1712 provided in Table 13. Details of the analytical studies are provided in Annex A.

1713 **Table 13:** Alkaloids content in root, root bark and bark of *B. vulgaris*

Alkaloid	Content range (% w/w DW)	Plant part	Reference
<b>Protoberberine alkaloids</b>			
<b>Berberine</b>	0.132–2.21	Root and root bark	(Ahmad et al., 2019; Miłek et al., 2024; Villinski et al., 2003)
	0.051–1.32	Bark	(Gird et al., 2017; Petruczynik et al., 2019)
<b>Jatrorrhizine</b>	0.149–0.460	Root	(Villinski et al., 2003)
	NQ	Bark	Unpublished data EHPM (Biagi, 2023)
<b>Palmatine</b>	0.064–0.208	Root	(Villinski et al., 2003)
	0.027	Bark	(Villinski et al., 2003)
<b>Thalifendine</b>	NQ	Root	(Nakonieczna et al., 2024)
<b>Epiberberine</b>	NQ	Root	(Nakonieczna et al., 2024)
<b>Demethyleneberberine</b>	NQ	Root	(Nakonieczna et al., 2024)
<b>Berberrubine</b>	NQ	Bark	Unpublished data EHPM (Biagi, 2023)
<b>Other alkaloids</b>			
<b>Berbamine</b>	0.465–0.631	Root	(Villinski et al., 2003)
<b>Oxyacanthine</b>	0.348–0.517	Root	(Villinski et al., 2003)

1714 Abbreviations: DW, dry weight; EHPM, European Federation of Associations of Health Product Manufacturers; NQ, detected but not  
 1715 quantified in the plant material; w; weight.

### 1716 3.4.2 Genotoxicity

1717 No eligible genotoxicity studies were retrieved.

### 1718 3.4.3 General toxicity

1719 No eligible toxicity studies in mammalian species were retrieved.

## 1720 3.5 *Chelidonium majus* (herb)

### 1721 3.5.1 Characterisation of the plants

1722 *C. majus* is a perennial herbaceous plant of the Papaveraceae family (poppies) native to the  
 1723 temperate-cold regions of Europe, Asia and America. Its common name in English is greater  
 1724 celandine. The list of synonyms is provided in Appendix A.

1725 The Panel notes that the terms “herb” and “aerial parts” are used interchangeably in analytical  
 1726 studies. For consistency in this assessment, these terms are treated as synonyms, referring  
 1727 collectively to the above-ground plant parts.

1728 The most abundant protoberberine alkaloid in aerial parts of *C. majus* is coptisine, while berberine  
 1729 content is typically lower. Among other alkaloids, stylophine, sanguinarine, chelerythrine, and  
 1730 chelidonine are also present in higher concentrations than berberine. Available analytical data  
 1731 regarding protoberberine alkaloids and other alkaloids are reported in Table 14. Details of the  
 1732 analytical studies are provided in Annex A.

1733 **Table 14:** Content of alkaloids in aerial parts of *C. majus*

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	0.0008–0.039	(Gird et al., 2017; Gu et al., 2010; Krizhanovska et al., 2021; Petruczynik et al., 2019; Sowa et al., 2018; Tuzimski et al., 2023; Zielinska et al., 2019)
<b>Coptisine</b>	0.021–0.813	(Gu et al., 2010; Krizhanovska et al., 2021; Sowa et al., 2018; Zielinska et al., 2019)
<b>Palmatine</b>	ND	(Petruczynik et al., 2019)
<b>Other alkaloids</b>		
<b>Sanguinarine</b>	0.0002–0.293	(Gu et al., 2010; Krizhanovska et al., 2021; Petruczynik et al., 2019; Sowa et al., 2018; Tuzimski et al., 2023; Zielinska et al., 2019)
<b>Chelidonine</b>	0.006–0.287	(Gu et al., 2010; Krizhanovska et al., 2021; Petruczynik et al., 2019; Sowa et al., 2018; Tuzimski et al., 2023; Zielinska et al., 2019)
<b>Stylophine</b>	0.109–0.319	(Gu et al., 2010)
<b>Protopine</b>	0.002–0.242	(Gu et al., 2010; Petruczynik et al., 2019; Sowa et al., 2018; Tuzimski et al., 2023; Zielinska et al., 2019)
<b>Chelerythrine</b>	0.0004–0.280	(Gu et al., 2010; Krizhanovska et al., 2021; Petruczynik et al., 2019; Sowa et al., 2018; Tuzimski et al., 2023; Zielinska et al., 2019)
<b>Allocriptopine</b>	0.004–0.010	(Krizhanovska et al., 2021; Zielinska et al., 2019)

1734 Abbreviations: DW, dry weight; ND, not detected; w, weight.

### 1735 3.5.2 Genotoxicity

1736 In 2024, the EFSA FEEDAP Panel raised concerns about the genotoxic potential of sanguinarine and,  
 1737 consequently, of the plant extract under assessment (EFSA FEEDAP Panel (EFSA Panel on Additives  
 1738 and Products or Substances used in Animal Feed) et al., 2024) (section 1.4.1.). These concerns were  
 1739 based on the ability of sanguinarine to intercalate into DNA, induce DNA adducts in vitro, cause DNA  
 1740 strand breaks in vivo, increase reactive oxygen species, and exert mutagenic and clastogenic effects  
 1741 in vitro and clastogenic effects in vivo observed after i.p. administration. The FEEDAP Panel extended  
 1742 the same conclusion to chelerythrine, owing to its structural similarity to sanguinarine.

1743 The NDA Panel also notes mechanistic data which indicate that sanguinarine inhibits DNA  
 1744 topoisomerase II via disrupted nucleocytoplasmic trafficking (Holy et al., 2006), consistent with  
 1745 induction of genome instability. Epidemiological reports linked its use in oral hygiene products to  
 1746 oral leucoplakia (Ansari & Das, 2010) a precancerous lesion, prompting voluntary U.S. market  
 1747 withdrawal (Lou et al., 2021; Mascarenhas et al., 2001).

1748 The QSAR analysis of sanguinarine and chelerythrine identified additional structural alerts for  
 1749 mutagenicity to those found in the protoberberines' family. The two compounds were predicted to  
 1750 be mutagenic by the VEGA consensus model, with a mutagenicity consensus score of 0.75 (good  
 1751 reliability). As for berberine and protoberberines, the results of the models for MN induction in vitro  
 1752 and in vivo provided no relevant additional information (low reliability of the prediction from the in  
 1753 vitro MN model; limited training sets for the in vivo MN model) (Annex C).

1754 Taken together, these findings are consistent with, and further support, the concerns expressed by  
 1755 the FEEDAP Panel regarding a potential genotoxicity of sanguinarine and chelerythrine.

### 1756 3.5.3 General toxicity

1757 From the systematic search of the literature one publication was retrieved which investigated  
 1758 hepatotoxicity of preparations of *C. majus* in rats (Mazzanti et al., 2009) (Table 15), following a case  
 1759 report of hepatitis in a man who had consumed a decoction of *C. majus* dried leaves for 1 month  
 1760 (Moro et al. (2009), see 3.5.4).

1761 **Table 15:** General characteristics of the sub-acute toxicity studies of *C. majus* in rats

Publication	Tier	Species	Sex, n/ group	Duration (days)	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>
Mazzanti et al. (2009)	2	Wistar rats	M, n=8	14, 28	Ethanollic extract (DER 1:5) from <i>C. majus</i> dried aerial parts	Gel <sup>b</sup>	0, 1500, 3000 <sup>c</sup>

1762 Abbreviations: bw, body weight; M, male; n, number.

1763 <sup>a</sup> Dose of test material.

1764 <sup>b</sup> Doses administered in 5 g of a palatable gel (water 99%).

1765 <sup>c</sup> Doses refer to the weight of the starting herbal material (dried aerial parts). The dose of 3000 mg/kg/bw per day corresponds  
 1766 approximately to 100 times the usual dose used in humans.

1767  
 1768 An ethanolic extract of dried aerial parts of *C. majus* was administered to male Wistar rats (n=8 per  
 1769 group) for two or four weeks. The extract was injected in a bolus of a “palatable gel” that was  
 1770 consumed daily by the rats. The total alkaloid content was 0.37±0.02% expressed as percentage of  
 1771 chelidonine. The administered doses of 0, 1500 or 3000 mg/kg bw per day of the ethanolic extract  
 1772 corresponded to 0, 5.5 and 11 mg/kg bw per day of total alkaloids, respectively. The authors stated  
 1773 that the doses corresponded to approximately 50 and 100 times the doses generally reported for  
 1774 humans. The study included a group of 8 male rats receiving a single dose of 2 g/kg bw  
 1775 acetaminophen at the end of the study, as a positive control for liver toxicity.

1776 No differences in body weight gain and food intake relative to body weight were reported compared  
 1777 to the controls at any dose or time point (data not shown). Weights of thymus, lungs, bronchus,  
 1778 trachea, heart, spleen, liver, pancreas, stomach, seminal vesicles and testis, urinary bladder, kidney  
 1779 and adrenal glands in the administered groups were reported as ‘not different’ from control at any  
 1780 dose or time point (data not shown). The weight of the intestine was significantly higher only in the  
 1781 group receiving 3000 mg/kg bw per day (+7.35%) after 4 weeks. Blood concentrations of ALT, AST  
 1782 or ALP, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholinesterase, amylase,  
 1783 lipase, bilirubin, prothrombine time (PT), partial thromboplastin time (PTT) and fibrinogen and liver  
 1784 histopathology (including immunohistochemical analysis of markers for fibrogenesis, cholangiocyte  
 1785 and hepatocyte proliferation and apoptosis) were also unaffected. In liver homogenates, slight but  
 1786 statistically significant decreases of reduced glutathione concentrations (high dose group) and  
 1787 superoxide dismutase activity (both dose groups) were observed after 4 weeks, while there were  
 1788 no statistically significant between-group differences in malondialdehyde concentrations.

1789 The Panel notes that the study did not show hepatotoxicity up to 3000 mg/kg bw per day of an  
 1790 ethanolic extract of *C. majus* (11 mg/kg bw per day total alkaloids) for up to 28 days in male rats.  
 1791 However, the Panel notes the limited number of animals tested (8 per group, only males tested)

1792 and the limited duration of the study. The Panel notes that this study is at moderate risk of bias (tier  
1793 2).

#### 1794 3.5.4 Human case reports

1795 The systematic review identified 16 publications-including case reports, case series, and reviews  
1796 that documented liver injury associated with *C. majus*-containing food supplements or herbal  
1797 medicinal products<sup>26</sup>, hereafter collectively called *C. majus* products (Benninger et al., 1999; BfArM,  
1798 2005; Crijns et al., 2002; Gerhardt et al., 2019; Greving et al., 1998; Hardeman et al., 2008; Im et  
1799 al., 2014; Moro et al., 2009; Rifai et al., 2006; Stickel et al., 2003; Tarantino et al., 2009; Teschke  
1800 et al., 2012a; Teschke et al., 2011; Teschke et al., 2012b; Valente et al., 2010; Whiting et al., 2002).  
1801 These publications identified 45 independent cases in total, described in Appendix G. In 21 of the  
1802 cases, *C. majus* was the only ingredient in the product, while in 24 cases, it was part of a multi-  
1803 ingredient preparation.

1804 The majority of cases were reported in Germany (n=38) (reviewed by BfArM (2005); Gerhardt et al.  
1805 (2019); Teschke et al. (2011); Teschke et al. (2012b)), two cases were reported in Italy (Moro et  
1806 al., 2009; Valente et al., 2010), one in Spain (Sáez-González et al., 2016), one in Australia (Whiting  
1807 et al., 2002), and one in South Korea (Im et al., 2014). Jaundice was the predominant symptom,  
1808 sometimes preceded by darkening of the urine, itching, fatigue, abdominal pain and/or nausea. The  
1809 latency periods to first symptoms varied from some weeks to some months. Liver injury was  
1810 identified in all cases, often presenting as acute hepatitis with a hepatocellular pattern, characterised  
1811 by elevated ALT concentrations with normal ALP concentrations. Most patients recovered after  
1812 discontinuing the *C. majus*-containing products. ALT concentrations typically normalised within a  
1813 few months following the cessation of the product. In one case, unintentional re-exposure to *C.*  
1814 *majus* led to a second episode of acute hepatitis (case 5 of Benninger et al. (1999).

1815 Teschke et al. have performed a causality assessment of 22 spontaneous reports recorded by the  
1816 German Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und  
1817 Medizinprodukte, BfArM) (Teschke et al., 2011) and 20 additional cases<sup>27</sup> published in the literature  
1818 (Teschke et al., 2012b), applying the liver-specific scale of the Council for International  
1819 Organizations of Medical Sciences (CIOMS). This scale considers causality criteria including the  
1820 latency period, course of alanine aminotransferase after discontinuation, risk factors, comedication  
1821 and alternative causes (Teschke et al., 2008). Causality for *C. majus* was rated as highly probable  
1822 in 3 cases, probable in 12 cases and possible in 20 cases, whereas it was considered unlikely in 1  
1823 case and a causal relationship was excluded in 6 cases.

1824 In addition to the cases reviewed by Teschke et al., five other cases were retrieved in the literature  
1825 (Im et al., 2014; Valente et al., 2010; Whiting et al., 2002) Sáez-González et al., 2016; Gerhardt et  
1826 al., 2019). A causal relationship between the consumption of *C. majus* products and liver injury was  
1827 rated as highly probable in the case reported by Im et al. (2014), applying the Council for  
1828 International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment method  
1829 (CIOMS/RUCAM). The cases reported by Valente et al. and Whiting et al. shared the same clinical  
1830 features as the other identified cases. However, in these two cases, *C. majus* was consumed as part

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<sup>26</sup> Preparations of *C. majus* have been regulated as herbal medicinal products in some countries.

<sup>27</sup> Teschke et al. (2012) identified 21 additional cases. However, the case reported by Strahl et al. (1998) is excluded by the Panel as the food supplement consumed contained Lesser Celandine (*Ranunculus ficaria* L.).

1831 of multi-ingredient preparations and the articles provided limited descriptions of the events and no  
1832 objective causality assessment scales were applied. Sáez-González et al. (2016) and Gerhardt et al.  
1833 (2019) reported two cases of liver failure following consumption of the same multi-ingredient food  
1834 supplement containing several plant extracts, including CM; both required liver transplantation and  
1835 one case was fatal. Based on CIOMS/RUCAM, hepatotoxicity was rated as probably and highly  
1836 probably due to the consumption of the food supplement, with *C. majus* identified as the most likely  
1837 causative component.

1838 The Panel notes that available case reports consistently indicate hepatotoxicity associated with the  
1839 consumption of *C. majus* products. Based on a liver specific CIOMS/RUCAM scale, a standardised  
1840 and validated causality assessment scale for use in clinical settings (Danan & Benichou, 1993;  
1841 Teschke et al., 2014), the causality for *C. majus* was rated as highly probable in 5 cases, probable  
1842 in 13 cases and possible in 20 cases (Im et al., 2014; Teschke et al., 2011; Teschke et al., 2012b)  
1843 Sáez-González et al., 2016; Gerhardt et al., 2019). The Panel considers it very likely<sup>28</sup> that cases of  
1844 liver injury can be attributed to the consumption of *C. majus* products. The Panel notes that the  
1845 nature of the reactions in these case reports is consistent with an idiosyncratic form of herb-induced  
1846 liver injury, i.e. may be specific to some individuals, characterised by unpredictability, lack of dose-  
1847 dependency, variable latency periods (Teschke et al., 2012a; Teschke et al., 2011; Teschke et al.,  
1848 2012b), and the failure to reproduce hepatotoxicity in one 28-day rat study (Mazzanti et al. (2009),  
1849 see section 3.5.3). Uncertainties, however, remain regarding the modes of action and potential  
1850 individual factors of susceptibility (Benninger et al., 1999; Mazzanti et al., 2013; Mazzanti et al.,  
1851 2009; Teschke et al., 2012b).

1852 The Panel considers that available case reports raise a safety concern regarding the consumption of  
1853 preparations of the aerial parts of *C. majus*. The Panel notes that the available evidence suggests  
1854 an idiosyncratic form of herb-induced liver injury and therefore a dose below which this form of liver  
1855 injury would not occur cannot be determined.

### 1856 3.5.5 Interactions with medicinal products

1857 Mazzanti et al. (2013) investigated whether *C. majus* could potentiate the hepatotoxic effect of  
1858 acetaminophen (paracetamol). Male and female rats were randomised into four groups (n=8 rats  
1859 per sex in each group) which were treated by oral route at 0 h, 12 h, 24 h, 36 h with a control  
1860 vehicle, an ethanolic extract of *C. majus* (1500 mg/kg bw), a control vehicle and a dose of  
1861 acetaminophen administered at 37 h (0.5 g/kg) or an ethanolic extract of *C. majus* extract (1500  
1862 mg/kg bw) and acetaminophen (0.5 g/kg) at 37 h. Based on histopathological examination of liver  
1863 samples and clinical chemistry, the experiment did not find evidence for a pharmacological  
1864 interaction between the ethanolic extract of *C. majus* and acetaminophen.

1865 The Panel notes that there is substantial uncertainty in extrapolating animal data to humans due to  
1866 potential species differences in drug metabolic pathways. The Panel considers that data are  
1867 insufficient to allow an assessment of the potential interactions between *C. majus* preparations and  
1868 medicinal products.

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<sup>28</sup> Very likely: 90-95% (subjective probability range), EFSA Scientific Committee. (2018a). *Guidance on uncertainty analysis in scientific assessments* (EFSA Journal 2018;16(1):5123, 39 pp. 10.2903/j.efsa.2018.5123).

### 1869 3.5.6 Concluding remarks

1870 In addition to the presence of berberine and other protoberberines in preparations of aerial parts of  
1871 *C. majus*, mechanistic, in vitro, in vivo data and QSAR analyses raise concern regarding the  
1872 genotoxicity of sanguinarine and chelerythrine.

1873 Human case reports indicate that consumption of *C. majus* products can result in liver injury, with  
1874 a pattern consistent with idiosyncratic herb-induced hepatotoxicity. Cases ranged from highly  
1875 probable to possible causality based on standardised CIOMS/RUCAM assessments.

1876 In contrast, a rat sub-acute study with *C. majus* extracts did not demonstrate hepatotoxicity, but  
1877 the study was limited in duration, number of animals, and sex tested. However, standard animal  
1878 toxicity studies cannot reliably reproduce human idiosyncratic reactions (EMA, 2010).

1879 The available evidence raises concerns regarding a risk for an idiosyncratic form of herb-induced  
1880 liver injury following consumption of preparations of the aerial parts of *C. majus*. The underlying  
1881 mechanisms are unknown, including whether it may be mediated by berberine or other substances  
1882 present in the preparations. Based on the current knowledge susceptible individuals in the general  
1883 population cannot be identified.

## 1884 3.6 *Coptis japonica* (rhizome)

### 1885 3.6.1 Characterisation of the plant

1886 *Coptis japonica* (Thunb.) (*C. japonica*) is an evergreen herbaceous perennial plant that belongs to  
1887 the Ranunculaceae family and is native to Japan. Its common name in English is Japanese  
1888 goldthread. The list of synonyms is provided in Appendix A.

1889 The main protoberberine alkaloids in *C. japonica* rhizome include berberine, coptisine, jatrorrhizine  
1890 and palmatine. Epiberberine and columbamine are present in lower amounts. Magnoflorine is also  
1891 present. Available analytical data regarding protoberberine alkaloids and other alkaloids are listed  
1892 in Table 16. Details of the analytical studies are provided in Annex A.

1893 **Table 16:** Alkaloids content in *C. japonica* rhizome

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
Berberine	4.32–7.48	(Kim et al., 2004; Liu et al., 1994)
Coptisine	0.19–2.22	(Kim et al., 2004; Liu et al., 1994)
Jatrorrhizine	0.53–1.23	(Liu et al., 1994)
Palmatine	0.01–1.76	(Kim et al., 2004; Liu et al., 1994)
Epiberberine	ND–0.18	(Liu et al., 1994)
Columbamine	0.02–0.15	(Liu et al., 1994)
Berberastine	ND	(Liu et al., 1994)
<b>Other alkaloids</b>		
Magnoflorine	0.43–0.97	(Liu et al., 1994)

1894 Abbreviations: DW, dry weight; ND, not detected; w, weight.

### 1895 3.6.2 Genotoxicity

1896 Akiyama et al. (2019) performed the Ames test on two water extracts of cultivated *C. japonica*  
1897 rhizome (berberine content of 18% and 22.2%, respectively) to compare the mutagenic potential  
1898 with two commercial extracts of the same plant species (berberine content of 12.6% and 20.5%,  
1899 respectively). Two *S. typhimurium* strains (TA102 and TA98) were exposed to up to 5,000 µg/plate  
1900 of the tested extracts using the pre-incubation method. None of the extracts was mutagenic in the  
1901 TA102 strain, while all the extracts induced dose-dependent and more than two-fold increases of  
1902 revertant colonies in the TA98 strain (with and without S9-mix) in comparison to control. The results  
1903 indicating mutagenic potential of the tested plant extracts in the TA98 strain, which is sensitive to  
1904 frameshift mutations, are of potential biological relevance. However, due to methodological  
1905 limitations (see Appendix E) and the lack of extract characterisation, the study is considered as  
1906 reliable with restrictions (Klimisch score 2) and the overall relevance of these results is considered  
1907 limited.

### 1908 3.6.3 General toxicity

1909 No eligible toxicity studies in mammalian species were retrieved.

## 1910 3.7 *Coptis teeta* (rhizome)

### 1911 3.7.1 Characterisation of the plant

1912 *Coptis teeta* Wall. (*C. teeta*) is a perennial evergreen herbaceous plant that belongs to the  
1913 Ranunculaceae family, native in temperate regions of Asia, especially in the Himalayan region. Its  
1914 common name in English is Indian goldthread. The list of synonyms is provided in Appendix A.

1915 Berberine is the predominant protoberberine alkaloid in the rhizomes of *C. teeta*. Other  
1916 protoberberines include coptisine, jatrorrhizine, palmatine, columbamine, epiberberine,  
1917 groenlandicine, demethyleneberberine, and berberrubine. Other alkaloids include magnoflorine and  
1918 tetrahydropalmatine. Available analytical data regarding protoberberine alkaloids and other  
1919 alkaloids are reported in Table 17. Details of the analytical studies are provided in Annex A.

1921 **Table 17:** Alkaloids content in the rhizome of *C. teeta*

Alkaloid	Content range (% w/w DW)	References
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	2.59–9.35	(Chen et al., 2017; Fan et al., 2012a; Fan et al., 2012b; He et al., 2014; Li et al., 2020; Li et al., 2016a; Lv et al., 2016; Misra et al., 2024; Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Coptisine</b>	0.746–2.260	(Chen et al., 2017; Fan et al., 2012a; Fan et al., 2012b; He et al., 2014; Li et al., 2020; Li et al., 2016a; Lv et al., 2016; Misra et al., 2024; Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Jatrorrhizine</b>	0.133–1.035	(Chen et al., 2017; Fan et al., 2012a; Fan et al., 2012b; He et al., 2014; Li et al., 2020; Li et al., 2016a; Lv et al., 2016; Misra et al., 2024; Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)

Alkaloid	Content range (% w/w DW)	References
<b>Palmatine</b>	0.03–0.695	(Chen et al., 2017; Fan et al., 2012a; Fan et al., 2012b; He et al., 2014; Li et al., 2020; Li et al., 2016a; Lv et al., 2016; Misra et al., 2024; Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Columbamine</b>	0.144–0.23	(Chen et al., 2017; Fan et al., 2012a; Fan et al., 2012b; He et al., 2014; Li et al., 2020; Lv et al., 2016; Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Epiberberine</b>	ND–0.414	(Chen et al., 2017; Fan et al., 2012a; Fan et al., 2012b; He et al., 2014; Li et al., 2020; Li et al., 2016a; Lv et al., 2016; Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Groenlandicine</b>	0.095–0.102	(Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Demethyleneberberine</b>	0.023	(Qi et al., 2022)
<b>Berberrubine</b>	0.002	(Qi et al., 2022)
<b>Other alkaloids</b>		
<b>Magnoflorine</b>	0.672–0.733	(Qi et al., 2018; Qi et al., 2022; Zhong et al., 2018)
<b>Tetrahydropalmatine</b>	0.004	(Misra et al., 2024)

1922 Abbreviations: DW, dry weight; ND, not detected; w, weight.

### 1923 3.7.2 Genotoxicity

1924 No eligible genotoxicity studies were retrieved.

### 1925 3.7.3 General toxicity

1926 No eligible toxicity studies in mammalian species were retrieved.

## 1927 3.8 *Coptis trifolia* (rhizome)

### 1928 3.8.1 Characterisation of the plant

1929 *Coptis trifolia* (L.) Salisb. (*C. Trifolia*) is an evergreen herbaceous perennial plant that belongs to the  
 1930 Ranunculaceae family, native to North America and Asia, across the subarctic region. Its common  
 1931 name in English is three-leaf goldthread or savoyane. The list of synonyms is provided in Appendix  
 1932 A.

1933 In one analytical study of the rhizomes of *C. trifolia*, berberine and coptisine were present in  
 1934 comparable amounts (Table 18). Details of the analytical studies are provided in Annex A.

1935

1936 **Table 18:** Alkaloids content in rhizome of *C. trifolia*

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	0.380	(Kamath et al., 2009)
<b>Coptisine</b>	0.344	(Kamath et al., 2009)

1937 Abbreviations: DW, dry weight; w, weight.

### 1938 3.8.2 Genotoxicity

1939 No eligible genotoxicity studies were retrieved.

1940 **3.8.3 General toxicity**

1941 No eligible toxicity studies in mammalian species were retrieved.

1942 **3.9 *Coscinium fenestratum* (root, stem)**

1943 **3.9.1 Characterisation of the plant**

1944 *Coscinium fenestratum* (Goetgh.) Colebr. (*C. fenestratum*) is a perennial, woody climber, that  
1945 belongs to the Menispermaceae family, native to South and Southeast Asia. Its common names in  
1946 English include tree turmeric and false calumba. The list of synonyms is provided in Appendix A.

1947 Berberine is the major protoberberine alkaloid in *C. fenestratum* stems, whereas jatrorrhizine and  
1948 palmatine have been reported in lower amounts. Berberrubine and thalifendine have also been  
1949 detected. No data were retrieved regarding the presence of berberine or other protoberberines in  
1950 the root of *C. fenestratum*. Available analytical data regarding protoberberine alkaloids are reported  
1951 in Table 19. Details of the analytical studies are provided in Annex A.

1952 **Table 19:** Alkaloids content in *C. fenestratum* stem

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	0.430–4.67	(Chitra et al., 2011; Deevanhxay et al., 2009a; Rojsanga et al., 2006)
<b>Jatrorrhizine</b>	0.21	(Deevanhxay et al., 2009a)
<b>Palmatine</b>	0.07	(Deevanhxay et al., 2009a)
<b>Berberrubine</b>	NQ	(Bajpai et al., 2015)
<b>Thalifendine</b>	NQ	(Deevanhxay et al., 2009b)

1953 Abbreviations: DW, dry weight; NQ, detected but not quantified in the plant material; w, weight.

1954 **3.9.2 Genotoxicity**

1955 No eligible genotoxicity studies were retrieved.

1956 **3.9.3 Acute toxicity**

1957 Four publications which reported on single dose administration of preparations of *C. fenestratum* in  
1958 mammalian species were retrieved from the systematic search of the literature (Shirwaikar et al.,  
1959 2005; Wattanathorn et al., 2006; Wongcome et al., 2007; Yibchok-anun et al., 2009) (Table 20Table  
1960 20:).

1961 One of them did not investigate acute toxicity and is not further considered (Yibchok-anun et al.,  
1962 2009). No deaths were reported and no LD50 could be determined in any of the studies.

1963 **Table 20:** LD50 for preparations of stems of *C. fenestratum* in rats

Publication	Species	Sex, n/ group	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>	LD50	Comment
Wongcome et al. (2007)	SD rats	F, M; n=5	Aqueous extract of <i>C. fenestratum</i> dried stems	NR	0, 5000	ND	No mortality
Wattanathorn et al. (2006)	SD rats	M; n=8	Ethanol extract of <i>C. fenestratum</i> dried stems	NR	0, 5, 10, 20	ND	No mortality
Shirwaikar et al. (2005)	Wistar rats	F, M; n=6	Ethanol extract of <i>C. fenestratum</i> stem powder	NR	100, 500, 1000, 3000	ND	No mortality

1964 Abbreviations: bw, body weight; F, female; M, male; n, number; ND, not determined; NR, not reported; SD, Sprague Dawley.

1965 <sup>a</sup> Dose of the test material.

1966

### 1967 3.9.4 General toxicity

1968 One study investigated toxicity of a preparation of *C. fenestratum* stems in rats (Wongcome et al.,  
1969 2007) (Table 21).

1970 **Table 21:** General characteristics of the sub-chronic toxicity studies of *C. fenestratum* in rats

Publication	Tier	Species	Sex, n/group	Duration (d)	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>
Wongcome et al. (2007)	3	SD rats	F (n=10) M (n=10)	90+28 (satellite group)	Aqueous extract of <i>C. fenestratum</i> dried stems	NR	0, 2500

1971 Abbreviations: bw, body weight; F, female; M, male; n, number; NR, not reported; SD, Sprague Dawley.

1972 <sup>a</sup> Dose of the test material.

1973 Wongcome et al. (2007) conducted a 90-day study in Sprague-Dawley rats (10 females and 10  
1974 males per group) administered with 0 or 2500 mg/kg/bw per day of an aqueous extract of dried  
1975 stem from *C. fenestratum* (no information on the yield) or distilled water. A satellite group was  
1976 administered the same dose levels of the same *C. fenestratum* extract for 90 days and was observed  
1977 for an additional 28 days post-treatment. The mode of oral administration was not reported. No  
1978 deaths or effects on body weight were reported. Organ weight or histopathology were said to be  
1979 unaffected, but the organs examined were not described and data were not shown. Spontaneous  
1980 motor activity was not affected. Statistically significant differences with control group were found  
1981 for some clinical chemistry parameters in the 90-day groups and the satellite group, which were not  
1982 considered treatment-related. The Panel notes that no treatment-related effects were reported at a  
1983 dose of 2500 mg/kg bw per day of the aqueous extract of dried stems of *C. fenestratum* for 90 days  
1984 in rats on the endpoints investigated. However, the Panel notes the poor reporting of this study,  
1985 which hampers a thorough assessment of the findings. The study is at high risk of bias (tier 3).

### 1986 3.9.5 Neurotoxicity

1987 **Table 22:** Neurotoxicity studies for *C. fenestratum* in rats

Publication	Tier	Species	Sex, n/group	Duration (d)	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>
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<b>Wattanathorn et al. (2006)</b>	<b>3</b>	SD Rats	M, n=8	14	Ethanollic extract of <i>C. fenestratum</i> dried stems (berberine content NR)	NR	0, 5, 10, 20
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1988 Abbreviations: bw, body weight; F, female; M, male; n, number; NR, not reported; SD, Sprague Dawley.

1989 (a) Dose of the test material

1990

1991 Wattanathorn et al. (2006) conducted a study to evaluate possible neurotoxic effects of oral doses  
 1992 of 0, 5, 10, and 20 mg/kg per day for 14 days of an ethanolic extract of dried stems of *C. fenestratum*  
 1993 on body weight in Sprague-Dawley (SD) rats (n=8) (Table 22)Table 22:.. The mode of oral  
 1994 administration was not reported. Body weight was statistically significantly increased at all doses  
 1995 compared to control (not dose-dependent). Food intake was not measured. Neuron density in  
 1996 coronal sections of brains was statistically significantly lower compared to control in all brain areas  
 1997 studied (striatum, frontal, parietal, temporal and occipital areas of the cortex and, hippocampus) at  
 1998 all tested doses, but not dose-dependently. The neurobehavioural parameters investigated  
 1999 (endurance time, immobility time in the forced swimming test and licking, grooming and rearing  
 2000 behaviour) were not affected, except for a significantly different licking behaviour in the mid dose  
 2001 group compared to the control group. The Panel notes that results of lower neuron density may be  
 2002 indicative of potential neurotoxic effect. However, the Panel notes the limitation of the test used (2D  
 2003 count), which could induce bias in the neuron count. In addition, the description of the study design  
 2004 and the reporting of neurobehavioural test results are inadequate, and the study is therefore  
 2005 considered at high risk of bias (tier 3). The Panel considers that no conclusion can be drawn from  
 2006 this study on a neurotoxic effect of up to 20 mg/kg bw per day of an ethanolic extract of dried stems  
 2007 of *C. fenestratum* for 14 days in rats.

### 2008 3.9.6 Other studies in mammalian animals

2009 From the systematic search of the literature one additional study was retrieved, which was primarily  
 2010 designed to evaluate pharmacological effects of an alcoholic stem extract of *C. fenestratum* on  
 2011 glucose metabolism (Shirwaikar et al., 2005). Male and female adult Wistar albino rats were divided  
 2012 into four groups (n=6) and received 2% acacia gum solution (control), 250 mg/kg bw of *C.*  
 2013 *fenestratum* extract, 500 mg/kg bw of *C. fenestratum* extract or 0.25 mg/kg glibenclamide (positive  
 2014 control), respectively, for 12 days. Lower fasting blood glucose concentrations were reported in the  
 2015 treated groups compared to the control group at day 5 and 12. Body weight, food and fluid intake  
 2016 were not reported; no other parameters were measured. The study was at moderate risk of bias  
 2017 (tier 2).

2018 The Panel considers that no conclusion can be drawn from this study regarding hazard identification  
 2019 of plant preparations from stems of *C. fenestratum*.

## 2020 3.10 *Hydrastis canadensis* (rhizome, root)

### 2021 3.10.1 Characterisation of the plant

2022 *Hydrastis canadensis* L. (*H. canadensis*) is an herbaceous perennial plant with a thick, yellow  
 2023 horizontal rhizome bearing numerous slender roots, a hairy purplish stem, and large, lobed serrated  
 2024 leaves that belongs to the Ranunculaceae family. The plant is native to North America. In English,

2025 its common name is goldenseal, but it is also known as orange root, Indian turmeric, and eye-balm.  
 2026 The list of synonyms is provided in Appendix A.

2027 Based on the European Pharmacopoeia, which defines *Hydrastis rhizoma* as 'whole or cut, dried  
 2028 rhizome and root of *Hydrastis canadensis* L.' (Council of Europe, 2023), the Panel considered the  
 2029 rhizome and root as the relevant parts of the plant for this assessment.

2030 Most analytical studies describe the plant parts tested as either 'rhizome' or 'rhizome and roots'. A  
 2031 few papers use the term 'roots'. In common usage, the term 'root' is often used to refer to *H.*  
 2032 *canadensis* rhizome or rhizome and roots and it is unclear whether the roots were truly separated  
 2033 from the rhizome in those studies. As analytical results were consistent across preparations from  
 2034 'rhizome', 'rhizome and roots' and 'roots', they are described together below. Detailed results can be  
 2035 found in Annex A.

2036 In the majority of the analytical studies, the concentration of the alkaloids was reported for roots  
 2037 and rhizome combined, with berberine and (–)-β-hydrastine as the most abundant alkaloids (Table  
 2038 23). *H. canadensis* only contains the (–)-β-hydrastine enantiomer, whereas the (+)-  
 2039 enantiomer is present in other plant species (Blaskó et al., 1982). One study specifically investigated  
 2040 the content of berberine and (–)-β-hydrastine in root and rhizomes separately (Douglas et al.,  
 2041 2010). For both compounds, they found marginally lower concentrations in roots (2.9–3.8%, 1.3-  
 2042 1.9%, respectively) than in rhizomes (3.4-4.6%, 1.7-2.8%, respectively). Minor alkaloids present  
 2043 include canadine (tetrahydroberberine) and hydrastinine. Berberastine was also detected in roots  
 2044 and rhizomes.

2045 **Table 23:** Alkaloids content in root and rhizome of *H. canadensis*

Alkaloid	Content range (% w/w DW)	Plant part/plant preparation	Reference
<b>Protoberberine alkaloids</b>			
<b>Berberine</b>	1.68–6.34	Root and rhizome	(Avula et al., 2012; Douglas et al., 2010; Kamath et al., 2009; Kim et al., 2004; Le et al., 2019; Villinski et al., 2003)
<b>Berberastine</b>	NQ	Root and rhizome	(Van Berkel et al., 2007; Weber et al., 2003)
<b>Jatrorrhizine</b>	ND	Root	(Avula et al., 2012; Villinski et al., 2003)
<b>Coptisine</b>	ND	Rhizome	(Avula et al., 2012; Kim et al., 2004)
<b>Palmatine</b>	ND	Root and rhizome	(Avula et al., 2012; Kim et al., 2004; Villinski et al., 2003)
<b>Other alkaloids</b>			
<b>(–)-β-hydrastine</b>	0.481–3.77	Root and rhizome	(Avula et al., 2012; Brown et al., 2008; Burkhart & Zuiderveen, 2019; Dawes & Brettell, 2012; Douglas et al., 2010; Etefagh et al., 2011; Kamath et al., 2009; Le et al., 2019; Villinski et al., 2003)
<b>Canadine</b>	ND–0.27	Root	(Avula et al., 2012; Burkhart & Zuiderveen, 2019; Le et al., 2019)
<b>Hydrastinine</b>	0.030–0.031	Root	(Avula et al., 2012)

2046 Abbreviations: DW, dry weight; ND, Not detected; NQ, detected but not quantified in the plant material; w, weight.

2047

## 2048 3.10.2 Genotoxicity

### 2049 3.10.2.1 In vitro studies

2050 Root powder of *H. canadensis* (berberine and (–)-β-hydrastine content NR) was studied in the  
2051 bacterial reverse mutation assay with *S. typhimurium* (TA98 and TA100) and *E. coli* (WP2 uvrA  
2052 pKM101) strains (NTP, 2010). The strains were exposed to concentrations of up to 10,000 µg/plate,  
2053 with and without metabolic activation (S9-mix). No cytotoxicity was observed at any tested  
2054 concentration. No increase in revertant colony numbers was observed at any dose tested indicating  
2055 no mutagenicity of the test material. The Panel notes that not all the bacterial strains required by  
2056 the OECD TG 471 were tested, thus the study is considered as reliable with restrictions (Klimisch  
2057 score 2) and the relevance of the study results as limited.

2058 Saha et al. (2013) conducted an in vitro comet assay with ethanolic root extract of *H. canadensis*  
2059 (the content of berberine was not reported). HeLa cells were treated with the test item up to 570  
2060 µg/mL for 24 h. The authors reported the formation of comet tails, indicative of DNA damage.  
2061 However, the results were shown as fluorescence images of only two cells and apoptosis was  
2062 observed at the same concentration, therefore the Panel considers these results as inconclusive.  
2063 Due to several other shortcomings in the methodological approach or missing information, the study  
2064 was scored as not reliable (Klimisch score 3), and the relevance of study results as low (Appendix  
2065 E).

2066 Chen et al. (2013) evaluated the genotoxic potential of eight commercial *H. canadensis* liquid  
2067 extracts in HepG2 cells using γ-H2AX expression as a marker for DNA DSBs. The extracts, which  
2068 varied in berberine content from 1.78 to 16.65 mg/mL, demonstrated a linear dose-response  
2069 relationship. Based on Western blot analysis, the magnitude of γ-H2AX induction was proportional  
2070 to the concentration of berberine present. However, as noted in section 3.1.3.1, assays which were  
2071 based solely on increased γ-H2AX expression by Western blot were omitted from the formal  
2072 evaluation since such measurements do not reliably distinguish direct DNA damage from secondary  
2073 cellular responses. Nevertheless, the high correlation between phosphorylated H2AX levels and  
2074 berberine content in the *H. canadensis* extracts is suggestive of a biologically relevant role of  
2075 berberine in these preparations.

### 2076 3.10.2.2 In vivo study

2077 Root powder of *H. canadensis* (3.45% of berberine and 3.02% (–)-β-hydrastine) was administered  
2078 to B6C3F1 mice (5 animals/sex and per group) via feed at concentrations of up to 50,000 mg/kg as  
2079 per protocol in a 90-day repeated dose toxicity study. After 3 months of exposure, peripheral blood  
2080 samples were used in a mammalian erythrocyte micronucleus test (NTP, 2010). In total, 2,000  
2081 normochromatic erythrocytes (NCEs) per animal were scored for the formation of micronuclei. In  
2082 addition, the percentage of PCEs in a population of 1,000 erythrocytes per animal was determined  
2083 as a measure of bone marrow toxicity. No increase in the frequency of micronucleated NCEs was  
2084 observed. No significant exposure-related changes in the percentages of PCEs were observed,  
2085 suggesting that no exposure-related bone marrow toxicity occurred.

2086 The Panel considers the study as reliable with restrictions (Klimisch score 2) because micronuclei  
2087 were scored in less sensitive NCEs instead of PCEs, no evidence of target-tissue exposure was  
2088 provided, and no historical positive control data were reported. It is noted that the target cells used  
2089 and the 90-day sub-chronic exposure period deviates from the internationally accepted acute test

2090 guideline for the MN assay (OECD TG 474). Although the Panel acknowledges that the integration  
2091 of the MN assay into a 90-day toxicity study represents an appreciable effort to reduce animal use  
2092 in line with the 3Rs principle, such a long-term design is problematic as clearance mechanisms (e.g.  
2093 apoptosis and autophagy) can effectively remove micronucleated cells, potentially resulting in false  
2094 negatives. Based on these limitations, the relevance of study results is considered low.

### 2095 3.10.2.3 QSAR predictions

2096 The Panel notes the lack of experimental data on (–)-β-hydrastine and canadine.

2097 The QSAR analysis of (–)-β-hydrastine and canadine did not identify structural alerts for  
2098 mutagenicity. Both compounds were predicted to be non-mutagenic by the VEGA consensus model,  
2099 with a mutagenicity consensus score of 0.5 and 0.4 for (–)-β-hydrastine and canadine (moderate  
2100 to low reliability), respectively. In contrast, in vitro MN induction model identified both compounds  
2101 as potentially genotoxic with moderate reliability, whereas the results of the in vivo MN induction  
2102 provided no relevant additional information (limited training sets for the in vivo MN model). A high  
2103 structural similarity was also identified between (–)-β-hydrastine and (S,R)-noscapine (CAS No. 128-  
2104 62-1), which exhibits aneugenic potential in cell cultures (Gatehouse et al., 1991; Schuler et al.,  
2105 1999, 2003) (Annex C). The Panel notes that (–)-β-hydrastine, and to a lesser extent canadine, may  
2106 require further investigation regarding their clastogenic/aneugenic potential.

### 2107 3.10.3 General toxicity

2108 Two publications were retrieved which reported on six multiple-dose studies conducted by the NTP,  
2109 i.e. 15-day sub-acute toxicity studies, 90-day sub-chronic toxicity studies and two-year  
2110 carcinogenicity studies, all performed in mice and rats, respectively. One publication was the study  
2111 report by NTP (NTP, 2010), while the other publication was a peer-reviewed article that reported  
2112 the results of two carcinogenicity studies (Dunnick et al., 2011). Results of the study report are  
2113 described and discussed in this section.

2114 The experiments were conducted in F344/N rats and B6C3F1 mice (5 to 7 weeks old at the start of  
2115 the study) fed a diet containing no (control) or various doses of *H. canadensis* root<sup>29</sup> powder. The  
2116 preparation used in the sub-acute and sub-chronic studies contained 3.45% berberine, 3.02% (–)-  
2117 β-hydrastine and 0.08% canadine with a total alkaloid content of 6.55%, while the root powder  
2118 used in the carcinogenicity study contained 3.89% berberine, 2.80% (–)-β-hydrastine and 0.17%  
2119 canadine, with a total alkaloid content of 6.86%. LC/MS analyses also detected hydrastinine,  
2120 tetrahydroberberastine, berberastine and canadine. The identity of the test material was confirmed  
2121 based on appearance and the characteristic alkaloid profile of *H. canadensis*. Palmatine, a  
2122 characteristic alkaloid of *Coptis* species, which are potential adulterants of *H. canadensis*  
2123 preparations, was not detected. The test material was also analysed for heavy metals, pesticides,  
2124 aflatoxins, nitrosamines and microbiological burden.

#### 2125 3.10.3.1 Sub-acute toxicity studies

2126 Key characteristics of the two eligible 2-week sub-acute toxicity studies in rats and mice are reported  
2127 in Table 24.

---

<sup>29</sup> The Panel notes that the term 'root' powder was very likely used as a synonym of powder of rhizome and roots, which is the typical preparation of *H. canadensis* available on the US market.

2128 **Table 24:** General characteristics of the sub-acute toxicity studies of *H. canadensis* in rodents

Author	Tier	Species	Sex, n/ group	Duration	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>
NTP (2010)	1	F344/N rats	M, n=5	2 weeks	<i>H. canadensis</i> 'root' powder	Feed- <i>ad libitum</i>	0, 155, 315, 630, 1190, 2465, 4815
NTP (2010)	1	F344/N rats	F, n=5	2 weeks	<i>H. canadensis</i> 'root' powder	Feed- <i>ad libitum</i>	0, 150, 290, 640, 1240, 2370, 4870
NTP (2010)	1	B6C3F1 mice	M, n=5	2 weeks	<i>H. canadensis</i> 'root' powder	Feed- <i>ad libitum</i>	0, 380, 840, 1760, 3435, 6700, 15710
NTP (2010)	1	B6C3F1 mice	F, n=5	2 weeks	<i>H. canadensis</i> 'root' powder	Feed- <i>ad libitum</i>	0, 330, 670, 1240, 2375, 4760, 8475

2129 *Note:* In the NTP study, average daily doses (mg/kg bw per day) were derived from feed concentrations of 1,560, 3,121, 6,250, 12,500,  
2130 25,000, and 50,000 ppm.

2131 Abbreviations: bw, body weight; F, female; M, male; n, number.

2132 <sup>a</sup> Doses of the test material.

2133  
2134 In rats, final mean body weights, body weight gains, and feed consumption of exposed animals  
2135 were similar to those of the controls. Dose-dependent increases in absolute and relative liver weights  
2136 were observed in both males and females across all doses. The authors reported minimal to  
2137 moderate hepatocellular hypertrophy in three males of the second highest dose group and in all  
2138 males of the highest dose group and in all females of the two highest dose groups.

2139 The exposed mice had final mean body weights, body weight gains, and feed consumption similar  
2140 to those of the control group. Mean relative and absolute liver weights were significantly higher in  
2141 the highest two dose groups in males and in the highest dose group in females compared to the  
2142 control group, accompanied by minimal hypertrophy of centrilobular hepatocytes in the highest dose  
2143 groups (both sexes). Relative thymus weights were decreased and relative and absolute lung  
2144 weights increased compared to the control group in some groups of males, with no dose-dependency  
2145 observed.

2146 The studies are at low risk of bias (Tier 1).

### 2147 3.10.3.2 Sub-chronic toxicity studies

2148 Key characteristics of the two eligible 90-day studies in rats and mice are reported in Table 25.

2149 **Table 25:** General characteristics of the sub-chronic toxicity studies of *H. canadensis* in rodents

Author	Tier	Species	Sex, n/ group	Duration	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>b</sup>
NTP (2010)	1	F344/N rats	M, n=10 <sup>a</sup>	90 days	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 255, 500, 1000, 2020, 4060
NTP (2010)	1	F344/N rats	F, n=10	90 days	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 260, 500, 1030, 2070, 4100
NTP (2010)	1	B6C3F1 mice	M, n=10	90 days	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 680, 1360, 2260, 5370, 10550
NTP (2010)	1	B6C3F1 mice	F, n=10	90 days	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 590, 1250, 2345, 4790, 10740

2150 *Note:* In the NTP study, average daily doses (mg/kg bw per day) were derived from feed concentrations of 3,121, 6,250, 12,500, 25,000,  
2151 and 50,000 ppm.

2152 Abbreviations: M, male; F, female; n, number; bw, body weight.

2153 <sup>a</sup> Regarding the number of rats in the control group, there is an unexplained discrepancy between the figure reported in the results table  
2154 (n=5) and the text of the publication (n=10).

2155 <sup>b</sup> Doses of the test material.

2156

2157 In rats, there were no relevant differences in final body weights, body weight changes and feed  
2158 consumption between the exposed groups and the control group. Dose-related increases in relative  
2159 and absolute liver weights were observed across all doses and were more pronounced in females  
2160 than males. The Panel notes that between-group differences in relative liver weight of 7% in males  
2161 and 17% in females were already observed at the lowest dose tested (255/260 mg/kg bw per day).  
2162 Increased incidences of hepatocellular hypertrophy were observed in both sexes, starting at a dose  
2163 of 500 mg/kg bw per day (2/10 in males and 3/10 in females) and reaching statistical significance  
2164 in the highest three dose groups (9/10, 10/10, 10/10 in males and 10/10, 10/10, 10/10 in females).  
2165 Cytoplasmatic vacuolisation of hepatocytes was observed in all males of all dose groups, while it  
2166 affected only one animal in the control group. Cytoplasmatic vacuolisation was not observed in the  
2167 female rats. Decreases in ALT, ALP and sorbitol dehydrogenase concentrations were observed in  
2168 most exposed groups (both sexes). The Panel notes that the toxicological relevance of the findings  
2169 regarding liver enzymes and their interpretation in relation to the other effects observed on the liver,  
2170 is unclear. Other findings included increased mean relative weights of the heart, kidneys, and testes  
2171 in males in the highest dose group compared to controls, as well as increased relative kidney weights  
2172 in the three highest dose groups and increased mean relative heart and lung weights in the two  
2173 highest dose groups in females. Differences in haematological parameters observed in the highest  
2174 dose groups early in the study (day 5) were consistent with a transient physiologic  
2175 haemoconcentration possibly related to a transient decrease in water intake. However, measures of  
2176 water intake were not reported. In both sexes, small increases in blood total protein concentrations  
2177 in the three highest dose groups, together with higher serum albumin in the two highest dose  
2178 groups, were noted, possibly due to dehydration. There were no relevant findings regarding  
2179 reproductive organ weights, sperm parameters and oestrous cyclicity. Benchmark dose (BMD)  
2180 modelling for the *H. canadensis* root powder was applied to the critical endpoints identified in this  
2181 study, resulting in BMDL<sub>10</sub> for relative liver weight of 89.8 mg/kg bw for females and 138.8 mg/kg  
2182 bw for males and BMDL<sub>10</sub> for the incidence of hepatocellular hypertrophy of 348.0 mg/kg bw for  
2183 females and 332.0 mg/kg bw for males (Annex B).

2184 In mice, final mean body weights and body weight gains in females were lower in the two highest  
2185 dose groups compared to control group. In males, a lower final mean body weight was reported in  
2186 the highest dose group. Feed consumption was generally similar in the control and exposed groups  
2187 (both sexes). Relative and absolute liver weights were higher compared to control in the highest  
2188 three dose groups in males and in the highest two dose groups in females. Increased incidences of  
2189 hepatocyte hypertrophy were observed in the highest three dose groups (both sexes). Increased  
2190 incidences of glycogen depletion in the liver of males exposed to the highest dose and females  
2191 exposed to the three highest doses were observed. They were considered exposure-related by the  
2192 study authors. Other findings included significantly lower left epididymal weight in male mice relative  
2193 to control in the highest dose, with no accompanying effects on reproductive parameters. In females,  
2194 relative thymus weights were increased in the highest three dose groups. In males, relative heart  
2195 and lung weights were higher in the same dose groups, with no dose-dependency observed.

2196 The Panel notes that, consistent with the subacute toxicological studies (section 3.10.3.1), the two  
2197 eligible 90-day subchronic toxicity studies in rodents identified the liver as the most sensitive organ  
2198 to the toxicity of preparations of rhizome/roots of *H. canadensis*, with rat as the most sensitive  
2199 species. The Panel also notes that exposure-related increases in liver weight were found, which  
2200 started at the lowest dose tested in the rat study, i.e. 255 and 260 mg/kg bw per day in males and

2201 females respectively. This was accompanied by an increased incidence of hepatocellular hypertrophy  
2202 at higher dose levels.

2203 The studies are at low risk of bias (tier 1).

### 2204 3.10.4 Developmental toxicity

2205 Key characteristics of the only available peer-reviewed study are reported in Table 26.

2206 **Table 26:** General characteristics of the developmental toxicity study of *H. canadensis* in rodents

Author	Tier	Species	Sex, n/group	Duration (days)	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>
Yao et al. (2005)	3	Sprague Dawley Rats	F, n=5	GD 1 to 8 (experiment 1) GD 8 to 15 (experiment 2)	Ethanol extract of <i>H. canadensis</i> (plant part not specified)	Gavage	0, 1860

2207 Abbreviations: bw, body weight; F, female; GD, gestational day; n, number.

2208 <sup>a</sup> Dose of the test material.

2209  
2210 In one pilot study investigating potential developmental toxicity (not compliant with standard  
2211 guidelines for reproduction toxicity and developmental studies), female rats were given an ethanolic  
2212 extract of *H. canadensis* (standardised to 9.6 mg/mL berberine and 5.4 mg/mL (–)-β-hydrastine) at  
2213 a dose of 1860 mg/kg bw daily vs control vehicle (ethanol solution), from GD 1–8 (5 animals) or GD  
2214 8–15 (5 animals) (Yao et al., 2005). The Panel assumes that the commercial extract used was  
2215 obtained from the root/rhizome of *H. canadensis*. Eight control rats were dosed with ethanol only.  
2216 There were no adverse effects on developmental outcomes and no effects on maternal body weight,  
2217 kidney and liver weights. No overt signs of toxicity or pathological changes in dams were reported  
2218 (parameters investigated not specified). The Panel notes that in this study no effects on the  
2219 endpoints investigated were detected in rats at 1860 mg/day for 7 days. The Panel, however, notes  
2220 the low number of animals, the short duration of the study and that the part of the plant used for  
2221 the preparation was not reported. The study is at high risk of bias (tier 3).

2222 Two additional studies, conducted in SD rats and Swiss CD-1 mice, respectively, were retrieved from  
2223 the NTP website (NTP, 2002<sup>30</sup>; NTP, 2003<sup>31</sup>). These studies have been conducted for the NTP by a  
2224 contract laboratory. The studies have not been peer-reviewed by NTP and no technical report was  
2225 available. Study results in abstract form and data sheets containing individual and summary data  
2226 for the different endpoints studied, were available. Feed containing *H. canadensis* root powder (5%  
2227 berberine and 4.5% (–)-β-hydrastine by weight) was given ad libitum to animals from GD 6 to GD  
2228 20 (rats) or GD 17 (mice). Administered doses were 0, 207, 415, 841 and 1215 mg/kg bw per day  
2229 in the rat study and 0, 514, 2048, 7738 mg/kg bw per day in the mice study (25 animals/group in  
2230 each study). In view of the lack of peer-reviewed publications and limited information on the study  
2231 protocol and conduct, a critical appraisal of those studies could not be conducted. The Panel  
2232 considers that no conclusion can be drawn from these studies in relation to developmental toxicity.

<sup>30</sup> Link to data sheet: <https://cebs.niehs.nih.gov/cebs/study/002-02221-0023-0000-4>,  
Abstract <https://ntp.niehs.nih.gov/publications/abstracts/dev/ter98007>

<sup>31</sup> Link to data sheet: <https://cebs.niehs.nih.gov/cebs/study/002-02221-0024-0000-5>;  
Abstract: <https://ntp.niehs.nih.gov/publications/abstracts/dev/ter99004>

2233 The Panel, however, notes that the two studies reported a dose-related increase in absolute and  
 2234 relative maternal liver weights, which is consistent with observations from other toxicity studies.

2235 The Panel considers that no conclusions can be drawn on potential developmental toxicity of  
 2236 rhizome/roots preparations of *H. canadensis* from the available toxicological database.

### 2237 3.10.5 Carcinogenicity

2238 Key characteristics of the two eligible 2-year carcinogenicity studies in rats and mice are reported in  
 2239 Table 27.

2240 **Table 27:** General characteristics of carcinogenicity studies of *H. canadensis* in rodents

Author	Tier	Species	Sex, n/ group	Duration	Test material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>
NTP (2010)	1	F344/N rats	M, n=50	2 years	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 135, 400, 1175
NTP (2010)	1	F344/N rats	F, n=50	2 years	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 150, 470, 1340
NTP (2010)	1	B6C3F1 mice	M, n=50	2 years	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 375, 1120, 3275
NTP (2010)	1	B6C3F1 mice	F, n=50	2 years	<i>H. canadensis</i> root powder	Feed- <i>ad libitum</i>	0, 330, 1000, 2875

2241 *Note:* In the NTP study, average daily doses (mg/kg bw per day) were derived from feed concentrations of 3,000, 9,000, 25,000 (mg/kg).  
 2242 Abbreviations: M, male; F, female; n, number; bw, body weight.

2243 <sup>a</sup> Dose of the test material.  
 2244

2245 In rats, mean body weights and feed consumption of exposed groups were similar to those of the  
 2246 control group throughout the study in males. Females in the highest dose had lower mean body  
 2247 weight compared to controls after week 8 with a between-group difference of 17% at the end of  
 2248 the study. A less pronounced trend was observed in the mid dose group (between-group difference  
 2249 of 5% at the end of study). Feed intake was similar in all groups of females. Dose-dependent  
 2250 increases in the incidence and severity of non-neoplastic lesions (hepatocyte hypertrophy,  
 2251 hepatocyte degeneration, and eosinophilic foci) were observed, starting at the lowest doses tested,  
 2252 i.e. 135 and 150 mg/kg bw per day in males and females, respectively. A statistically significant  
 2253 increase in the incidence of hepatocellular adenomas was observed in males and females of the high  
 2254 dose groups. Hepatocellular carcinoma was observed in one male of the highest dose group. An  
 2255 increased incidence of pituitary gland adenomas in males in the mid and high dose groups was  
 2256 observed. However, this was not accompanied by an increase in preneoplastic pituitary gland  
 2257 lesions, the incidence of pituitary gland carcinoma was not increased, and the pituitary gland lesion  
 2258 rates were within the historical control ranges. Other findings included increased haematopoietic cell  
 2259 proliferation in the spleen in the low and high dose groups of males, decreased incidences of  
 2260 fibroadenoma in mammary glands in all exposed groups of females with a decrease in incidence of  
 2261 hyperplasia in the high dose group, decreased incidences of cardiomyopathy in males (all doses)  
 2262 and females (high dose), decreased incidences of chronic lung inflammation (high dose) and nasal  
 2263 inflammation (all groups) in males. BMD modelling was applied to the critical endpoints identified in  
 2264 this study. For females, a BMDL<sub>10</sub> for the incidence of hepatocellular hypertrophy of 45.5 mg/kg bw  
 2265 and a BMDL<sub>10</sub> for the incidence of hepatocyte degeneration of 183.1 mg/kg bw were derived. In  
 2266 males, data on these endpoints were not suitable for BMD modelling. Modelling of the incidence of  
 2267 hepatocellular adenomas and carcinomas resulted in BMDL<sub>10</sub> of 769.0 mg/kg bw per day in females  
 2268 and 528.5 mg/kg bw in males (Annex B).

2269 In mice, mean body weights and feed consumption of exposed groups were similar to those of the  
2270 control group throughout the study in males. Females in the highest dose had slightly lower mean  
2271 body weight compared to controls (between-group difference of 5% at the end of the study). Feed  
2272 consumption of exposed groups of males and females was generally similar to that of the controls  
2273 throughout the study. Survival of female mice in the mid dose group was significantly less than that  
2274 of the controls. In males, increased incidences of hepatoblastoma (high dose group) and of multiple  
2275 hepatocellular adenoma (mid and high dose groups) were found. The incidences of hepatocellular  
2276 carcinoma were increased, but not significantly, in all dose groups. Small and not statistically  
2277 significant increases in the incidences of hepatocellular adenoma occurred in all exposed groups of  
2278 females.

2279 The Panel considers that the two 2-year carcinogenicity studies provide evidence of carcinogenic  
2280 activity of preparations of rhizome/root of *H. canadensis* in the liver of male and female rats and in  
2281 male but not in female mice. The Panel also notes that dose-dependent increases in the incidence  
2282 and severity of hepatic non-neoplastic lesions (hepatocyte hypertrophy and degeneration, and  
2283 eosinophilic foci) were found, which started at the lowest dose tested in the rat study, i.e. 135 and  
2284 150 mg/kg bw per day in males and females respectively. The study is at low risk of bias (tier 1).

### 2285 3.10.6 Human case reports

2286 Three human case reports associated with the consumption of preparations of *H. canadensis*  
2287 rhizome/roots were identified (Bhowmick et al., 2007; Patel et al., 2015; Weissman et al., 2020)  
2288 (Appendix G).

2289 An 11-year-old girl was admitted to the hospital with a history of lethargy, polyuria, polydipsia, and  
2290 a weight loss of approximately 9 kg over a period of 3 weeks (Bhowmick et al., 2007). Type I  
2291 diabetes mellitus with an episode of ketoacidosis, was diagnosed. Severe hypernatremia was also  
2292 observed and the authors thought this to be exacerbated by diuretic properties of a preparation of  
2293 *H. canadensis* that she had taken for at least two weeks prior to admission to hospital at a dose of  
2294 500 mg 2-3 times per day.

2295 A 60-year-old woman with an history of paranoid schizophrenia and diabetes mellitus, who had  
2296 taken *H. canadensis* L. root powder for 4 months (unspecified dose), presented with delusional  
2297 behaviour, jaundice, hepatomegaly (Patel et al., 2015). Liver function test showed elevated total  
2298 bilirubin, AST, ALT and ALP. Viral hepatitis serology, antinuclear antibody, anti-mitochondrial  
2299 antibody, anti-smooth muscle antibody, liver/kidney microsome antibodies, ceruloplasmin level, and  
2300 iron panel were normal. Liver biopsy revealed cholestasis with canalicular and intraductal bile plugs.  
2301 Liver function improved upon cessation of the supplement. No other aetiology of hepatitis was  
2302 identified.

2303 A 53-year-old woman reported abdominal pain two days after consumption of a food supplement  
2304 containing a mixture of herbal preparations, including *H. canadensis* (unspecified dose) (Weissman  
2305 et al., 2020). The patient was diagnosed with acute pancreatitis following a CT scan which showed  
2306 pancreatic inflammation and peri-pancreatic oedema and reported having had another episode of  
2307 pancreatitis in the past after taking the same supplement.

2308 The Panel notes three case reports associated with the consumption of *H. canadensis* supplement  
2309 reporting severe hypernatremia, acute hepatitis and pancreatitis, respectively. No standardised

2310 causality assessment was performed. In the three cases, there is high uncertainty regarding the  
2311 identification of the causing factor, due to the poor description of the *H. canadensis* preparations or  
2312 concurrent ingestion of other substances or medications. The Panel considers that these case reports  
2313 do not allow to conclude on hazards associated with the consumption of preparations of  
2314 rhizome/root of *H. canadensis*.

### 2315 3.10.7 Interactions with medicinal products

2316 A total of eight publications were retrieved which investigated the possible interactions between  
2317 berberine and medicinal products in humans (seven clinical studies) or animals (one study).

#### 2318 3.10.7.1 Human clinical studies

2319 Sandhu et al. (2003) found no effect on the pharmacokinetics of indinavir, a substrate of CYP3A4,  
2320 upon administration of a daily dose of 2280 mg of a preparation of *H. canadensis* 'root' (2.96%  
2321 berberine and 2.0% (-)- $\beta$ -hydrastine) for 14 days in 10 healthy volunteers. In contrast, in a 28-day  
2322 supplementation of *H. canadensis* 'root' extract (2700 mg/day; 2.86% berberine and 2.4% (-)- $\beta$ -  
2323 hydrastine) in 12 healthy volunteers using two probe drug cocktails, midazolam+caffeine and  
2324 chlorzoxazone+debrisoquine, an inhibition of approximately 40% of the activities of CYP2D6 and  
2325 CYP3A4/5 was found, while CYP1A2 and CYP2E1 activities were not affected (Gurley et al., 2005).  
2326 Inhibitory effects of 'root' preparations of *H. canadensis* on CYP3A and CYP2D6 were confirmed in  
2327 further studies using midazolam and debrisoquine as probe drugs (Gurley et al., 2008a; Gurley et  
2328 al., 2008b; Nguyen et al., 2021).

2329 Physiologically based pharmacokinetic (PBPK) modelling in humans, (Nguyen et al., 2023) predicted  
2330 (-)- $\beta$ -hydrastine as the principal constituent responsible for the interaction with midazolam, acting  
2331 through an inhibition of intestinal CYP3A. Although berberine was found to inhibit intestinal CYP3A  
2332 enzymes at high concentrations ( $\geq 50 \mu\text{M}$ ), it was less potent than (-)- $\beta$ -hydrastine.

2333 Upon investigation of the interaction between *H. canadensis* root extract (3210 mg daily for 14 days;  
2334 1.8% isoquinoline alkaloids) and membrane transport protein P-glycoprotein in 20 healthy human  
2335 volunteers, (Gurley et al., 2007) observed a 14% increase in the  $C_{\text{max}}$  of digoxin ( $p < 0.05$ ), used as  
2336 probe drug, while other pharmacokinetics parameters were not affected.

2337 Upon oral administration of 3000 mg 'root' extract of *H. canadensis* daily for 5 days in 16 healthy  
2338 individuals, metformin  $\text{AUC}_{0-\text{inf}}$  and  $C_{\text{max}}$  decreased by 23% and 27% (both  $p < 0.05$ ), while its half-  
2339 life and renal clearance were unchanged, indicating an inhibition of metformin absorption, possibly  
2340 through an inhibition of uptake transporters OCT1/2 (Nguyen et al., 2021). In the same study, no  
2341 effects were found on the pharmacokinetics of rosuvastatin, as a probe drug for efflux transporter  
2342 breast cancer resistance protein (BCRP) and uptake organic anion transporting (OAT) polypeptide  
2343 1B1/3 (P1B1/3), and furosemide, as probe drug for uptake transporter OAT1/3.

#### 2344 3.10.7.2 Animal studies

2345 Oyanna et al. (2023) compared the effects of orally administered berberine (71 mg/kg bw per day),  
2346 (-)- $\beta$ -hydrastine (46 mg/kg bw per day) and *H. canadensis* 'root' extract (500 mg/kg bw per day)  
2347 on metformin pharmacokinetics in mice. *H. canadensis* 'root' extract substantially decreased  
2348 metformin  $C_{\text{max}}$  with no effect on its half-life, whereas berberine or (-)- $\beta$ -hydrastine alone had no  
2349 effect.

### 2350 3.10.7.3 Conclusions on interactions with medicinal products

2351 The Panel notes that there is evidence that preparations of *H. canadensis* rhizome/root can interact  
2352 with various drugs due to its inhibitory effect on CYP enzymes, particularly CYP3A and CYP2D6, and  
2353 possibly intestinal uptake transporters such as OCTs. Experimental data indicated that, apart from  
2354 berberine (Section 3.1.9), (-)- $\beta$ -hydrastine could significantly contribute to these interactions. Other  
2355 constituents of *H. canadensis* rhizome/root may also play a role.

### 2356 3.10.8 Concluding remarks

2357 The oral toxicity of preparations of rhizome/roots of *H. canadensis* has been investigated in a  
2358 comprehensive set of standard 2-week subacute, 90-day subchronic and 2-year carcinogenicity  
2359 toxicity studies in rats and mice conducted by NTP. The studies were conducted with adequately  
2360 characterised preparations, according to standard toxicological guidelines and were at low risk of  
2361 bias (tier 1).

2362 The Panel considers that the subacute, subchronic and carcinogenicity toxicity studies in rodents  
2363 identify the liver as the target organ of the toxicity of *H. canadensis* rhizome/root preparations. In  
2364 addition to the hepatocytic hypertrophy that was observed during the 90-day study in rats,  
2365 hepatocytic degeneration was observed after a 2-year daily exposure. The Panel notes that relevant  
2366 hepatic effects were observed at the lowest dose tested in the rat studies.

2367 Available human data do not allow characterisation of the risk of hepatotoxicity following  
2368 consumption of preparations from the rhizome or root of *H. canadensis*.

2369 An increased incidence of hepatocellular adenoma, an uncommon tumour in F344/N rats that is  
2370 known to progress to malignancy, was observed in both sexes. A positive trend in the incidence of  
2371 hepatoblastoma and hepatocellular adenoma was also found in male mice, whereas no evidence of  
2372 carcinogenicity was found in female mice. Altogether, the Panel considers that available  
2373 carcinogenicity studies provide evidence of carcinogenic activity of *H. canadensis* rhizome/root  
2374 preparations in rodents.

2375 The mechanisms underlying the carcinogenic activity of rhizome/root preparations of *H. canadensis*  
2376 are not fully elucidated. No definitive conclusion on mutagenicity or clastogenicity can be drawn  
2377 from the available studies conducted with preparations of rhizome/root powder of *H. canadensis*  
2378 (section 3.10.2). In vitro genotoxicity findings supported by mechanistic data, indicate a biologically  
2379 plausible genotoxic potential of berberine, one of the major alkaloids of *H. canadensis* rhizome/root  
2380 powder (section 3.10.2.1). The Panel, therefore, considers that a genotoxic MoA is possible.

2381 The Panel notes that the contribution to liver toxicity and carcinogenic activity of other substances  
2382 present in the rhizome/root of *H. canadensis* are not elucidated. Based on the outcome of the QSAR  
2383 analysis, the Panel notes that (-)- $\beta$ -hydrastine, and to a lesser extent canadine, may require further  
2384 investigation regarding their clastogenic/aneugenic potential.

2385 BMD modelling was applied to characterise the dose-response between oral intake of rhizome/root  
2386 powder of *H. canadensis* and critical endpoints identified in the 90-day subchronic and 2-year  
2387 carcinogenicity toxicity studies in rats. However, due to unresolved genotoxicity concerns, the  
2388 derived BMDLs cannot be used to establish a safe level of intake in humans.

2389 The Panel considers that no conclusions can be drawn on potential developmental toxicity of  
2390 rhizome/root preparations of *H. canadensis* from the available toxicological database.

### 2391 3.11 *Jateorhiza palmata* (root)

#### 2392 3.11.1 Characterisation of the plant

2393 *Jateorhiza palmata* (Lam.) Miers (*J. palmata*) is a perennial climbing plant that belongs to the  
2394 Menispermaceae family and is native to East Tropical Africa (Kenya, Tanzania), South Tropical Africa  
2395 (Malawi, Mozambique, Zimbabwe) and Western Indian Ocean (Mauritius). Its common name in  
2396 English is Calumba. The list of synonyms is provided in Appendix A.

2397 Berberine, palmatine and jatrorrhizine have been detected in *J. palmata* roots, but no quantitative  
2398 data were retrieved. Available analytical data regarding protoberberine alkaloids are reported in  
2399 Table 28. Details of the analytical studies are provided in Annex A.

2400 **Table 28:** Alkaloids content in *J. palmata* root

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	NQ	(Sturm & Stuppner, 1998)
<b>Palmatine</b>	NQ	(Sturm & Stuppner, 1998)
<b>Jatrorrhizine</b>	NQ	(Sturm & Stuppner, 1998)

2401 Abbreviations: DW, dry weight; NQ, detected but not quantified in the plant material; w, weight.

#### 2402 3.11.2 Genotoxicity

2403 No eligible genotoxicity studies were retrieved.

#### 2404 3.11.3 General toxicity

2405 No eligible toxicity studies in mammalian species were retrieved.

### 2406 3.12 *Phellodendron amurense* (bark)

#### 2407 3.12.1 Characterisation of the plant

2408 *Phellodendron amurense* Rupr. (*P. amurense*) is a deciduous tree that belongs to the Rutaceae  
2409 family. The plant is native to Asia. Its common name in English is Amur cork tree. The list of  
2410 synonyms is provided in Appendix A.

2411 The main protoberberine alkaloids quantified in *P. amurense* bark include berberine and palmatine,  
2412 with lower amounts of jatrorrhizine, coptisine and columbamine. Other alkaloids include  
2413 magnoflorine, menisperine, phellodendrine, and tetrahydropalmatine, with contents  $\leq 1\%$  w/w, and  
2414 canthin-6-one, noroxyhydrastinine, (-)-(R)-platydesmin, pteleine, and skimmianine, with contents  
2415  $< 0.01\%$ . Available analytical data regarding protoberberine alkaloids and other alkaloids are  
2416 reported in Table 29. Details of the analytical studies are provided in Annex A.

2417 **Table 29:** Alkaloids content in *P. amurense* bark

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	0.05–4.83	(Chen et al., 2010; Hu et al., 2010; Kim et al., 2004; Li et al., 2013; Li et al., 2016a; Liu et al., 1993; Peng et al., 2021; Ryuk et al., 2012; Sheu, 1997; Uzaşçi & Erim, 2014; Xian et al., 2014; Xian et al., 2011; Yang et al., 2010)
<b>Palmitine</b>	0.03–1.55	(Chen et al., 2010; Hu et al., 2010; Kim et al., 2004; Li et al., 2016a; Liu et al., 1993; Peng et al., 2021; Ryuk et al., 2012; Sheu, 1997; Xian et al., 2014; Yang et al., 2010)
<b>Jatrorrhizine</b>	ND–0.313	(Hu et al., 2010; Li et al., 2016a; Liu et al., 1993; Peng et al., 2021; Ryuk et al., 2012; Sheu, 1997; Xian et al., 2014; Yang et al., 2010)
<b>Coptisine</b>	ND–0.11	(Kim et al., 2004; Li et al., 2016a)
<b>Columbamine</b>	0.008–0.052	(Xian et al., 2014)
<b>Epiberberine</b>	ND	(Li et al., 2015)
<b>Other alkaloids</b>		
<b>Magnoflorine</b>	0.08–1.04	(Hu et al., 2010; Liu et al., 1993; Peng et al., 2021; Sheu, 1997; Xian et al., 2014)
<b>Menisperine</b>	0.17–0.57	(Hu et al., 2010)
<b>Phellodendrine</b>	0.07–0.47	(Liu et al., 1993; Peng et al., 2021; Sheu, 1997; Xian et al., 2014; Yang et al., 2010)
<b>Tetrahydropalmatine</b>	0.14–0.29	(Hu et al., 2010)
<b>Canthin-6-one</b>	0.009	(Li et al., 2013)
<b>Noroxyhydrastinine</b>	0.008	(Li et al., 2013)
<b>(–)-(R)-platydesmin</b>	0.006	(Li et al., 2013)
<b>Pteleine</b>	0.006	(Li et al., 2013)
<b>Skimmianine</b>	0.004	(Li et al., 2013)

2418 Abbreviations: DW, dry weight; ND, not detected; w, weight.

### 2419 3.12.2 Genotoxicity studies

2420 An immunofluorescence analysis of DSB markers such as  $\gamma$ -H2AX and 53BP1 foci in human fibroblast  
 2421 cell line (MRC5sv) was performed with a bark extract of *P. amurense* (Inoue et al., 2021). Tested  
 2422 item induced accumulation of DSBs which was confirmed by pulse field gel electrophoresis (PFGE)  
 2423 and through fluorescence microscopy. Since this assay is not an OECD recognised assay for  
 2424 assessing genotoxicity and due to several other shortcomings in the methodological approach or  
 2425 missing information, the study was scored as not reliable (Klimisch score 3), and the relevance of  
 2426 study results as low.

### 2427 3.12.3 Acute toxicity

2428 One publication which reported on single dose administration of preparations of *P. amurense* was  
 2429 retrieved from the systematic search of the literature (Alam et al., 2021) (Table 30).

2430 **Table 30:** LD50 for preparations of bark of *P. amurense* in rats

Publication	Species	Sex, n/group	Test material	Exposure route	Dose (mg/kg bw per day) <sup>a</sup>	LD50 (mg/kg bw)	Comment
<b>Alam et al. (2021)</b>	Wistar rats	M (n=5)	Nexrutine®, (extract of <i>P.amurense</i> bark)	NR	2000	>2000	No mortality

2431 Abbreviations: bw, body weight; LD50; M, male; n, number; NR, not reported.  
 2432 <sup>a</sup> Dose of the test material.

### 2433 3.12.4 General toxicity

2434 One study investigated toxicity of a preparation of *P. amurense* stems in rats (Alam et al., 2021)  
 2435 (Table 31).

2436 **Table 31:** General characteristics of the sub-acute toxicity study of preparations of bark of *P.*  
 2437 *amurense* in rats

Publication	Tier	Species	Sex, n/group	Duration (days)	Test material	Exposure route	Dose (mg/kg bw per day) <sup>(a)</sup>
Alam et al. (2021)	2	Wistar rats	M (n=5)	28	Nexrutine <sup>®</sup> (extract of <i>P. amurense</i> bark)	Gavage	0, 250, 500, 750

2438 Abbreviations: bw, body weight; M, male; n, number.  
 2439 <sup>a</sup> Dose of the test material.

2440  
 2441 A sub-acute toxicity study was conducted in male Wistar rats (n/group=5) to evaluate the effects of  
 2442 a commercial extract of *P. amurense* bark. Rats were administered doses of 0 (control vehicle), 250,  
 2443 500, and 750 mg/kg bw per day via gavage in cellulose containing saline. No deaths were reported  
 2444 during the study. There were no statistically significant differences in body weight changes and food  
 2445 intake. Histopathology of cardiac tissue from treated animals revealed occasional dispersed  
 2446 lymphocytes and congested blood vessels, without evidence of oedema. Lung sections showed  
 2447 minimal prebronchial lymphocytic infiltration and mild vascular congestion, also without oedema. In  
 2448 both control and treated groups, hepatic tissue demonstrated slight lymphatic infiltration  
 2449 surrounding the central vein, in the portal tract, and parenchyma. No treatment-related pathological  
 2450 alterations were observed in the spleen or intestinal tissues. At the highest dose level (750 mg/kg  
 2451 bw), renal tissue exhibited slight tubular degeneration. Differences in relative organ weights of liver,  
 2452 colon, heart, lungs, and spleen between the dosed group and control were not significant. Among  
 2453 all clinical chemistry and haematological parameters tested, small but significant changes were  
 2454 reported for RBC, Hb and HCT levels in the low and median dose (250 and 500 mg/kg bw per day).  
 2455 Values in the highest dose were not significantly different from control. Based on histopathological  
 2456 findings in the kidney and the overall evidence the authors derived a NOAEL of 500 mg/kg bw per  
 2457 day. The Panel concurs with the NOAEL identified by the study authors of 500 mg/kg bw per day of  
 2458 an extract of bark of *P. amurense* for up to 28 days in rats. The Panel however notes the low  
 2459 number of animals and that only one sex was exposed. The study is at moderate risk of bias (tier  
 2460 2).

### 2461 Other studies in mammalian animals

2462 From the systematic search of the literature one additional study was retrieved, which was primarily  
 2463 designed to evaluate the effect of an aqueous bark extract of *P. amurense* on growth and pubertal  
 2464 development in female SD rats (Lee et al., 2018). Animals received either 100 or 300 mg/kg bw per  
 2465 day of the *P. amurense* extract, or distiller water (control). Other groups received recombinant  
 2466 growth hormone, estradiol and triptorelin. Longitudinal bone growth was statistically significantly  
 2467 higher in the low dose group compared to control, but not in the high dose group. The study is at  
 2468 moderate risk of bias (Tier 2).

2469 The Panel considers that no conclusion can be drawn from this study regarding hazard identification  
 2470 of plant preparation of the bark of *P. amurense*.

### 2471 3.13 *Thalictrum flavum* (root)

#### 2472 3.13.1 Characterisation of the plant

2473 *Thalictrum flavum* L. (*T. flavum*) is a perennial stoloniferous and glabrous herbaceous plant that  
 2474 belongs to the Ranunculaceae family and is native to Europe and temperate Asia. Its common name  
 2475 in English is common meadow-rue. The list of synonyms is provided in Appendix A.

2476 Two independent studies have investigated the presence of berberine in the roots of *T. flavum*. One  
 2477 study reported no detectable levels, whereas the other isolated berberine in root samples collected  
 2478 during the flowering stage. The other alkaloid magnoflorine is also present in low amount (<0.01%).  
 2479 Available analytical data regarding protoberberine alkaloids and other alkaloids are reported in  
 2480 Table 32. Details of the analytical studies are provided in Annex A.

2481 **Table 32:** Alkaloids content in *T. flavum* root

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	NQ	(Petruczynik et al., 2018; Ropivia et al., 2010)
	ND	(Petruczynik et al., 2018)
<b>Other alkaloids</b>		
<b>Magnoflorine</b>	0.001	(Petruczynik et al., 2018)

2482 Abbreviations: DW, dry weight; ND, not detected; NQ, detected but not quantified in the plant material; w, weight.

#### 2483 3.13.2 Genotoxicity

2484 No eligible studies were retrieved.

#### 2485 3.13.3 General toxicity

2486 No eligible studies in mammalian species were retrieved.

### 2487 3.14 *Tinospora sinensis* (root, stem, leaf)

#### 2488 3.14.1 Characterisation of the plant

2489 *Tinospora sinensis* (Lour.) Merr. (*T. sinensis*) is a deciduous, climbing shrub that belongs to the  
 2490 Menispermaceae family. The species is native to Asia, with a primary distribution across tropical  
 2491 regions including India, China, Indochina, Myanmar, and Sri Lanka. Its common name in English is  
 2492 Chinese tinospora. The list of synonyms is provided in Appendix A.

2493 The stem of *T. sinensis* contains the protoberberine alkaloids berberine, jatrorrhizine, and palmatine  
 2494 (detected), as well as the alkaloid magnoflorine. No data were retrieved regarding the presence of  
 2495 berberine or other protoberberines in the root and leaf of *T. sinensis*. Available analytical data  
 2496 regarding protoberberine alkaloids and other alkaloids are reported in Table 33. Details of the  
 2497 analytical studies are provided in Annex A.

2498 **Table 33:** Alkaloids content in *T. sinensis* stem

Alkaloid	Content range (% w/w DW)	Reference
<b>Protoberberine alkaloids</b>		
<b>Berberine</b>	0.097	(Srinivasan et al., 2008)
<b>Jatrorrhizine</b>	ND–0.028	(Parveen et al., 2020)
<b>Palmatine</b>	NQ	(Maurya et al., 2009)
<b>Other alkaloids</b>		
<b>Magnoflorine</b>	0.041–0.102	(Parveen et al., 2020)

2499 Abbreviations: DW, dry weight; ND, not detected; NQ, detected but not quantified in the plant material; w, weight.

### 2500 3.14.2 Genotoxicity

2501 No eligible genotoxicity studies were retrieved.

### 2502 3.14.3 Acute toxicity

2503 Three publications reporting on single dose administration of extract of dried stems of *T. sinensis* in  
 2504 rodents were retrieved from the systematic search of the literature (Devbhuti et al., 2009; Khayum  
 2505 et al., 2009; Sharma et al., 2007). The LD50 derived from these studies are reported in Table 34.

2506 **Table 34:** LD50 for preparations of stems of *T. sinensis* in mice

Publication	Species	Sex, n/ group	Test Material	Mode of administration	Dose (mg/kg bw per day) <sup>a</sup>	LD50 (mg/kg bw)
<b>Khayum et al. (2009)</b>	Swiss albino mice	F (NR)	Aqueous, ethanolic extract of dried stems of <i>T. sinensis</i>	NR	5000	>5000
			Petroleum ether extract of dried stems of <i>T. sinensis</i>	NR	2000	>2000
<b>Devbhuti et al. (2009)</b>	Swiss albino mice	M (NR)	Methanolic extract of dried stems of <i>T. sinensis</i>	NR	500-3500	>3500
			Aqueous extract of dried stems of <i>T. sinensis</i>	NR	500-3500	>3500
<b>Sharma et al. (2007)</b>	Swiss albino mice	F (n=6)	Aqueous extract of dried stem of <i>T. sinensis</i>	NR	5000	>5000
			Ethanolic extract of dried stems of <i>T. sinensis</i>	NR	5000	>5000
			Petroleum ether extract of dried stems of <i>T. sinensis</i>	NR	2000	>2000

2507 Abbreviations: bw, body weight; LD50, median lethal dose; M, male; F, female; n, number; NR, not reported.

2508 <sup>a</sup> Dose of the test material.

### 2510 3.14.4 General toxicity

2511 No eligible studies were retrieved.

### 2512 3.14.5 Human case reports

2513 One publication was retrieved reporting two cases of jaundice and acute hepatitis in two adult men  
 2514 who had consumed "*T. sinensis*" (no further information provided) for 3 months and 18 days,  
 2515 respectively (Wu et al., 2013). In the two cases, marked elevation of liver enzymes and bilirubin,  
 2516 leucocytosis, monocytosis, coagulopathy and duodenal ulcer with bleeding were reported. In one  
 2517 case, eosinophilia was also found. The patients were said to have "subsequently recovered after  
 2518 supportive care". No information was given regarding the medical history of the patients.

2519 The Panel notes 2 cases of acute hepatitis associated with the consumption of *T. sinensis*  
 2520 supplement. No standardised causality assessment was performed. In the two cases, there is high  
 2521 uncertainty regarding the identification of the causing factor due to the poor description of the  
 2522 supplements, the lack of information regarding the medical history of the patients or concurrent  
 2523 medications, and the limited description of the course of events. The Panel considers that these  
 2524 case reports do not allow to conclude on hazards associated with the consumption of *T. sinensis*.

## 2525 3.15 Exposure assessment

### 2526 3.15.1 Occurrence data

2527 Occurrence data of berberine and other protoberberine alkaloids in food supplements containing  
 2528 preparations of *B. aristata* root or bark, based on data submitted through the ad-hoc call for data  
 2529 (Section 7) (Unpublished data (SISTE, 2023)), are provided in Table 35. No data were received on  
 2530 the other plant species (section 2.2.3).

2531 **Table 35:** Concentration of berberine and protoberberine alkaloids in food supplements containing  
 2532 preparations of *B. aristata* root or bark

Alkaloid	N samples	Max LOQ	%LC	Mean (mg/g <sup>a</sup> )	
				LB <sup>b</sup>	UB <sup>b</sup>
<b>Berberine</b>	47	4	-0	402	402
<b>Jatrorrhizine</b>	41	0.5	19.5	6.78	6.86
<b>Thalifendine</b>	34	0.442	14.7	1.57	1.63
<b>Palmatine</b>	38	0.5	34	1.08	1.21
<b>Berberrubine</b>	40	0.5	20	1.07	1.15
<b>Berberastine</b>	34	0.442	97.0	0.019	0.290
<b>Demethyleneberberine</b>	40	0.5	70	0.174	0.467
<b>Corysamine</b>	34	0.442	100	0	0.143
<b>Fissisaine</b>	34	0.442	100	0	0.143
<b>Stephabine</b>	34	0.442	100	0	0.143
<b>Thalidastine</b>	34	0.442	100	0	0.143

2533 Abbreviations: LB, lower bound; LOQs, limits of quantification; N, number of available analytical results; %LC, percentage of left  
 2534 censorship; UB, upper bound.

2535 <sup>a</sup> A dose form may be, for instance, 1 tablet, 1 capsule, 5 g powder, as defined by the data provider.

2536 <sup>b</sup> The lower bound (LB) estimate is obtained by assigning a value of zero to all samples reported as lower than the limit of detection (<  
 2537 LOD) or limit of quantification (< LOQ); the upper bound (UB) estimate is obtained by assigning the numerical value of LOD to values  
 2538 reported as < LOD and LOQ to values reported as < LOQ, depending on whether LOD or LOQ is reported by the data provider (EFSA,  
 2539 2010b). Further explanations are provided in Annex D.

2540

2541 3.15.2 Data from the Mintel’s Global New Products Database and from manufacturers

2542 **Food supplements**

2543 The search for the category “Vitamins & Dietary supplements” identified 55 multiherbal food  
 2544 supplements which, according to the information in the ingredient labels, contained preparations of  
 2545 the parts of the plants from the mandate. These products were marketed in 14 EU countries. The  
 2546 number of products by plant species (and parts, when provided) and the amount of berberine  
 2547 content or the plant preparation per serving, as reported on the label, is provided in Table 36.

2548 The majority of the products mentioned berberine in their ingredients list or product description,  
 2549 with about half of the products reporting berberine content in mg/serving. The level of descriptions  
 2550 was heterogeneous regarding the form of berberine salt (chloride salts reported on 27% of the  
 2551 products; not specified on other products) and berberine purity (ranging from 2 to 98%; not  
 2552 specified on 35% of the products).

2553 Findings from a survey carried out by the European Federation of Associations of Health Product  
 2554 Manufacturers (EHPM) submitted to EFSA through the call for data are consistent with the Mintel  
 2555 data (Biagi, 2023). Information collected among manufacturers reported more than 60 food  
 2556 supplements containing berberine (as berberine hydrochloride). Content of other protoberberine  
 2557 alkaloids was not reported in any of these products. The most common preparation were *B.*  
 2558 *aristata* bark extracts (59%), followed by *B. vulgaris* bark extracts (17%). The remaining food  
 2559 supplements contained *P. amurense* bark extracts (8%), or *H. canadensis*, *C. majus*, or other  
 2560 *Berberis* species (8%). Most of the products’ labels recommended a daily berberine dose of 500  
 2561 mg/day.

2562 **Table 36:** Food supplements with berberine containing plant preparations as ingredient

Plant species	n total products	Plant part specified (n products)	Labelled compound (n products)	Labelled purity of the compound (n products)	Labelled content (range min-max, mg/serving <sup>a</sup> )	
					BBR/ BBR Cl (n products)	Plant preparation (n products)
<i>B. aristata</i>	39	bark/root (30)	BBR (25) BBR Cl (11) NR (3)	2% (1), 8% (1), 23% (3), 85% (10), 95% (1), 97% (5), 98% (1), NR (17)	10-1000 (28)	27-1190 (32)
<i>B. vulgaris</i>	6	Bark (2), root/root bark (3), NR (1)	BBR Cl (3)	85% (1), 97% (1), NR (4)	200-1164 (3)	75-1200 (4)
<i>H. canadensis</i>	6	Root (2), NR (4)	BBR Cl (1), NR (5)	NR (5)	NR (5)	30-65 (2)
<i>C. teeta</i>	2	Root/rhizome (2)	BBR (1)	8%	6-8 (2)	75-100 (2)
<i>B. aquifolium</i>	1	root (1)	NR (1)	NA	NA	50 (1)
<i>P. amurense</i>	1	bark (1)	NR (1)	NA	NA	50 (1)

2563 Abbreviations: BBR, berberine; Cl, chloride; n, number; NA, not applicable; NR, not reported.

2564 <sup>a</sup> Servings are defined by the food manufacturer and vary across products (e.g. 1 serving = 1 tablet or 3 tablets, depending on the products).

2565

2566 **Other food products**

2567 The search in the other food sub-categories than "Vitamins & Dietary supplements" yielded 13 food  
 2568 products mentioning a preparation of one of the plant species under assessment among their  
 2569 ingredients:

- 2570 • 4 products of the sub-category "tea", containing *C. fenestratum* (stem; 1 product), *C.*  
 2571 *japonica* (part not specified; 1 product), and *C. majus* (herb; 2 products);
- 2572 • 1 product of the sub-category "malt & other hot beverages", containing *C. japonica* (part not  
 2573 specified);
- 2574 • 7 products of the sub-categories "gin" and "liqueur", containing *J. palmata* root;
- 2575 • 1 product of the sub-category "decongestive, Cough, Cold & Flu Relief", containing *J. palmata*  
 2576 root
- 2577 • 1 product of the sub-category "nutritional & meal replacement drinks", containing *B. aristata*  
 2578 bark.

2579 The berberine content was not labelled on any of these products.

2580 The Panel notes that the search in the Mintel GNPD captured only those products (food supplements  
 2581 and other food products) that were introduced on the market between October 2015 and October  
 2582 2025, or for which the packaging was changed during this period. Therefore, the information  
 2583 collected is indicative and does not necessarily represent a comprehensive overview of the products  
 2584 available on the market nor does it reflect their market share. The database does not cover products  
 2585 sold in pharmacies.

2586 **3.15.3 Exposure estimates and associated uncertainties**

2587 The Panel notes that, based on available information, preparations of the plants under assessment  
 2588 are not part of the habitual diet of the European populations. Preparations of some of the plants  
 2589 may be used as ingredients of specific food products present in the EU market, other than food  
 2590 supplements. In view of the limited number of products identified in Mintel and the lack of eating  
 2591 occasion reported in the EFSA comprehensive database regarding those products, the dietary  
 2592 exposure to berberine through the background diet is expected to be negligible in the general  
 2593 European population.

2594 A theoretical daily exposure to berberine via food supplements was estimated based on information  
 2595 received from stakeholders or reported on the labels of products identified in the Mintel GNPD  
 2596 database and are reported in Table 37. The Panel notes that these estimates represent the range  
 2597 of average daily exposures to berberine among consumers of berberine-containing food  
 2598 supplements. They are indicative as they are not based on actual consumption data and rely on the  
 2599 assumption that consumers follow the use levels recommended by the manufacturers. In addition,  
 2600 regarding data collected in the Mintel GNP database, the estimates rely on the assumption that the  
 2601 actual content of the product complies with the content declared on the food labels.

2602 **Table 37:** Mean, minimum and maximum theoretical daily exposure to berberine (mg/day) via  
 2603 food supplements containing preparations of berberine-containing plants

Plant species	Plant part	n total products	Mean	Median	Minimum <sup>a</sup>	Maximum <sup>a</sup>
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**Based on content data received through the call for data**

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<i>B. aristata</i>	Bark, root	47 <sup>(b)</sup>	405	445	73	496
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**Based on content data from the Mintel GNPD**

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<i>B. aristata</i>	Bark, root or unspecified part	30	332	274	10	1000
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<i>B. vulgaris</i>	Root or root bark	3	621	500	200	1164
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<i>C. teeta</i>	Root/rhizome	2	NA	NA	6	8
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2604 Abbreviations: n, number; NA, not applicable.

2605 <sup>a</sup> The minimum and maximum values correspond to the food supplements providing the lowest and highest daily exposure respectively,  
2606 as calculated based on the content information reported on product labels and use levels recommended by the manufacturers.

2607 <sup>b</sup> Data were provided for 47 individual analytical samples. Some analytical values may relate to the same commercial product.

## 2608 4 Integration of the evidence and uncertainties

2609 A summary of the body of evidence available for hazard identification and characterisation of  
2610 berberine and other protoberberines tested as single substances, and as preparations of plant  
2611 species included in the mandate, is provided in Table 38 and Table 39, respectively.

2612  
2613

**Table 38:** Summary of the body of evidence available for hazard identification and characterisation on berberine and other protoberberines tested as single substances, by study type

	<b>In silico, in vitro and animal in vivo genotoxicity studies<sup>a</sup></b>	<b>Animal carcinogenicity studies</b>	<b>Animal sub-chronic toxicity studies</b>	<b>Animal developmental toxicity studies</b>	<b>Human case reports</b>	<b>Human RCTs</b>
Berberine	Consistent positive findings in vitro regarding DNA damage and gene mutation (HPRT mammalian cell and <i>S. typhimurium</i> TA98) supported by mechanistic data (e.g. DNA intercalator, inhibitor of topoisomerases I and II). In vivo findings inconclusive	No data	<u>In rats:</u> No effect at a dose of 156 mg/kg bw per day (90-day study; tier 2, moderate RoB)	<u>In rats:</u> Maternal NOAEL 223 mg/kg bw per day; Developmental NOAEL 792 mg/kg bw per day (20-d study; tier 2, moderate RoB) <u>In mice:</u> Maternal NOAEL 450 mg/kg bw per day; Developmental NOAEL 666 mg/kg bw per day (17-d study; tier 2, moderate RoB)	3 cases; Causal relationship cannot be ascertained	Gastrointestinal symptoms
Berberastine	No data High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	No data	No data	No data	No data
Berberrubine	Inhibitor of topoisomerase II. High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	<u>In rats:</u> Signs of hepatotoxicity at a dose of 100 mg/kg bw per day (42-day study; tier 2, moderate RoB)	No data	No data	No data
Columbamine	No data. High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	<u>In rats:</u> No effect at a dose of 154 mg/kg bw per day (90-day study; tier 2, moderate RoB)	No data	No data	No data
Coptisine	Inconclusive results regarding DNA damage (comet assay); inhibitor of topoisomerase I. High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	<u>In rats:</u> No effect at a dose of 156 mg/kg bw per day (90-day study; tier 2, moderate RoB)	No data	No data	No data
Corysamine	No data	No data	No data	No data	No data	No data

	<b>In silico, in vitro and animal in vivo genotoxicity studies<sup>a</sup></b>	<b>Animal carcinogenicity studies</b>	<b>Animal sub-chronic toxicity studies</b>	<b>Animal developmental toxicity studies</b>	<b>Human case reports</b>	<b>Human RCTs</b>
	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model					
	No data					
Demethylene-berberine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	No data	No data	No data	No data
	No data.					
Epiberberine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts	No data	<u>In rats</u> : No effect at a dose of 156 mg/kg bw per day (90-day study; tier 2, moderate RoB)	No data	No data	No data
	No data					
Fissisaine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	No data	No data	No data	No data
	No data					
Groenlandicine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	No data	No data	No data	No data
	No data.					
Jatrorrhizine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts	No data	<u>In rats</u> : No effect at a dose of 70 mg/kg bw per day (90-day study; tier 2, moderate RoB)	No data	No data	No data
	Limited evidence suggestive of DSB in vitro; inhibitor of topoisomerases I and II.					
Palmatine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts	No data	<u>In rats</u> : No effect at a dose of 156 mg/kg bw per day (90-day study; tier 2, moderate RoB)	No data	No data	No data
	No data					
Stephabine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	No data	No data	No data	No data
	No data					
Thalidastine	High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model	No data	No data	No data	No data	No data
	No data					
Thalifendine	No data	No data	No data	No data	No data	No data

In silico, in vitro and animal in vivo genotoxicity studies <sup>a</sup>	Animal carcinogenicity studies	Animal sub-chronic toxicity studies	Animal developmental toxicity studies	Human case reports	Human RCTs
High structural similarity with BBR and shared QSAR predicted mutagenicity alerts. Predicted mutagenicity by VEGA model					

2614 Abbreviations: BBR, berberine; bw, body weight; DNA, Deoxyribonucleic Acid; HPRT, Hypoxanthine-Guanine Phosphoribosyltransferase; NOAEL, No Observed Adverse Effect Level; QSAR, Quantitative  
2615 Structure-Activity Relationship; RCTs, Randomised controlled trials; RoB, Risk of Bias.-Observed-Adverse-Effect Level; QSAR  
2616 <sup>a</sup> For conciseness, a summary of the key findings from genotoxicity studies is provided, reflecting the weight of the evidence. Please refer to the relevant section for information regarding the reliability and  
2617 relevance of individual studies.

2618 **Table 39:** Summary of the body of evidence available for hazard identification and characterisation on preparations of plant species included in the  
2619 mandate, by study type

Plant species (part)	In silico, in vitro and animal in vivo genotoxicity studies	Animal carcinogenicity studies	Animal sub-acute and sub-chronic toxicity studies	Animal developmental toxicity studies	Human case reports	Human RCTs
<i>B. aquifolium</i> (root)	No data	No data	No data	No data	No data	No data
<i>B. aristata</i> (root, bark)	Aqueous extract not mutagenic in <i>S. typhimurium</i> MTCC 1251; study not reliable; relevance low	No data	No data	No data	No data	No data
<i>B. vulgaris</i> (root, bark)	No data	No data	No data	No data	No data	No data
<i>C. majus</i> (herb)	<u>Sanguinarine</u> : evidence of chromosomal and DNA-damaging effects in vivo (i.p.); inhibition of Topoisomerase II; epidemiological study (mouthwash); Predicted mutagenicity by VEGA model. <u>Chelerythrine</u> : no experimental data; high structural similarity with sanguinarine	No data	<u>In rats</u> : No effect on liver of an ethanolic extract of aerial parts up to a dose of 3000 mg/kg bw per day for 28 days (28-day study; tier 2, moderate RoB)	No data	43 cases; Hepatotoxicity very likely attributed to consumption of <i>C. majus</i> commercial product, compatible with idiosyncratic form of HILI	No data
<i>C. japonica</i> (rhizome)	Mutagenic in <i>S. typhimurium</i> TA98; study reliable with restrictions; relevance limited	No data	No data	No data	No data	No data
<i>C. teeta</i> (rhizome)	No data	No data	No data	No data	No data	No data
<i>C. trifolia</i> (rhizome)	No data	No data	No data	No data	No data	No data

Plant species (part)	In silico, in vitro and animal in vivo genotoxicity studies	Animal carcinogenicity studies	Animal sub-acute and sub-chronic toxicity studies	Animal developmental toxicity studies	Human case reports	Human RCTs
<i>C. fenestratum</i> (root, stem)	No data	No data	<u>In rats</u> : No effect of an aqueous stem extract at a dose of 2500 mg/kg bw per day (90-day study: tier 3, high RoB)	No data	No data	No data
<i>H. canadensis</i> (root/rhizome)	Not mutagenic in <i>S. typhimurium</i> TA98 and TA100; study reliable with restrictions, relevance limited. Inconclusive results in mice erythrocyte micronucleus test in vivo; study reliable with restrictions, relevance low. VEGA: Predicted in vitro MN genotoxicity of (-)- $\beta$ -hydrastine and canadine; high structural similarity between (-)- $\beta$ -hydrastine and (S,R)-noscapine, which has aneugenic potential	Root powder carcinogenic in male and female rats and female mice  <u>In rats</u> : BMDL10 of 45.5 mg/kg bw for females; ND for males - based on hepatocellular hypertrophy in the 2-year study (tier 1, low RoB)	Root powder hepatotoxic in male and female rats and mice (14-day, 90-day and 2-year study)	Inconclusive (tier 3, high RoB)	3 cases; Causal relationship cannot be ascertained	No data
<i>J. palmata</i> (root)	No data	No data	No data	No data	No data	No data
<i>P. amurense</i> (bark)	Induction of DSBs; study not reliable; relevance low	No data	NOAEL of 500 mg/kg bw per day of a bark extract (28-day study; tier 2, moderate RoB)	No data	No data	No data
<i>T. flavum</i> (root)	No data	No data	No data	No data	No data	No data
<i>T. sinensis</i> (root, stem, leaf)	No data	No data	No data	No data	2 cases; Causal relationship cannot be ascertained	No data

2620  
2621

Abbreviations: BBR, berberine; bw, body weight; DNA, Deoxyribonucleic Acid; DSB, double strand breaks; HILI, Herb-Induced Liver Injury; i.p, intraperitoneal; MN, micronucleus; MTCC, Microbial Type Culture Collection; NOAEL, No-Observed-Adverse-Effect Level; QSAR, Quantitative Structure-Activity Relationship; RCTs, Randomised controlled trials; RoB, Risk of Bias.

## 2622 4.1. Berberine and protoberberines content of the plant species included in 2623 the assessment

2624 The amount of analytical data on the berberine content of the plant species (and relevant parts  
2625 thereof) included in the mandate is heterogeneous. Quantitative analytical data were particularly  
2626 scarce on some of the plant species, with only one study quantifying berberine content in *C. trifolia*  
2627 rhizome (section 3.8.1) and *T. sinensis* root (section 3.14.1), and no quantification available on *T.*  
2628 *sinensis* stem and leaves (section 3.14.1), *J. palmata* root (section 3.11.1), or *T. flavum* root (section  
2629 3.13.1). The highest contents of berberine were reported in *B. aristata* root, *C. japonica* rhizome, *C.*  
2630 *teeta* rhizome, *C. fenestratum* stem, *H. canadensis* root/rhizome and *P. amurense* bark, with wide  
2631 content variations across different samples of the same plant species. Differences among values  
2632 reflect variability due to both intrinsic natural factors (e.g. botanical origin, plant part, developmental  
2633 stage, harvesting time) and extrinsic methodological factors. The latter include sample preparation  
2634 and extraction methods (e.g. solvent type, temperature, extraction time, drug-to-solvent ratio), as  
2635 well as analytical methods and quantification strategies.

2636 There is also heterogeneity in the identification and characterisation of other protoberberines in  
2637 these plant species, as they were not systematically investigated in the available analytical studies.  
2638 The most comprehensive chemical profiling is available for preparations of *B. aristata* root and bark,  
2639 for which analytical data were received through the call for data.

## 2640 4.2. ADME

2641 The absorption of berberine in the intestinal tract is low, and the intestine serves as the primary site  
2642 of phase I metabolism. The gut microbiota can metabolise berberine into dihydroberberine, which  
2643 is reconverted into berberine once in the enterocytes, thereby contributing to berberine absorption.  
2644 The gut microbiota may also generate other metabolites, such as oxyberberine, and contribute to  
2645 the enterohepatic recirculation of berberine and its metabolites, therefore influencing the  
2646 toxicokinetics of berberine (section 3.1.2.1).

2647 After absorption, berberine and its metabolites undergo further metabolism in the liver, where they  
2648 are rapidly converted into glucuronidated and sulphated forms. As a result, systemic exposure to  
2649 berberine is low, whereas its phase II metabolites and, to a lesser extent, the phase I metabolite  
2650 berberrubine, are the predominant forms found in the systemic circulation (sections 3.1.2.2,  
2651 3.1.2.3).

2652 The metabolism of berberine is further complicated when co-occurring protoberberines from plant  
2653 preparations are involved. This is due to shared absorption pathways and common metabolic routes,  
2654 which can lead to complex interactions and formation of overlapping downstream metabolites  
2655 (section 3.1.2.6).

## 2656 4.3. Genotoxicity

2657 When tested as a single substance, berberine was found to be mutagenic in an in vitro HPRT  
2658 mutation assay in mouse cells and in the Ames test TA98 *S. typhimurium* strain. The mutagenic  
2659 effect was observed only in the absence of metabolic activation (S9-mix), suggesting that berberine  
2660 has direct mutagenic potential (section 3.1.3). Regarding plant extracts containing berberine,  
2661 mutagenic activity of several extracts of *C. japonica* rhizome (berberine content 12.6%-22.2%)

2662 (section 3.6.1) and of a powdered extract of *C. chinensis* rhizome (berberine content 21.2%)  
2663 (section 3.1.3) was also found in the TA98 strain without S9 mix. Comparative analysis indicated  
2664 that the genotoxic responses observed with the extract of *C. chinensis* rhizome were associated with  
2665 its berberine content in the TA98 Ames assay and the TK6 in vitro MN assay in a dose dependent  
2666 manner.

2667 In contrast, no mutagenic activity in the Ames test was found with *H. canadensis* root powder in  
2668 T98 and the other bacterial strains tested (berberine content NR) (section 3.10.2). However, in one  
2669 study it was found that several commercial products of *H. canadensis* increased levels of the  $\gamma$ -  
2670 H2AX protein (marker of DNA damage) in HepG2 cells, with the effect correlating with berberine  
2671 content, suggesting a potential activation of a DNA damage response. One mutagenicity study on  
2672 an aqueous extract of the root bark of *B. aristata* (berberine content not reported) was inconclusive  
2673 due to methodological limitations (section 3.3.2).

2674 Overall, there is convincing evidence from in vitro assays on the mutagenicity of berberine, especially  
2675 its ability to cause frameshift mutations. This is in line with mechanistic evidence regarding the  
2676 capacity of berberine to intercalate into DNA, which may also involve inhibition of topoisomerases.

2677 Regarding clastogenic and/or aneugenic effects of berberine, limited evidence was found in two in  
2678 vitro MN assays in mammalian cells. One in vivo experiment in mice was inconclusive (section 3.1.3).  
2679 Regarding berberine-containing plant extracts, inconclusive results were found for a *H. canadensis*  
2680 rhizome/roots powder (berberine content 3.45%) in an in vivo MN test in mice (section 3.10.2). No  
2681 data is available on the other plant species included in the mandate.

2682 In vitro studies in human and murine cell lines provided consistent evidence regarding the potential  
2683 of berberine to induce both DNA SSBs and DSBs (section 3.1.3).

2684 Mechanistic data indicated several potential modes of action for genotoxicity, including berberine's  
2685 ability to inhibit DNA topoisomerases I and II, intercalate into DNA, cause oxidative DNA damage,  
2686 and impair DNA repair pathways. Berberrubine, the main primary metabolite of berberine, was also  
2687 found to specifically inhibit topoisomerase II. These findings collectively support plausible and  
2688 potentially interconnected mechanisms of genotoxicity.

2689 Regarding berberine, while the results of in vitro tests provide evidence of genotoxicity, the main  
2690 uncertainties relate to the lack of reliable in vivo studies to confirm or refute genotoxic effects at  
2691 both systemic and first-contact sites of exposure.

2692 Regarding genotoxicity of berberine-containing plant preparations, there is further uncertainty  
2693 regarding the genotoxicity of other compounds that may be present in the preparations, including  
2694 other protoberberine alkaloids, which share high structural similarity with berberine and similar  
2695 predictions in QSAR analyses. Sparse data on coptisine and palmatine suggest genotoxic potential,  
2696 but the interpretation of findings is limited by the lack of standardised testing protocols, and the  
2697 genotoxicity of other protoberberines remains largely unknown.

#### 2698 4.4. Carcinogenicity

2699 There is evidence of carcinogenic activity of *H. canadensis* rhizome/root preparations in rodents  
2700 (section 3.10.5). In rats, an increased incidence of hepatocellular adenoma, an uncommon tumour  
2701 in F344/N rats that is known to progress to malignancy, was observed in both sexes. A positive

2702 trend in the incidence of hepatoblastoma and hepatocellular adenoma was also found in male mice,  
2703 whereas no evidence of carcinogenicity was found in female mice. The Panel notes that evidence  
2704 for carcinogenicity was consistently found in male and female rats and male mice. The Panel further  
2705 notes that the evidence is derived from two studies compliant with established standards for  
2706 carcinogenicity testing, both considered at low risk of bias (tier 1), strengthening the reliability of  
2707 these findings.

2708 The Panel notes that the carcinogenic mechanisms of *H. canadensis* rhizome/root powder are not  
2709 elucidated. A MoA involving genotoxic effects of berberine and/or its metabolites is possible but  
2710 requires confirmation in vivo. Data regarding other, non-genotoxic pathways, are lacking. Also, it is  
2711 unknown whether alkaloids or other compounds present in the plant's rhizome/root could be  
2712 involved.

2713 *H. canadensis* rhizome/root powder is the only plant preparation for which carcinogenicity data are  
2714 available. The Panel notes that the carcinogenic potential of preparations of the other plants included  
2715 in the mandate has not been investigated.

#### 2716 4.5. Liver toxicity

2717 No sign of liver toxicity was reported at a dose of 156 mg/kg bw per day berberine (extracted from  
2718 *Coptis* species, purity NR) in a 90-day toxicity study in rats but the number of animals examined  
2719 was small. Pharmacological and mechanistic studies on berberine involving mammalian species,  
2720 which included liver examinations, did not indicate liver toxicity (section 3.1.5.1). In addition, no  
2721 increases in liver weight were seen in the dams in developmental toxicity studies with rats and mice  
2722 at dose levels up to 792 mg/kg bw per day berberine chloride (section 3.1.6). In contrast, some  
2723 signs of liver toxicity were found in a 42-day study on rats exposed to synthetic berberrubine (purity  
2724 >98%), the main primary metabolite of berberine, at the highest dose tested of 100 mg/kg bw per  
2725 day, whereas results at lower doses were inconclusive (section 3.1.5.2). In RCTs on berberine  
2726 supplementation, clinically meaningful elevations in serum transaminases have been reported in two  
2727 participants receiving berberine, of which one was judged probably related to the consumption of  
2728 the supplement. The Panel notes that liver function was tested in a limited number of trials, many  
2729 of which were also of limited duration (median 8 weeks). Overall, the Panel considers that, in view  
2730 of the methodological limitations of the available studies, the hepatotoxicity of berberine cannot be  
2731 ascertained based on available animal and human studies.

2732 Evidence on hepatotoxic effect of berberine-containing plant preparations is heterogeneous. Some  
2733 animal studies on preparations of *P. amurense* (section 3.12.4) and *C. fenestratum* (section 3.9.4)  
2734 did not indicate liver toxicity, but serious methodological limitations prevent definitive conclusions.

2735 In contrast, liver toxicity was consistently observed in available toxicity studies of *H. canadensis*  
2736 rhizome/root preparations in rodents, following subacute (14 days), subchronic (90 days), chronic  
2737 (2 years) exposure and in the dams in developmental toxicity studies (sections 3.10.3 and 3.10.4).  
2738 Increased liver weight and hepatocellular hypertrophy were observed in shorter term studies. Upon  
2739 chronic exposure, hepatocyte degeneration and eosinophilic foci were also observed in rats. As  
2740 discussed above, an increased incidence of adenomas was also found in hepatic tissues. The Panel  
2741 notes that the evidence on hepatotoxicity of *H. canadensis* rhizome/root preparations is consistent  
2742 and is derived from several toxicity studies that complied with established standards for toxicity  
2743 testing and were at low risk of bias (tier 1), thereby strengthening the reliability of these findings.

2744 The Panel notes that increased liver weight, the most sensitive endpoint, was found at the lowest  
2745 doses tested, i.e. 155 and 150 mg/kg bw per day in male and female rats, respectively, in a subacute  
2746 study. Considering that the root preparation used in this experiment contained 3.45% berberine,  
2747 the corresponding exposure to berberine was about 5 mg/kg bw per day. The low exposure to  
2748 berberine resulting from the *H. canadensis* preparation in this study suggests that berberine is  
2749 unlikely to be the constituent responsible for the observed hepatotoxicity, given the absence of liver  
2750 toxicity reported in studies examining berberine alone at substantially higher doses. Nevertheless,  
2751 uncertainty remains due to the methodological limitations of the available studies investigating  
2752 berberine hepatotoxicity. Regarding human data, there is only one published case report of liver  
2753 injury associated with the consumption of *H. canadensis* L. root powder (section 3.10.6).

2754 In humans, available case reports raise concerns regarding a risk for an idiosyncratic form of herb-  
2755 induced liver injury following consumption of preparations of aerial parts of *C. majus*. Jaundice was  
2756 the predominant symptom, sometimes preceded by darkening of the urine, itching, fatigue,  
2757 abdominal pain and/or nausea. The latency periods to first symptoms varied from some weeks to  
2758 some months. The underlying mechanisms are unknown, including whether it may be mediated by  
2759 berberine or other substances present in the preparations. No report of clinically apparent liver injury  
2760 associated with berberine supplementation was found. Only two cases of acute hepatitis associated  
2761 with the consumption of *T. sinensis* supplement are published.

2762 Overall, the Panel considers that there is evidence that preparations of rhizome/root of *H. canadensis*  
2763 and of aerial parts of *C. majus* can cause liver injury. Regarding *H. canadensis* rhizome/root, the  
2764 hepatotoxic effect was found to be consistent and dose-dependent in rodents. For *C. majus* aerial  
2765 parts, cases of liver injury have been observed in humans and appear to depend on individual  
2766 susceptibility factors. The Panel notes that data are suggestive of different mechanisms of liver  
2767 toxicity. Notably, the plant preparations have distinct alkaloid profiles (sections 3.5.1 and 3.10.1).  
2768 The role of berberine in these effects remains uncertain.

#### 2769 4.6. Reproductive and developmental toxicity

2770 Signs of maternal and foetal toxicity have been found in developmental toxicity studies in rats and  
2771 mice of berberine. The lowest NOAELs were 223 mg/kg bw per day for maternal toxicity determined  
2772 in rats (based on increased water consumption), and 666 mg/kg bw per day for foetal toxicity (based  
2773 on increased incidence of malformations), with mice as the most sensitive species (section 3.1.6).  
2774 No maternal or foetal toxicity was observed in one study in rats administered a commercial extract  
2775 of *H. canadensis* at a dose of 1860 mg/kg bw daily. However, due to its methodological limitations,  
2776 no firm conclusions can be drawn from this study on potential developmental toxicity of  
2777 rhizome/roots preparations of *H. canadensis* (section 3.10.4). Data are lacking regarding the  
2778 developmental toxicological potential of preparations of the other plant species.

2779 No studies on reproductive toxicity are available.

#### 2780 4.7. Other adverse effects

2781 Regarding other adverse effects identified in the protocol, the Panel did not find data indicating  
2782 hypoglycaemic or hypotension episodes in consumers of berberine or berberine-containing plant  
2783 preparations, based on the human studies included in this assessment (clinical trials and case

2784 reports). No evidence of immunotoxicity following oral administration of berberine or berberine-  
2785 containing plant preparations was found in animal or human studies.

2786 The Panel notes that, except for the toxicity studies conducted on root powder of *H. canadensis*, all  
2787 animal studies investigating the toxicity of preparations of other plant species were at moderate  
2788 (tier 2) to high (tier 3) risk of bias (Appendix D). Critical methodological limitations included the poor  
2789 description of the test material, the limited number of organs and endpoints tested, and incomplete  
2790 reporting. Therefore, for most plant species included in the mandate, there is substantial uncertainty  
2791 in using available animal toxicity studies for hazard identification and hazard characterisation and  
2792 their toxicity profiles remain largely unknown.

#### 2793 4.8. Interactions with medicinal products

2794 Available data indicate that berberine has the potential to inhibit CYP3A4 and possibly CYP2D6 and  
2795 CYP2C9 (section 3.1.9). There is also evidence that preparations of *H. canadensis* rhizome/root  
2796 extracts have inhibitory effects on CYP3As and CYP2D6, and possibly intestinal uptake transporters,  
2797 e.g. OCTs. Apart from berberine, (-)- $\beta$ -hydrastine could significantly contribute to these interactions,  
2798 as well as other uncharacterised compounds in these preparations (section 3.10.7).

2799 Overall, berberine-containing plant preparations may interact with various medicinal products.

#### 2800 4.9. Gastrointestinal symptoms

2801 The consumption of berberine-containing food supplements may cause transient gastrointestinal  
2802 symptoms. Across available RCTs on berberine supplementation, constipation, diarrhoea, nausea,  
2803 abdominal pain/discomfort were more frequently reported in participants receiving berberine (daily  
2804 doses of between 400 mg and 1500 mg) compared to placebo (section 3.1.7). In most studies, the  
2805 specific forms and sources of berberine were not reported.

### 2806 5 Conclusions

2807 The Panel concludes that:

- 2808 • There is evidence for berberine genotoxicity in vitro, indicating gene mutation and  
2809 chromosomal damage, which are supported by mechanistic evidence. Confirmation in vivo  
2810 is lacking, especially at contact sites of exposure, namely the gastrointestinal tract and the  
2811 liver.
- 2812 • In view of their high structural similarity with berberine, the genotoxic potential of other  
2813 protoberberine alkaloids also requires consideration. Available experimental data are sparse  
2814 and inconclusive.
- 2815 • Other alkaloids present in the plant preparations included in the mandate, including  
2816 sanguinarine and chelerythrine in *C. Majus*, also raise some genotoxic concerns.
- 2817 • There is no adequate repeated dose toxicity study on berberine alone that would allow  
2818 identification of a reference point.
- 2819 • The consumption of berberine-containing food supplements may lead to transient  
2820 gastrointestinal symptoms such as constipation, diarrhoea, nausea, and abdominal pain.

- 2821 • Berberine may inhibit CYP3A4 and possibly other CYP450 enzymes, indicating a risk for  
2822 interaction between berberine-containing plant preparations and various medicinal products.
- 2823 • Except for *H. canadensis*, the toxicity profiles of preparations of the plant species included  
2824 in the mandate, beyond their berberine content, remain largely unknown due to the lack of  
2825 adequate toxicity studies, resulting in significant uncertainty in the identification and  
2826 characterisation of hazards associated with these preparations.
- 2827 • Preparations from rhizome/root of *H. canadensis* showed evidence of carcinogenic activity in  
2828 rodents, particularly causing liver adenomas. The mechanisms remain unclear, including  
2829 potential genotoxic modes of action. The consumption of preparations of rhizome/root of *H.*  
2830 *canadensis* represents a carcinogenic risk for humans.
- 2831 • The carcinogenic activity of berberine-containing preparations from plants other than *H.*  
2832 *canadensis* has not been studied.
- 2833 • In humans, consumption of preparations of *C. majus* aerial parts has been linked to  
2834 idiosyncratic herb-induced liver injury; susceptible individuals cannot currently be identified,  
2835 nor can a dose be established below which such reactions would not occur.
- 2836 • The available data do not allow establishing a safe intake for humans for any preparations  
2837 of the plant species, and plant parts thereof, included in the assessment.

## 2838 6 Data gaps and information needed to address them

2839 This chapter describes the additional data needed to address identified concerns, and the stepwise  
2840 approach to generating these data (Figure 4).

2841 All studies generated should follow OECD TGs if existing and be conducted in accordance with GLP.  
2842 The test material should be well characterised and representative of the plant preparation to be  
2843 used for the European market. For newly generated studies, full study reports should be provided.  
2844 If additional searches were conducted, a complete description of the literature search strategy and  
2845 results should be given.

### 2846 *Step 1 Characterisation*

2847 For the characterisation of the plant preparation, the requirements of the EFSA Guidance on safety  
2848 assessment of botanicals and botanical preparations intended for use as ingredients in food  
2849 supplements should be applied (EFSA Scientific Committee, 2009). Single substances should be  
2850 identified by their chemical name and CAS number. Information on their purity is to be provided.  
2851 For all analyses, validated methods should be used. The methods of analysis should be described  
2852 alongside their references, and proof of their validation should be provided.

2853 The conditions of use of the plant preparation of interest, including the target population and  
2854 recommended doses, should be provided.

### 2855 *Step 2a Genotoxicity of berberine*

2856 Given the positive findings in vitro regarding mutagenicity and chromosomal damage, the Panel  
2857 considers that the genotoxic potential of berberine in vivo requires elucidation, both at the level of

2858 systemic exposure and at first-contact sites of exposure, namely the liver and the GI tract, which  
2859 represent the major site of metabolism and the highest local exposure, respectively. Follow-up  
2860 experiments in vivo are needed to address mutagenicity and chromosomal damage, in accordance  
2861 with the EFSA Scientific Committee opinion on genotoxicity testing strategies (EFSA Scientific  
2862 Committee, 2011a). The Panel notes that, in view of berberine toxicokinetics and the proposed  
2863 modes of action, the following battery of assays is necessary (illustrated in Figure 5, Step 2a):

- 2864 • A transgenic rodent (TGR) mutation assay (OECD TG 488) to address berberine's mutagenic  
2865 potential. The assay must be capable of detecting a broad spectrum of mutation types, including  
2866 deletions and frameshift mutations, which are commonly associated with DNA intercalation and  
2867 topoisomerase inhibition. The assay should be performed in both the liver and gastrointestinal  
2868 tract tissues (duodenum and/or jejunum).
- 2869 • An in vivo MN assay to address berberine's potential for chromosomal damage upon systemic  
2870 exposure. The assay should be performed in the liver of male juvenile rats (ideally 6-week-old  
2871 rats, not older than 8 weeks) to assess clastogenic and aneugenic potential at a relevant systemic  
2872 target tissue. It is advised to sample the tissues 72 hours (3 days) after the last oral dose to  
2873 allow sufficient MN expression while minimising loss of damaged cells. Diethylnitrosamine (DEN)  
2874 or dimethylnitrosamine (DMN) should be used as positive controls.
- 2875 • An in vivo comet assay (OECD TG 489) to address berberine's potential for DNA damage at first-  
2876 contact sites of exposure. The Panel emphasises the need for a careful strategy regarding the  
2877 timing of tissue sampling (i.e. 3–6 hours after the last dose to capture transient DNA strand  
2878 breaks). The Panel advises the use of an Fpg-modified comet assay to adequately detect  
2879 oxidative DNA damage. The assay should be performed in the liver and the GI tract tissues  
2880 (duodenum and/or jejunum). As recommended in OECD TG 489, tissue toxicity and apoptosis  
2881 must be assessed to ensure DNA damage is not secondary to cytotoxicity.

2882 The Panel notes that negative results on genotoxicity are only considered valid if exposure of the  
2883 target tissue is demonstrated (OECD TG 488 (TGR), OECD TG 489 (comet assay) (OECD, 2017)).  
2884 Therefore, given the low bioavailability of berberine, toxicokinetic measurements will be critical to  
2885 demonstrate relevant target tissue exposure in all assays (e.g. measurements of berberine and its  
2886 main metabolites, e.g. berberrubine, concentrations in plasma and in tissue homogenates from both  
2887 the liver and the GI tract).

#### 2888 *Step 2b Genotoxicity of other protoberberines*

2889 Additionally, the genotoxic potential of other protoberberines present in the relevant plant  
2890 preparations requires consideration, given their high structural similarity with berberine. The Panel  
2891 considers that read-across approaches can be applied provided that the principles of the SC guidance  
2892 are followed (EFSA Scientific Committee et al., 2025). The Panel notes that the applicability of a  
2893 read-across approach is endpoint-specific and requires evaluation of suitability and associated  
2894 uncertainties for each genotoxic endpoint. The Panel considers that there is a sufficient basis to  
2895 justify a read-across approach regarding the outcome of the in vivo mutagenicity study on berberine,  
2896 considering the consistent pattern of structural alerts for mutagenicity identified in other  
2897 protoberberines and the reliability of the predictions obtained regarding this endpoint from the QSAR  
2898 analysis in VEGA conducted in this assessment (section 3.1.3.4). With respect to chromosomal  
2899 damage, the Panel notes a high level of uncertainty in the application of a read-across approach,  
2900 due to limitations of the VEGA regarding in vitro and in vivo MN models (section 3.1.3.4). To reduce

2901 these uncertainties, the Panel considers that additional experimental data are needed for at least  
2902 one other member of the protoberberines' family, starting with an in vitro MN assay (OECD TG 487)  
2903 (EFSA Scientific Committee, 2011a). The selected compound should represent a worst-case  
2904 scenario, based on considerations of physio-chemical properties, available toxicokinetics data (e.g.  
2905 systemic exposure, PBPK modelling), and the presence of structural alerts (e.g. identified in the  
2906 literature) regarding the respective protoberberines (illustrated in Figure 5, Step 2b).

### 2907 *Step 3 Genotoxicity of the plant preparations*

2908 Finally, irrespectively of outcome of genotoxicity assays conducted with berberine and  
2909 protoberberines, the genotoxic potential of the respective plant preparations needs to be further  
2910 addressed on a case-by-case basis, according to the principles outlined in the SC guidance of  
2911 genotoxicity assessment of chemical mixtures, i.e. considering other substances present beside  
2912 berberine and other protoberberines, as well as the unidentified fraction (Figure 4, Step 3). With  
2913 respect to substances present in the mixture, literature searches need to be conducted in order to  
2914 identify additional potential concerns for genotoxicity. Should concerns be identified, genotoxicity  
2915 testing for these additional substances need to be carried out. When component-based genotoxicity  
2916 of the mixture is ruled out, testing can proceed to investigating genotoxicity of the whole mixture  
2917 (EFSA Scientific Committee et al., 2019)<sup>32</sup>.

2918 In particular, the Panel notes that:

- 2919 • regarding preparations of *C. majus*, the genotoxicity potential of sanguinarine and chelerythrine  
2920 raises additional concerns and requires investigation at first-contact sites of exposure in vivo  
2921 (section 3.5.2);
- 2922 • regarding preparations of *H. canadensis*, the mechanisms underlying the carcinogenic activity  
2923 are not elucidated, including genotoxic modes of actions of other relevant substances contained  
2924 in preparations of rhizome/roots of *H. canadensis*, aside berberine and protoberberines. Based  
2925 on the outcome of the QSAR analysis, the Panel notes that (–)-β-hydrastine, and to a lesser  
2926 extent canadine, may require further investigation regarding their clastogenic/aneugenic  
2927 potential (section 3.10.2.3).

### 2928 *Step 4 Subchronic toxicity study and other studies, in case genotoxicity is ruled out*

2929 The Panel considers that available data is inadequate to establish safe doses for the plant  
2930 preparations included in the mandate. A 90-day study in the rat (OECD TG 408) with the test material  
2931 administered via gavage is the minimum requirement to identify a reference point and establish safe  
2932 dose for the general population (Figure 4, Step 4).

2933 In case the plant preparations are intended for pregnant and/or lactating women, the Panel notes  
2934 that a 90-day study does not cover the period of pregnancy and lactation. The reproductive and  
2935 developmental toxicological potential of the plant preparation should be assessed through a  
2936 standard reproduction and developmental toxicity study (OECD TG 422), unless justified by the food  
2937 business operators.

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<sup>32</sup> The Panel notes that the EFSA Scientific Committee opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA SC, 2011), which is mentioned in the guidance of genotoxicity assessment of chemical mixtures (EFSA SC, 2019), is under revision (EFSA Mandate M-2024-00152, question number EFSA-Q-2025-00166). The updated version is expected to be released in 2027. The latest applicable version should be considered in preparing data submission.

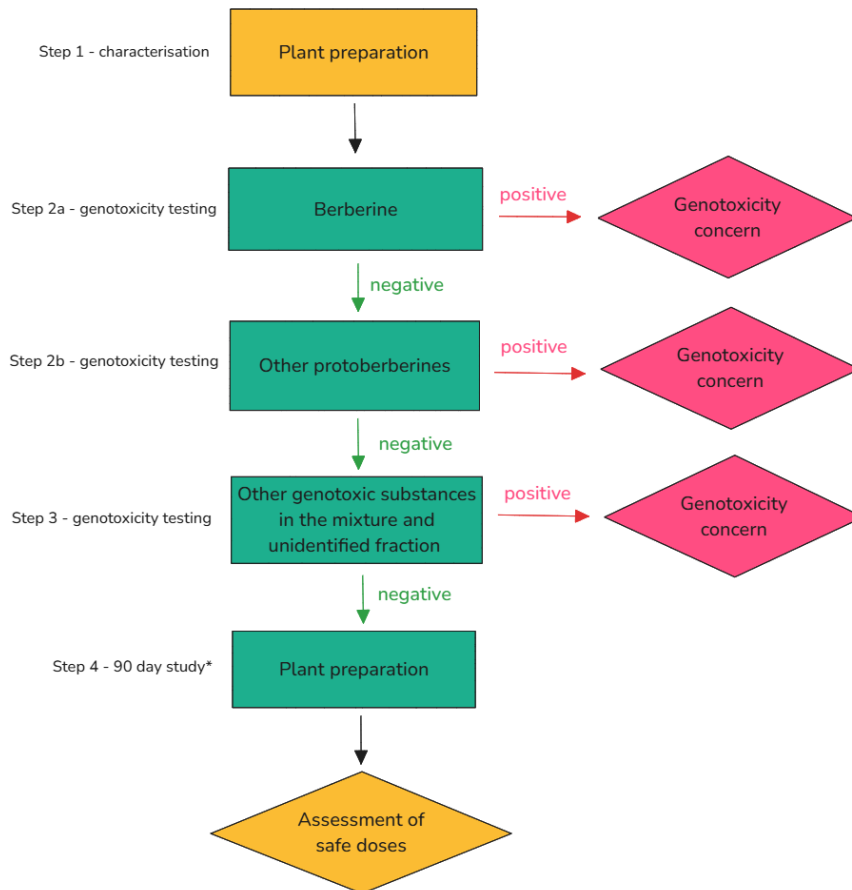
2938 To what extent results from the submitted studies can be extrapolated to other plant preparations  
2939 will need to be evaluated based on the data provided and will be an expert judgement.

2940 *Specific considerations regarding preparations of aerial parts of rhizomes/root of H. canadensis*

2941 The consumption of preparations of rhizome/root of *H. canadensis* represents a carcinogenic risk  
2942 for humans. Without evidence excluding a genotoxic mechanism, a safe level of exposure cannot  
2943 be determined. In case a genotoxic mechanism was reliably ruled out, the available toxicity studies  
2944 on rhizomes/root of *H. canadensis* can be used to identify a reference point for establishing a safe  
2945 level of intake in humans (section 3.10).

2946 *Specific considerations regarding preparations of aerial parts of C. majus*

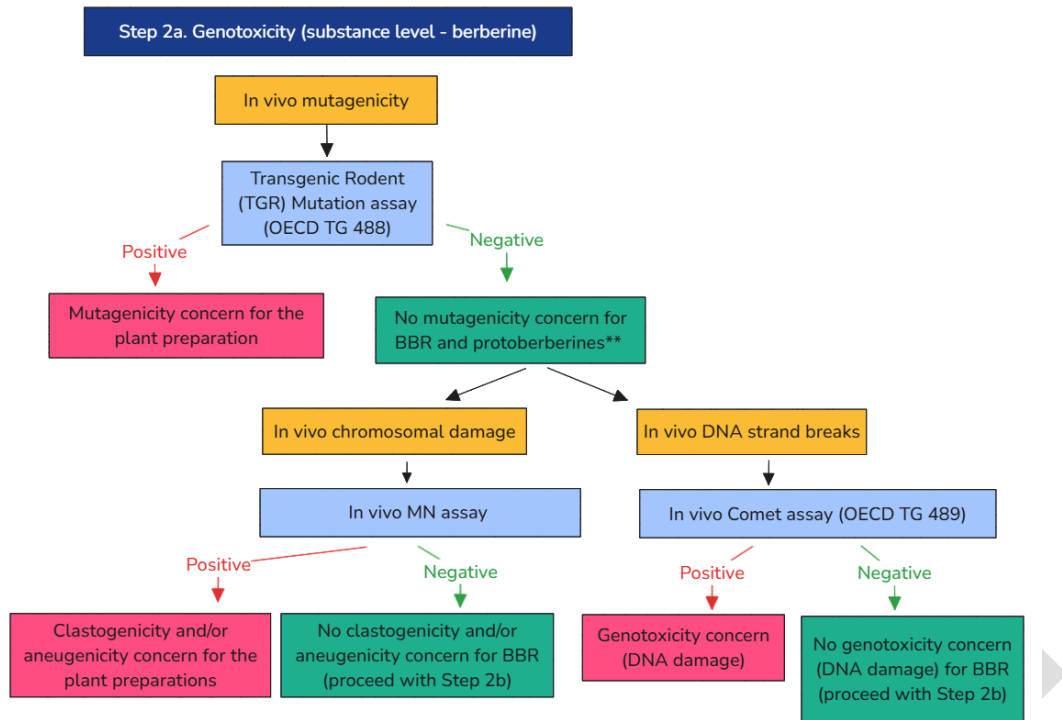
2947 Idiosyncratic drug reactions are rare, unpredictable adverse responses that typically lack a clear  
2948 dose-response relationship and are not reliably reproduced in animal models (EMA (European  
2949 Medicines Agency), 2010). The Panel did not identify an experimental approach that could be used  
2950 to address this concern.



\* In case the plant preparation is intended for pregnant and/or lactating women, the Panel notes that a 90-day study does not cover the period of pregnancy and lactation. The reproductive and developmental toxicological potential of the plant preparation should be assessed through a standard reproduction and developmental toxicity study (OECD TG 422), unless justified.

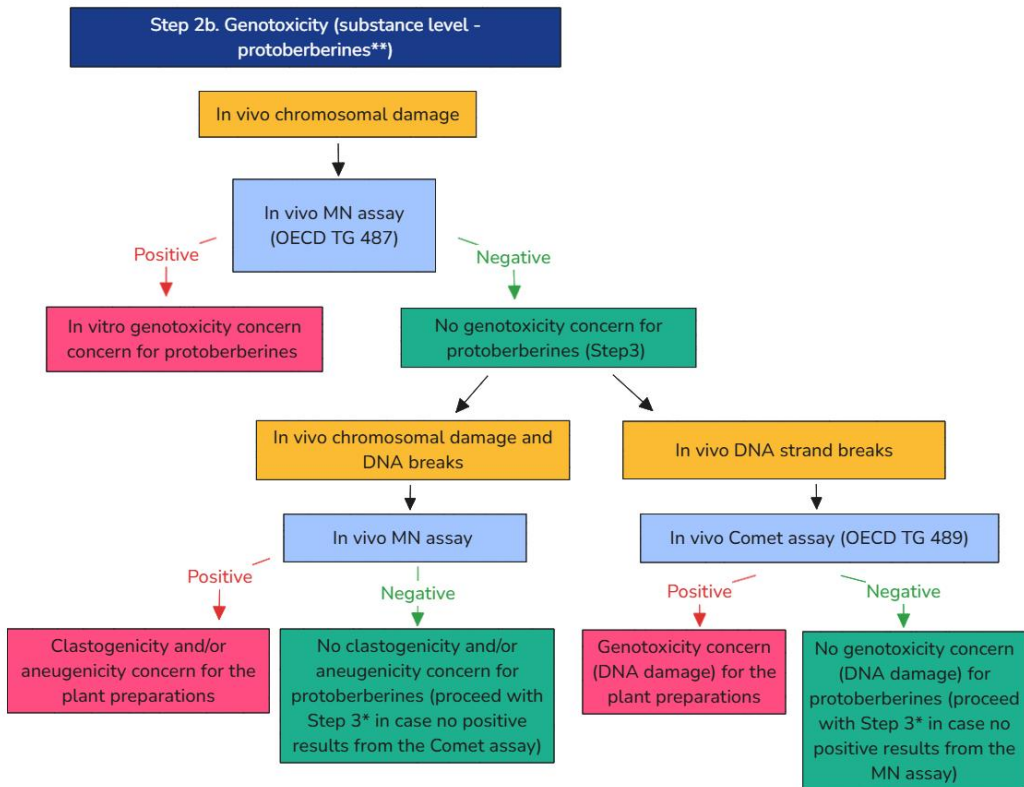
2951

2952 **Figure 4.** Stepwise flowchart for generating data to address concerns related to the consumption of berberine-  
2953 containing plant preparations, following the principles of EFSA SC Scientific opinion on genotoxicity testing strategies  
2954 applicable to food and feed safety assessment (EFSA SC, 2011) and EFSA SC statement on the genotoxicity assessment  
2955 of chemical mixtures (EFSA SC, 2019).



\*Step 2 (mixture level) in accordance with EFSA GD on genotoxicity testing strategy (EFSA SC, 2011) and EFSA GD on genotoxicity assessment of chemical mixtures (EFSA SC, 2019)  
 \*\*For mutagenicity as the endpoint, read-across from berberine to the entire protoberberine family is possible

2956



\*Step 2 (mixture level) in accordance with EFSA GD on genotoxicity testing strategy (EFSA SC, 2011) and EFSA GD on genotoxicity assessment of chemical mixtures (EFSA SC, 2019)  
 \*\*Only one protoberberine alkaloid to be tested based on the worst-case scenario

2957

2958

2959

**Figure 5.** Flowchart of data requirements for assessing the genotoxicity of berberine and other protoberberines

2960 **7 Documentation as provided to EFSA**

2961 Details about the type of data provided by interested parties following the call for data are given in  
 2962 the following table.

2963 **Table 40:** Data provided by interested parties

Interested party	Type of document	Consideration on the data in the assessment
<b>European Federation of Associations of Health Product Manufacturers (EHPM)</b>	Full study report: "Update on the bioavailability and safety assessment of berberine; in vitro and in silico insights"	Data were used in the following sections <ul style="list-style-type: none"> <li>• 3.3.1. Characterisation of the plant (<i>B. aristata</i>) (Table 11)</li> <li>• 3.4.1 Characterisation of the plant (<i>B. vulgaris</i>)</li> </ul>
<b>Società italiana di scienze applicate alle piante officinali e ai prodotti per la salute (SISTE)</b>	Analytical data on 396 samples of <i>B. aristata</i> bark extract and on 78 samples of food supplement containing <i>B. aristata</i> bark extracts, including recommended levels.	Data were used in the following Sections: 3.3.1. Characterisation of the plant ( <i>B. aristata</i> ) (Table 11)
	Analytical data on 62 samples of <i>B. aristata</i> root extracts.	3.15.1. Occurrence data
	Analytical data on 7 food supplement samples, including recommended levels.	
	Narrative review of studies on ADME (absorption, distribution, metabolism, and excretion)	Individual references cited in the provided review were assessed and included if relevant.
	Bibliographic search on toxicological data (UniMi)	Individual references cited in the provided review were assessed and included if relevant.
<b>Federal Public Service (FPS) – Health Good Chain Safety Environment</b>	Analytical data on food supplement samples, including recommended levels	The data provider could not supply further details upon EFSA's request. Therefore, these data were not used in the assessment.

2964

2965

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4059 **Appendices**

4060 Appendix A – List of plants included in the scientific assessment

4061 Appendix B – Study selection process to address sQ2

4062 Appendix C – Reporting reliability and relevance of genotoxicity studies

4063 Appendix D – Risk of bias appraisal methodology and outcome

4064 Appendix E – Genotoxicity studies on berberine and berberine-containing plant preparations

4065 Appendix F – Randomised controlled reporting adverse events trials on berberine and berberine-  
4066 containing plant preparations

4067 Appendix G – Summary of human case reports on berberine and berberine-containing plant  
4068 preparations

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## Appendix A. List of plant species included in the scientific assessment

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**Table A1.** Accepted scientific names and synonyms, according to the POWO database

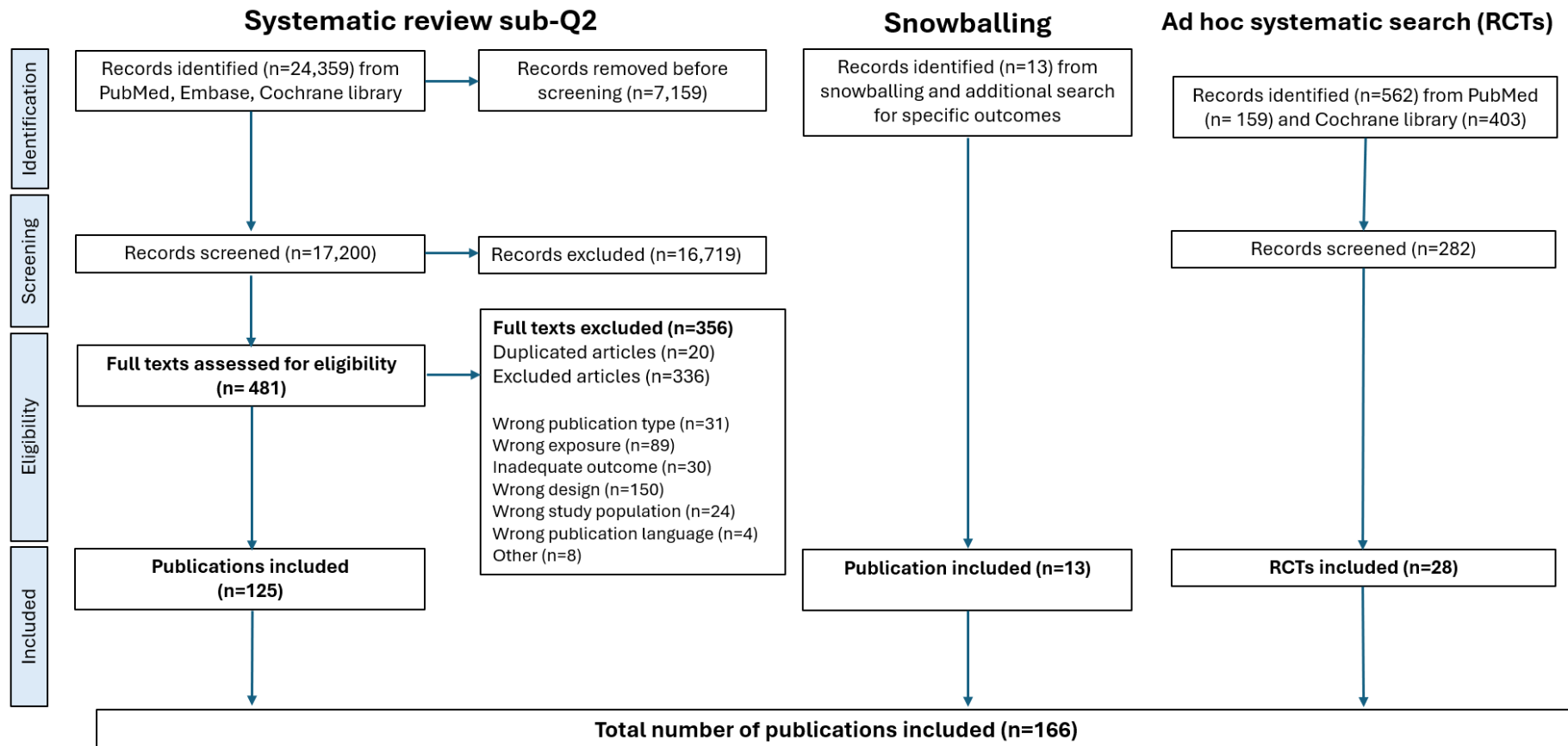
Scientific name	Synonyms		
<b><i>Berberis aquifolium</i> Pursh</b>	<i>Mahonia aquifolium</i> (Pursh) Nutt. <i>Odostemon aquifolius</i> (Pursh) Rydb. <i>Berberis aquifolium</i> var. <i>juglandifolia</i> (Jouin) Rehder <i>Berberis aquifolium</i> var. <i>lyallii</i> (Ahrendt) Marroq. & Laferr <i>Berberis aquifolium</i> var. <i>nutkana</i> (DC.) Marroq. & Laferr. <i>Berberis brevipes</i> Greene <i>Berberis pinnata</i> Banks ex DC. <i>Berberis pinnata</i> var. <i>hortensis</i> (Fedde) Marroq. & Laferr. <i>Mahonia aquifolium</i> f. <i>albovariegata</i> Schwer.	<i>Mahonia aquifolium</i> f. <i>amabilis</i> Schwer <i>Mahonia aquifolium</i> f. <i>aucubifolia</i> Schwer. <i>Mahonia aquifolium</i> f. <i>aureovariegata</i> Schwer. <i>Mahonia aquifolium</i> var. <i>juglandifolia</i> Jouin <i>Mahonia aquifolium</i> f. <i>lutescens</i> Schwer. <i>Mahonia aquifolium</i> var. <i>lyallii</i> Ahrendt <i>Mahonia aquifolium</i> var. <i>nutkana</i> DC. <i>Mahonia brevipes</i> (Greene) Rehder <i>Mahonia diversifolia</i> Sweet	<i>Mahonia latifolia</i> Dippel <i>Mahonia moseri</i> Ahrendt <i>Mahonia moseriana</i> Moser ex Martinet <i>Mahonia murrayana</i> Dippel <i>Mahonia pinnata</i> var. <i>hortensis</i> Fedde <i>Mahonia undulata</i> Ahrendt <i>Odostemon brevipes</i> (Greene) <i>Odostemon nutkanus</i> (DC.) Rydb
<b><i>Berberis aristata</i> DC</b>	<i>Berberis chitria</i> Buch.-Ham. ex Ker Gawl. <i>Berberis aristata</i> var. <i>sinensis</i> K.Koch <i>Berberis bussmul</i> K.Koch ex Miq. <i>Berberis ceratophylla</i> G.Don <i>Berberis chitria</i> Ahrendt <i>Berberis chitria</i> var. <i>occidentalis</i> Ahrendt	<i>Berberis chitria</i> var. <i>sikkimensis</i> C.K.Schneid. <i>Berberis coccinea</i> K.Koch <i>Berberis coerulescens</i> G.Nicholson <i>Berberis elegans</i> K.Koch <i>Berberis gracilis</i> Lindl. <i>Berberis gracillima</i> K.Koch ex Miq.	<i>Berberis macrophylla</i> K.Koch <i>Berberis serratifolia</i> K.Koch <i>Berberis sikkimensis</i> (C.K.Schneid.) Ahrendt <i>Berberis sikkimensis</i> var. <i>baileyi</i> Ahrendt <i>Berberis umbellata</i> Lindl. <i>Berberis undulata</i> K.Koch
<b><i>Berberis vulgaris</i> L.</b>	<i>Berberis racemosa</i> Stokes		
<b><i>Chelidonium majus</i> L.</b>	-		
<b><i>Coptis japonica</i> (Thunb.) Makino</b>	<i>Thalictrum japonicum</i> Thunb.		
<b><i>Coptis teeta</i> Wall.</b>	-		
<b><i>Coptis trifolia</i> (L.) Salisb.</b>	<i>Helleborus trifolius</i> L. <i>Anemone groenlandica</i> Oeder <i>Anemone groenlandica</i> O.F.Müll. <i>Chryza borealis</i> Raf. <i>Coptis daisetsuensis</i> Miyabe & Tatew	<i>Coptis groenlandica</i> (Oeder) Fernald <i>Coptis trifolia</i> var. <i>groenlandica</i> (Oeder) Fassett <i>Coptis trifolia</i> subsp. <i>groenlandica</i> (Oeder) Hultén <i>Coptis trifolia</i> f. <i>plena</i> K.Imai <i>Coptis trifolia</i> f. <i>sempierna</i> (Miyabe & Tatew.) Okuyama	<i>Coptis trifolia</i> var. <i>sempierna</i> Miyabe & Tatew. <i>Helleborus pumilus</i> Salisb. <i>Helleborus trifoliatus</i> Houtt. <i>Helleborus trilobus</i> Lam. <i>Isopyrum trifolium</i> Britton
<b><i>Cosciniium fenestratum</i></b>	<i>Menispermum fenestratum</i> Gaertn. <i>Cosciniium fenestratum</i> var. <i>macrophyllum</i> Yamam.	<i>Cosciniium miosepalum</i> Diels <i>Cosciniium peltatum</i> Merr.	<i>Cosciniium wightianum</i> Miers ex Diels <i>Pereiria medica</i> Lindl.

Scientific name	Synonyms		
<b>(Goetgh.) Colebr.</b>	<i>Cosciniium fenestratum</i> var. <i>ovalifolium</i> <i>Cosciniium maingayi</i> Pierre	<i>Cosciniium usitatum</i> Pierre <i>Cosciniium wallichianum</i> Miers	
<b><i>Hydrastis canadensis</i> L.</b>	<i>Warneria canadensis</i> Mill. <i>Warneria diphylla</i> Raf.	<i>Warneria tinctoria</i> Raf. <i>Hydrastis trifolia</i> Raf.	<i>Warneria canadensis</i> Mill.
<b><i>Jateorhiza palmata</i> (Lam.) Miers</b>	<i>Chasmanthera palmata</i> (Lam.) Baill. <i>Cocculus palmatus</i> (Lam.) DC. <i>Jateorhiza miersii</i> Oliv.	<i>Menispermum palmatum</i> Lam. <i>Chasmanthera columba</i> (Roxb.) Baill. <i>Jateorhiza columba</i> (Roxb.) Miers	<i>Menispermum calumba</i> Stokes <i>Menispermum columba</i> Roxb.
<b><i>Phellodendron amurense</i> Rupr.</b>	<i>Phellodendron amurense</i> f. <i>insulare</i> (Nakai) H.S.Kim <i>Phellodendron amurense</i> var. <i>lavalleeii</i> (Dode) Sprague <i>Phellodendron amurense</i> var. <i>molle</i> (Nakai) <i>Phellodendron amurense</i> f. <i>molle</i> (Nakai) <i>Phellodendron amurense</i> var. <i>sachalinense</i> F.Schmidt <i>Phellodendron amurense</i> var. <i>suberosum</i> (H.Hara) H.Hara	<i>Phellodendron amurense</i> f. <i>suberosum</i> (H.Hara) H.Hara <i>Phellodendron insulare</i> Nakai <i>Phellodendron japonicum</i> Maxim. <i>Phellodendron kodamanum</i> Makino <i>Phellodendron lavalleeii</i> Dode <i>Phellodendron molle</i> Nakai	<i>Phellodendron nikkomontanum</i> Makino <i>Phellodendron piriforme</i> E.L.Wolf <i>Phellodendron sachalinense</i> (F.Schmidt) <i>Phellodendron sachalinense</i> f. <i>longipes</i> Y.C.Wu <i>Phellodendron sachalinense</i> var. <i>suberosum</i> H.Hara <i>Zanthoxylum kibada</i> Siebold
<b><i>Thalictrum flavum</i> L.</b>	<i>Thalictrum controversum</i> K.F.Schimp. & Spenn.	<i>Thalictrum controversum</i> subsp. <i>flavum</i> (L.) K.F.Schimp. & Spenn.	<i>Thalictrum linnaeanum</i> Rouy & Foucaud
<b><i>Tinospora sinensis</i> (Lour.) Merr.</b>	<i>Campylus sinensis</i> Lour. <i>Cocculus chondodendrum</i> DC. <i>Cocculus malabaricus</i> (Lam.) DC. <i>Cocculus tomentosus</i> Colebr.	<i>Epibaterium tomentosum</i> (Colebr.) Pers. <i>Menispermum chondrodendron</i> (DC.) Spreng. <i>Menispermum malabaricum</i> Lam. <i>Menispermum tomentosum</i> (Colebr.) Roxb.	<i>Tinospora malabarica</i> (Lam.) Hook.f. & Thomson <i>Tinospora malabarica</i> var. <i>tomentosa</i> (Colebr.) Trimen <i>Tinospora tomentosa</i> (Colebr.) Hook.f. & Thomson

4075

Source: Plants of the World Online (POWO), available at <https://powo.science.kew.org/>

4076 Appendix B. Study selection process to address sub-question Q2 (sub-Q2) of the  
 4077 systematic review



4078  
 4079  
 4080 **Figure B1:** Flow diagram of the study selection process

4081 **Table B1.** Total number of publications included, by exposure and type

	<b>Berberine</b>	<b>Other protoberberines</b>	<b><i>B. aristata</i></b>	<b><i>C. majus</i></b>	<b><i>C. japonica</i></b>	<b><i>C. fenestratum</i></b>	<b><i>H. canadensis</i></b>	<b><i>P. amurense</i></b>	<b><i>T. sinensis</i></b>	<b>Total</b>
<b>In vitro and in vivo genotoxicity studies</b>	26	3	1	0	1	0	3	1	0	35
<b>Animal studies</b>										
<b>General toxicity</b>	31	4	2	1	0	3	5	2	3	51
<b>Interactions with medicinal products</b>	12	0	0	1	0	0	1	0	0	14
<b>Human studies</b>										
<b>Case reports/series</b>	7 <sup>a</sup>	0	0	16	0	0	3	0	1	27
<b>Randomised controlled trials</b>	28	0	0	0	0	0	0	0	0	28
<b>Clinical studies on interactions with medicinal products</b>	4	0	0	0	0	0	7	0	0	11

4082 <sup>a</sup> Of which 2 reported possible interactions between berberine and medicinal products.

4083

4084 **Appendix C. Reporting reliability and relevance of**  
 4085 **genotoxicity studies**

4086 **Table C1.** Numerical scores used for the reliability assessment of genotoxicity studies and their  
 4087 meaning

<b>Reliability score (Klimisch scoring)</b>	<b>Definitions in the technical Report for reporting reliability and relevance of genotoxicity studies</b>
1 - Reliable without restriction	'This includes studies or data from the literature or reports which were carried out or generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline (preferably performed according to GLP) or in which all parameters described are closely related/comparable to a guideline method.'
2 - Reliable with restrictions	'This includes studies or data from the literature, reports (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.'
3 - Not reliable	'This includes studies or data from the literature/reports in which there are interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (...) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert judgment.'
4 - Not assignable	'This includes studies or data from the literature, which do not give sufficient experimental details, and which are only listed in short abstracts or secondary literature (books, reviews, etc).'

4088  
 4089 **Scoring of the relevance of the test system**

4090 The relevance of the test system is based on the genetic endpoint (e.g. higher relevance should be  
 4091 given to studies providing information on apical endpoints in in vivo studies, i.e. gene mutations,  
 4092 structural and numerical chromosomal alterations). Supporting information may be obtained from  
 4093 indicator assays; exception is the in vivo comet assay that is considered with high relevance when  
 4094 applied as follow-up to a positive in vitro result (as recommended by the EFSA Scientific Committee,  
 4095 2011).

4096 Tests with high relevance for hazard identification:

- 4097 • Bacterial reverse mutation test
- 4098 • Mammalian cell gene mutation tests in vitro
- 4099 • Micronucleus tests in vitro and in vivo
- 4100 • Chromosomal aberration tests in vitro and in vivo
- 4101 • Comet assay in vivo
- 4102 • Mutation tests in vivo (e.g. in transgenic rodents and Pig-a)

4103 Other test systems although potentially considered of limited or low relevance may provide useful  
 4104 supporting information.

4105 **The relevance of the study results depends on:**

- 4106 - the route of administration (e.g., higher relevance should be given to oral vs. parenteral  
 4107 (intravenous, subcutaneous or intraperitoneal injection) or inhalation exposure in case of in

4108 vivo studies, as such routes are not physiological and not recommended by OECD test  
4109 guidelines),  
4110 - the biological relevance of the study results (e.g., purity of the test substance; the metabolic  
4111 capabilities of the test system; the bioavailability of the test substance, with particular  
4112 consideration on the evidence of target tissue exposure in in vivo tests (EFSA Scientific  
4113 Committee, 2017a; OECD, 2017); the interference of high cytotoxicity; the reproducibility of  
4114 study results),  
4115 - the reliability of the study (e.g., studies with a Klimisch score of 3 or 4 are of low relevance).  
4116  
4117  
4118

DRAFT

## 4119 Appendix D. Risk of bias appraisal, methodology and outcome

4120 The internal validity of eligible studies for which data were extracted in relation to sQ2 (i.e., addressed through systematic reviews) was assessed  
4121 in duplicate by two independent reviewers using a customised version of the OHAT RoB tool developed by the US NTP (OHAT-NTP, 2015). Any  
4122 discrepancies in the RoB assessment for each bias domain were discussed among the assessors. If there was disagreement, a third reviewer  
4123 was consulted for resolution. Studies were allocated to RoB tiers reflecting their overall internal validity, i.e. at low (tier 1), moderate (tier 2)  
4124 or high (tier 3) RoB, according to the algorithm presented in Table D1 (OHAT/NTP, 2019).

4125

4126 **Table D1.** Algorithm applied for the allocation of studies to tiers of internal validity

Tier 1	Study must be rated as “definitely low” or “probably low” risk of bias for all key criteria <b>AND</b> have a maximum of two other applicable criteria rated as “probably high” (-/NR) risk of bias	Low RoB
Tier 2	Study does not meet criteria for Tier 1 or Tier 3	Moderate RoB
Tier 3	Study must be rated as “definitely high” (--) or “probably high” (-/NR) risk of bias for at least two key criteria <b>AND</b> have at least three other applicable criteria rated as “definitely high” (--) or “probably high” (-/NR) risk of bias	High RoB

4127

4128

4129 **Table D2.** RoB rating instructions for each question

Rating	Description
<b>Q1. Can we be confident in the exposure characterisation? [KEY QUESTION]</b>	
++	<p>For single substances: the purity of the substance is reported and the remainder is characterised or the purity of the substance is high (&gt;90%)</p> <p>For plant preparations: A (if applicable) +B+C+D+E needs to be adequate</p> <p>A. In case the raw material was bought/collected, the plant of interest is correctly identified by trained staff/botanist</p> <p>B. An analysis with respect to the compounds of interest was conducted (for BBR: if the total alkaloid fraction analysed is sufficient)</p> <p>C. Analytical methods used were appropriate</p> <p>D. Description of the extraction process (if the investigational product is an extract)</p> <p>E. At least one quantified value of the substance(s) of interest is reported</p>
+	<p>For single substances: the purity of the substance as percentage needs to be reported</p> <p>For plant preparations: A, B and C are replaced by the reporting in the publication on raw material specifications given by the provider of the investigational material OR there were some minor limitations in any of the points A, B, C, D and E</p>
- or NR	<p>For single substances: the purity of the substance is reported and the remainder is characterised or the purity of the substance is high (&gt;90%)</p> <p>if E is not reported, score "-/ NR" or "- -" (not possible to score "+" or "+ +")</p> <p>missing information on any of the other items results in a scoring of 'NR'</p>
--	<p>For single substances: the purity of the substance is &lt; 90%</p>
<p>NB If uncertainty exists as which score is to be given, the following items could be used to increase/decrease confidence.</p> <ul style="list-style-type: none"> <li>- Appropriate stability testing and/or storage; preparations made for the purpose of the study: were they made every day or stored?</li> <li>- In case of known issues with adulteration (e.g. palmatine in <i>H. canadensis</i> coming from <i>C. chinensis</i>)</li> </ul>	
<b>Q2. Can we be confident in the outcome assessment?</b>	
++	<p>Haematology, clinical chemistry, organ weight and body weight measurements are always to be judged as '++'. It is assumed that routine methods are applied even if this is not reported. For body weight measurements it is assumed that even if the scale might not have been calibrated, any measurement error would affect groups equally</p> <p>Other outcomes which are assessed using the 'gold standard'/routine methods are to be judged against OECD guidelines: in order to give a '++' fully compliant with OECD guidelines</p> <p>For outcomes derived from data analysis prone to bias (e.g. histopathology, neurotoxicity/behavioural tests) the following is to be considered:</p> <p>A. blinding of outcome assessors</p> <p>B. assessment performed by experienced personnel (single outcome assessor)</p> <p>in order to give a '++' A+B reported</p>

Rating	Description
+	<p>Haematology, clinical chemistry, organ weight and body weight measurements are always to be judged as '++'. It is assumed that routine methods are applied even if this is not reported. For body weight measurements it is assumed that even if the scale might not have been calibrated, any measurement error would affect groups equally</p> <p>Other outcomes which are assessed using the 'gold standard'/routine methods are to be judged against OECD guidelines: in order to give a '+': some minor deviations from OECD guidelines</p> <p>For outcomes derived from data analysis prone to bias (e.g. histopathology, neurotoxicity/behavioural tests) the following is to be considered: in order to give a '+': A is reported</p>
- or NR	<p>For outcomes derived from data analysis prone to bias (e.g. histopathology, neurotoxicity/behavioural tests) the following is to be considered: in order to give a 'NR': A, B not reported</p> <p>in order to give a '-': A is not reported, but B available</p>
--	<p>For outcomes derived from data analysis prone to bias (e.g. histopathology, neurotoxicity/behavioural tests) the following is to be considered: in order to give a '--': A is not reported and the method if not valid</p>
<p>Non-blinding of outcome assessors may be acceptable on a case-by-case basis, depending on whether other safeguard measures were implemented to avoid bias, e.g. assessment by a Panel of trained assessors, or if non-blinding has been judged not to affect the outcome.</p> <p>Feed intake is not included as an outcome in the question 2. It is considered in the question 5)c).</p>	
<p><b>Q3. Was the number of animals per sex and dose group in line with internationally recognised guidelines? [KEY QUESTION]</b></p>	
++	<p>number and sex of animal in the study correspond to guidelines:</p> <ul style="list-style-type: none"> <li>• For studies investigating general toxicity, the following minimum number of animals should be considered: <ul style="list-style-type: none"> <li>- 50 per sex group and dose for chronic studies for rodents (<math>\geq 1</math>)</li> <li>- 10 per sex group and dose for sub-chronic studies for rodents (&gt;28 d to &lt; 1 y)</li> <li>- 5 per sex group and dose for sub-acute studies for rodents (&gt; 24h to <math>\leq 28</math> d)</li> <li>- 4 per sex and dose for any non-rodent studies</li> </ul> </li> <li>• For prenatal developmental studies: 20 female rats or rabbits with implantation sites, less than 16 females may be inappropriate</li> <li>• For developmental neurotoxicity studies: preferred species is the rat, 20 litters per dose level, on postnatal day 4 litters need to be standardised for size with similar numbers of male and female pups, litter size should not exceed average litter size of the strain (8-12 pups).</li> <li>• For reproduction and developmental toxicity screening tests: 10 male and 12-13 female rats. Female rats should be screened to exhibit 4-5-day oestrous cycles to yield. This should result in at least 10 females included of which at least 8 should become pregnant.</li> <li>• For reproduction toxicity studies: Both males and females should be studied. Each test and control group should contain a sufficient number of mating pairs to yield at least 20 pregnant females per dose group.</li> </ul>
+	<p>number of animals corresponds to guidelines &amp; only one sex is included in the study, but it is judged that this will have a minor impact on the risk of bias according to expert judgement</p>
- or NR	<ul style="list-style-type: none"> <li>• number of animals corresponds to guidelines &amp; only one sex is included in the study, with the possibility to upgrade to (+) due to an expert judgement. But it is unclear if an effect at a lower dose in the other sex group was missed</li> </ul> <p>OR</p> <ul style="list-style-type: none"> <li>• number of animals marginally insufficient</li> </ul> <p>OR</p> <ul style="list-style-type: none"> <li>• If the initial number of allocated animals (total number and number per group) is missing, then -&gt; 'NR'</li> </ul>

Rating	Description
--	Number of animals largely insufficient OR Number of animals corresponds to guidelines but no information on sex
<b>Q4. Was the study adequately randomised and/or baseline characteristics of animals similar?</b>	
++	Randomisation mentioned, not described in detail, and baseline weight are similar OR Randomisation described in detail without information on baseline weight
+	Randomisation is mentioned but baseline weight is not given or are given just as range across groups OR Randomisation is not mentioned but baseline weight is given per group and are similar between groups
- or NR	Randomisation is not mentioned but baseline weight is described and somewhat different between groups: '-' If nothing is reported: 'NR'
--	Direct evidence that they did not conduct randomisation (large differences in baseline weight or allocation is not adequate) OR Direct evidence of an imbalance of the body weight (also if no SD provided)
<p>Example of randomization described in detail: "physical randomization using random order of cages into the group and animals were randomized into cage. Mice were randomly divided into thirteen groups."</p> <p>Examples of randomization only mentioned, not described in detail:            "...randomly placed rats into three groups."            "mice were randomly divided into thirteen groups."            "Wistar rats were randomly categorized into 4 groups"</p>	
<b>Q5. Were the experimental conditions adequate? [KEY QUESTION]</b>	
++	A+B+C+D+E are adequate
+	A+B+C+D+E are all adequate, but some uncertainty persists and assumptions are made by the RoB assessors
- or NR	One or two out of A+B+C+D+E is inadequate; or if there is evidence that housing conditions were different between groups, irrespective of the assessment of the other criteria listed above. If no rationale for dose selection, 5A should be scored "-". If in gavage studies, no information on feed intake is reported
--	At least three out of A+B+C+D+E are inadequate
<p>List of criteria for Q5</p> <p>A. How were the doses and dose spacing (for multiple-dose studies) selected and was there a rationale for the doses given? Were they adequate for the purpose? The rationale should be written and justified in the text, otherwise it is inadequate. If the rationale is not adequate, it should be "inadequate". We do not consider the number of dosed groups</p> <p>B. Vehicle/negative control used is appropriate? Is the vehicle the same across groups?</p> <p>C. Consistency of exposure administration across groups (e.g. for feeding studies: differences in the amount of feed consumed e.g. owing to palatability issues, differences in the eating behaviour). For feeding studies: If no information on feed or water intake is reported, C is to be judged to be inadequate. If gavage study, adequate by default.</p>	

Rating	Description
	<p>D. Exposure frequency and duration in line with OECD guidelines if applicable (e.g. carcinogenicity or specific duration of exposure in developmental/reprotox. studies; see links in Appendix A). When the study duration is less than 28 days (with a margin of -3 days), it is inadequate. For example, a study of 45 days could be considered as a longer subacute study. If the daily administration is not mentioned and the exposure is not expressed per day, it is inadequate.</p> <p>E. Description of housing condition provided (temperature, humidity, photoperiod). Housing conditions are assumed to have been similar between the groups, even if not explicitly stated unless there is evidence to the contrary. In the OECD TG 407 and TG408, they require 22°C (± 3°C), humidity between 30% and 70% and cycles of 12h light and 12 hours dark.</p> <p>In case of single dose level studies, if uncertainty exists as to which score is to be given, this design (i.e. that only a single dose level was used) could be used as a reason to decrease confidence.</p>
<p><b>Q6. Were outcome data complete without attrition or exclusion from analysis?</b></p>	
++	no missing outcome data AND the number of animals analysed is reported in the results (text, tables) and there was no unexplained loss of animals
+	<p>reasons for the missing data or exclusion of animals from the analysis are given and the reasons are not related to the treatment, and the missing data are similar between groups. There is no suspicion of missing outcome data, but the number of animals analysed is NOT reported in the results</p> <p>OR</p> <p>If there is no indication of missing data but the number of animals included in the final analysis of results is not reported</p>
- or NR	<p>The number of animals allocated to the groups is higher than the number of animals mentioned in the result section (assessed by outcome) AND missing data are not similar between groups, BUT missing numbers are not substantial (&lt;20%)</p> <p>If the number of analysed animals is not reported in the results (text, tables), give a "NR"</p>
--	The number of animals allocated to the groups is higher than the number of animals mentioned in the result section (assessed by outcome) AND substantial number of animals (>20%) are missing without reasons
<p><b>Q7. Were there other potential threats to internal validity?</b></p>	
<p>Q7 is based on Expert judgement.</p> <ul style="list-style-type: none"> <li>• Is the statistical analysis appropriate for the type of data analysed?</li> <li>• Is it likely that the statistical method used could have led to type I or type II errors? (e.g. performing many t-tests increases the likelihood of finding a significant effect (type I error), correcting 'excessively' for multiple comparisons may mask an adverse effect (type II error))</li> <li>• Are the main outcomes mentioned in the method section also addressed in the result section and are data shown? Could a non-reporting be related to the findings?</li> <li>• If results on the main outcomes are not shown in numerical form -&gt; '-' irrespective of the other items judged under this sub-question</li> <li>• Do any other procedures in the study raise a concern which is not yet addressed under any other question?</li> <li>• For developmental studies: was the litter effect appropriately considered?</li> <li>• Feed should not be restricted.</li> </ul>	

<b>List of OECD guidelines</b>	
<b>Summary of Considerations in the Report from the OECD Expert Groups on Short Term and Long Term Toxicology</b> <a href="https://www.oecd.org/en/publications/summary-of-considerations-in-the-report-from-the-oecd-expert-groups-on-short-term-and-long-term-toxicology_9789264035447-en.html">https://www.oecd.org/en/publications/summary-of-considerations-in-the-report-from-the-oecd-expert-groups-on-short-term-and-long-term-toxicology_9789264035447-en.html</a>	
<b>Sub-acute toxicity</b>	Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents <a href="https://www.oecd.org/en/publications/test-no-407-repeated-dose-28-day-oral-toxicity-study-in-rodents_9789264070684-en.html">https://www.oecd.org/en/publications/test-no-407-repeated-dose-28-day-oral-toxicity-study-in-rodents_9789264070684-en.html</a>
<b>Sub-chronic toxicity</b>	Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents <a href="https://www.oecd.org/en/publications/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en.html">https://www.oecd.org/en/publications/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en.html</a> Test No. 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents <a href="https://www.oecd.org/en/publications/test-no-409-repeated-dose-90-day-oral-toxicity-study-in-non-rodents_9789264070721-en.html">https://www.oecd.org/en/publications/test-no-409-repeated-dose-90-day-oral-toxicity-study-in-non-rodents_9789264070721-en.html</a>
<b>Chronic toxicity and Carcinogenicity</b>	Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies <a href="https://www.oecd.org/en/publications/test-no-453-combined-chronic-toxicity-carcinogenicity-studies_9789264071223-en.html">https://www.oecd.org/en/publications/test-no-453-combined-chronic-toxicity-carcinogenicity-studies_9789264071223-en.html</a> Test No. 452: Chronic Toxicity Studies <a href="https://www.oecd.org/en/publications/test-no-452-chronic-toxicity-studies_9789264071209-en.html">https://www.oecd.org/en/publications/test-no-452-chronic-toxicity-studies_9789264071209-en.html</a> Test No. 451: Carcinogenicity Studies <a href="https://www.oecd.org/en/publications/test-no-451-carcinogenicity-studies_9789264071186-en.html">https://www.oecd.org/en/publications/test-no-451-carcinogenicity-studies_9789264071186-en.html</a>
<b>Developmental toxicity</b>	Test No. 414: Prenatal Developmental Toxicity Study <a href="https://www.oecd.org/en/publications/test-no-414-prenatal-development-toxicity-study_9789264070820-en.html">https://www.oecd.org/en/publications/test-no-414-prenatal-development-toxicity-study_9789264070820-en.html</a> Test No. 426: Developmental Neurotoxicity Study <a href="https://www.oecd.org/en/publications/test-no-426-developmental-neurotoxicity-study_9789264067394-en.html">https://www.oecd.org/en/publications/test-no-426-developmental-neurotoxicity-study_9789264067394-en.html</a>
<b>Reproduction toxicity</b>	Test No. 415: One-Generation Reproduction Toxicity Study <a href="https://www.oecd.org/en/publications/test-no-415-one-generation-reproduction-toxicity-study_9789264070844-en.html">https://www.oecd.org/en/publications/test-no-415-one-generation-reproduction-toxicity-study_9789264070844-en.html</a> Test No. 416: Two-Generation Reproduction Toxicity <a href="https://www.oecd.org/en/publications/test-no-416-two-generation-reproduction-toxicity_9789264070868-en.html">https://www.oecd.org/en/publications/test-no-416-two-generation-reproduction-toxicity_9789264070868-en.html</a> Test No. 443: Extended One-Generation Reproductive Toxicity Study <a href="https://www.oecd.org/en/publications/test-no-443-extended-one-generation-reproductive-toxicity-study_9789264185371-en.html">https://www.oecd.org/en/publications/test-no-443-extended-one-generation-reproductive-toxicity-study_9789264185371-en.html</a>
<b>Reproduction and developmental toxicity screening</b>	Test No. 421: Reproduction/Developmental Toxicity Screening Test <a href="https://www.oecd.org/en/publications/test-no-421-reproduction-developmental-toxicity-screening-test_9789264264380-en.html">https://www.oecd.org/en/publications/test-no-421-reproduction-developmental-toxicity-screening-test_9789264264380-en.html</a> Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test <a href="https://www.oecd.org/en/publications/test-no-422-combined-repeated-dose-toxicity-study-with-the-reproduction-developmental-toxicity-screening-test_9789264264403-en.html">https://www.oecd.org/en/publications/test-no-422-combined-repeated-dose-toxicity-study-with-the-reproduction-developmental-toxicity-screening-test_9789264264403-en.html</a>
<b>Neurotoxicity</b>	Test No. 424: Neurotoxicity Study in Rodents <a href="https://www.oecd.org/en/publications/test-no-424-neurotoxicity-study-in-rodents_9789264071025-en.html">https://www.oecd.org/en/publications/test-no-424-neurotoxicity-study-in-rodents_9789264071025-en.html</a> Test No. 426: Developmental Neurotoxicity Study <a href="https://www.oecd.org/en/publications/test-no-426-developmental-neurotoxicity-study_9789264067394-en.html">https://www.oecd.org/en/publications/test-no-426-developmental-neurotoxicity-study_9789264067394-en.html</a>

4131 **Table D3.** Risk of bias evaluation for the eligible animal studies

Authors	Tier	Q1-key	Q3-key	Q5-key	Q2	Q4	Q6	Q7
<b>Berberine</b>								
<b>Jahnke et al., 2006</b>	2	(--)	(++)	(++)	Body weight: (++) , Organ weight: (++) ; Histopathology: NR Fetal, implantation and resorption examinations: NR.	(++)	(++)	(++)
<b>Alagal et al., 2023</b>	3	NR	(-)	(-)	Body weight: (++) ; Clinical chemistry: (++) ; Histopathology: (++) .	(+)	(-)	(-)
<b>Akhzari M. et al., 2022</b>	3	NR	(--)	(--)	Clinical chemistry: (++) ; Histopathology: NR.	(+)	(+)	(+)
<b>Ghavipanje et al., 2021a,b ; Ghavipanje et al., 2022a,b</b>	2	NR	(++)	(-)	Body weight:(++) ; Clinical chemistry: (++) ; Glucose homeostasis (++) .	(+)	(+)	(+)
<b>Adefegha et al., 2021</b>	2	NR	(++)	(-)	Clinical chemistry: (++) ; Sexual parameters: NR ; Histopathology: NR.	(+)	(+)	(-)
<b>Jia et al., 2020</b>	2	NR	(++)	(- -)	Clinical chemistry: (++) ; Histopathology: NR	(+)	(++)	(+)
<b>Tian et al., 2019</b>	3	NR	(-)	(- -)	Body weight: (++) , Clinical chemistry: (++) ; Histopathology: NR	(+)	(++)	(+)
<b>Gholampour et al., 2017</b>	3	(--)	(-)	(- -)	Body weight:(++) , Clinical chemistry: (++) ; Histopathology: (+)	(+)	(++)	(+)
<b>Maurya et al., 2016</b>	3	NR	(- -)	(++)	Body weight: (++) , Clinical chemistry: (++) ; Haematology ; (++) ; Histopathology: NR	(-)	(+)	(-)
<b>Hasanein et al., 2017</b>	2	NR	(- -)	(+)	Body weight: (++) , Clinical chemistry: (++) , Histopathology: (+)	(+)	(++)	(++)
<b>Heidarian et al., 2014</b>	3	NR	(- -)	(-)	Clinical chemistry: (++) , Histopathology: NR	(+)	(++)	(++)
<b>Moghaddam et al., 2013</b>	3	NR	(- -)	(-)	Body weight: (++) ; Glucose homeostasis: (++)	(++)	(+)	(+)
<b>Gu et al., 2012</b>	2	(+)	(-)	(+)	Body weight: (++) ; Clinical chemistry: (++) ; Glucose homeostasis ; Immunohistochemistry: NR	(++)	NR	(++)
<b>Zhang et al., 2012</b>	3	NR	(-)	(-)	Body weight: (++) ; Clinical chemistry: (++)	(++)	(+)	(++)
<b>Hu et al., 2012</b>	2	(++)	(- -)	(--)	Body weight: (++) ; Clinical chemistry: (++) ; Glucose homeostasis (++) .	(+)	(+)	(+)
<b>Chatuphonprasert et al., 2012</b>	3	NR	(-)	(--)	Body weight: (++) ; Glucose homeostasis: (++) .	(-)	NR	(++)
<b>Hassanein et al., 2019</b>	3	NR	(- -)	(- -)	Body weight: (++) , Clinical chemistry: (++) , Histopathology: (+)	NR	(+)	(++)

Authors	Tier	Q1-key	Q3-key	Q5-key	Q2	Q4	Q6	Q7
<b>Akhzari et al., 2019</b>	3	NR	(- -)	(- -)	Clinical chemistry: (++) ; Histopathology: NR.	(+)	(+)	(++)
<b>Guo et al., 2011</b>	3	NR	(-)	(-)	Clinical chemistry: (++) ; Organ weight: (++) ; Histopathology: (+).	(-)	NR	(-)
<b>Yi et al., 2013</b>	3	(+)	(--)	(-)	Body weight: (++) , Clinical chemistry: (++) ; Organ weight: (++) ; Histopathology: NR.	NR	(+)	(--)
<b>Other protoberberine alkaloids</b>								
<b>Berberrubine</b>								
<b>Wang et al., 2020</b>	2	(+)	(-)	(-)	Clinical chemistry: (++) ; Histopathology: NR	(+)	(++)	(+)
<b>Jatrorrhizine</b>								
<b>Wu et al., 2014</b>	2	(- -)	(- -)	(+)	Body weight: (++) ; Clinical chemistry: (++) ; Haematology: (++) ; Organ weight: (++) ; Histopathology: NR	(+)	(+)	(++)
<b>Columbamine</b>								
<b>Wang et al, 2016</b>	2	(++)	(++)	(-)	Body weight: (++) ; Clinical chemistry: (++) ; Organ weight: (++) , Histopathology: NR	NR	(++)	(+)
<b>Coptisine, epiberberine, palmatine</b>								
<b>Yi et al., 2013</b>	3	(--)	(--)	(-)	Body weight: (++) , Clinical chemistry: (++) ; Organ weight: (++) ; Histopathology: NR.	NR	(+)	(--)
<b>Plant preparations</b>								
<b><i>P. amurensis</i></b>								
<b>Alam et al., 2021</b>	2	(--)	(-)	(+)	Clinical chemistry: (++) ; Haematology: (++) ; Histopathology: (+)	(+)	(++)	(-)
<b>Lee et al., 2018</b>	2	(+)	(++)	(-)	Body weight: (++) ; Organ weight: (++) ; Histopathology: NR ; Vaginal opening: NR ; Bone length: (++) .	(+)	(++)	(+)
<b><i>H. canadensis</i></b>								
<b>NTP, 2010 ; Dunnick et al., 2011</b>	1	(++)	(++)	(++)	Body weight: (++) ; Haematology: (++) ; Clinical chemistry: (++) ; Organ weight: (++) ; Histopathology: (++) ; Sperm morphology, counting (+) ; Vaginal cytology: (+).	(++)	(++)	(++)
<b>Yao et al., 2005</b>	3	(-)	(-)	(-)	Body weight: (++) ; Fetal, placental weight: (++) ; Organ weight: (++) ; Fetal external and internal examinations (malformations), embryonic/fetal development: NR.	NR	(+)	(-)
<b><i>C. fenestratum</i></b>								
<b>Shirwaikar et al., 2005</b>	2	(++)	(-)	(--)	Glucose homeostasis: (++) .	(-)	(++)	(--)
<b>Wattanathorn et al., 2006</b>	3	(--)	(-)	(-)	Body weight: (++) ; Neuron density: (-) , Stereotyped behaviour blinding: NR.	(+)	(+)	(+)

Authors	Tier	Q1-key	Q3-key	Q5-key	Q2	Q4	Q6	Q7
<b>Wongcome et al., 2006</b>	3	NR	(++)	(--)	Body weight: (++) ; Clinical chemistry; Haematology: (++) ; Organ weight: (++) ; Histopathology: NR; Spontaneous motor activity: (++)	NR	(++)	-
<b><i>C. majus</i></b>								
<b>Mazzanti et al., 2009</b>	2/3	(-)	(-)	(-)	Body weight: (++) ; Clinical chemistry; Haematology: (++) ; Organ weight: (++) ; Histopathology: NR.	(+)	(+)	(--)

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4135 Appendix E. Genotoxicity studies on berberine, protoberberines and berberine-  
 4136 containing plant preparations

4137 **Table E1.** Evidence table on in vitro genotoxicity studies on berberine

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
<b>Gene mutation<sup>a</sup></b>							
Bacterial reverse mutation assay - "Fit-for-purpose" study -GLP compliance: no	<i>S. typhimurium</i> TA98 strain; - S9-mix, only - Vehicle: DMSO control: not used - Positive control: not used	Berberine chloride - CAS: 633-65-8 - purity: 99%	15.39, 31.8, 63.5, 136.9, 273.8, 547.5 and 1,095 µg/plate	Positive Concentration-dependent increase up to 547.5 µg/plate (approx. 2.5-fold increase)	2 - negative and positive controls were not used	High - although this was "fit-for-purpose" Ames study, the relevance is high  Limited	Sun et al., 2025
Mammalian cell gene mutation test (HPRT) - OECD 476 GLP compliance: yes	L5178Y tk +/- (3.7.2C) mouse lymphoma cells; +/- S9-mix - Vehicle: DMSO - Negative control: (vehicle) - Positive control: NOQ (-S9-mix) and B[a]P (+S9-mix)	Berberine chloride - CAS: 633-65-8 - purity: 99%	(-S9-mix): 0, 322.7, 376.5, 403.4, 430.3, 457.2, 484.1 and 511 µM for 3h  (+S9-mix): 0, 134.5, 376.5, 403.4, 430.3, 457.2 and 484.1 µM for 3h  Cytotoxicity ≤80%	Positive Concentration-dependent, statistically significant increase in HPRT mutation frequencies in tested cell-line (without S9-mix).  Negative No stat. significant increase in HPRT mutation frequencies with S9-mix	1 The study was conducted in accordance with OECD TG 476 and in compliance with GLP	High/High	Sun et al., 2025

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Bacterial reverse mutation assay (pre-incubation method for 20 min) - GLP compliance: NR	<i>S. typhimurium</i> (TA97, TA98, TA100 and TA1535); +/- S9-mix - Vehicle: NR - Negative control: (solvent) - Positive control (sodium azide, 9-aminoacridine, 4-nitro- <i>o</i> -phenylenediamine and 2-aminoanthracene)	Berberine chloride - CAS: 633-65-8 - Purity: NR	0, 0.33, 1, 3.3, 10, 33 and 100 µg/plate (- S9-mix)  0, 0.33, 1, 3.3, 10, 33, 100, 333 and 1,000 µg/plate (+ S9-mix)	Negative Non-mutagenic in strains TA97, TA98, TA100 and TA1535, with or without S9 mix	2 Deviations from the OECD TG 471: not all required bacterial strains were tested - purity of berberine chloride not reported - the possible presence of toxic impurities could have restricted the range of concentrations tested	High/Limited	NTP, 2010
Bacterial reverse mutation assay (pre-incubation method) -GLP compliance: NR	<i>S. typhimurium</i> TA100 and TA98; +/- S9-mix - Vehicle: DMSO - Negative control: (vehicle) - Positive control: (benzo[a]pyrene and furylfuramide (AF-2))	Berberine hydrochloride - CAS: 633-65-8 - Purity: no impurities were found	NR	Inconclusive - with S9 mix  Inconclusive - without S9 mix weak mutagenicity on TA98 strain as reported by the authors	3 Deviation from the OECD TG 471: Not all required bacterial strains were tested; concentrations used are not reported; unclear reporting of results	High/Low	Nozaka et al., 1990
SOS chromotest -GLP compliance: NR	<i>E. coli</i> (PQ37) - Vehicle: DMSO - Negative control: vehicle - Positive control: AFB1 (+S9-mix) and 4-NQO (-S9-mix)	Berberine chloride -purity: NR	0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10 µg/test for 2h.	Negative - no induction of the synthesis of $\beta$ -galactosidase with and without S9-mix	2 This assay is not an OECD recognised assay for genotoxicity testing.	Limited/Limited	Pasqual et al., 1993

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Frameshift Mutation Assay (hom3-10) - GLP compliance: NR	<i>S. cerevisiae</i> haploid strain XV-185-14c (hom3-10 marker) - Vehicle: Water or saline - Negative control: Untreated cells (vehicle only) - Positive control: 8-MOP + UVA	Berberine chloride -purity: NR	- Nondividing cells: 0, 75, 150, 225, 300 µg/mL (21 h) - Dividing cells: 0, 10, 20, 30, 40, 50 µg/mL (21 h)  No cytotoxic effects in non dividing cells Survival >50% in dividing cells	Positive Significant increase in frameshift revertants in dividing cells only.	2 This assay is not an OECD recognised assay for genotoxicity testing.	Limited/Limited	Pasqual et al., 1993
Point Mutation Assay (lys1-1, his1-7) - GLP compliance: NR	<i>S. cerevisiae</i> haploid strain XV-185-14c (lys1-1 ochre, his1-7 missense) - Vehicle: Water or saline - Negative control: Untreated cells (vehicle only) - Positive control: 8-MOP + UVA	Berberine chloride -purity: NR	- Nondividing cells: 0, 75, 150, 225, 300 µg/mL (21 h) - Dividing cells: 0, 10, 20, 30, 40, 50 µg/mL (21 h)	Negative No induction of point mutations in either dividing or nondividing cells.	2 This assay is not an OECD recognised assay for genotoxicity testing.	Limited/Limited	Pasqual et al., 1993

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Cytoplasmic "Petite" Mutation Assay - GLP compliance: NR	<i>S. cerevisiae</i> haploid strain XV-185-14c (detection of respiratory-deficient "petite" mutants) - Vehicle: Water or saline - Negative control: Untreated cells (vehicle only) - Positive control: 8-MOP + UVA	Berberine chloride -purity: NR	- Nondividing cells: 0, 75, 150, 225, 300 µg/mL (21 h) - Dividing cells: 0, 10, 20, 30, 40, 50 µg/mL (21 h)	Negative Modest not significant increase of mitochondrial "petite" mutants in dividing cells  Negative No effect under nondividing conditions.	2 This assay is not an OECD recognised assay for genotoxicity testing.	Limited/Limited	Pasqual et al., 1993
Chromosomal instability <sup>a</sup> Micronucleus assay - GLP compliance: NR	L5178Y tk +/- mouse lymphoma and human TK6 cells: -S9-mix only -Vehicle: DMSO  - Negative control: (vehicle)  - Positive control: not used	Berberine chloride - CAS: 633-65-8 - purity: 99%	Human TK6 cells: Six concentrations in total, but only 0,100 and 200 µM mentioned (4h treatment + 20h recovery)  Cytotoxicity did not exceed 60%  L5178Y tk +/- mouse lymphoma: 0, 134, 194, 254, 314 and 376 µM (4h treatment + 20h recovery)  Cytotoxicity exceeded 60%	Human TK6 cells: Positive Significant increases at top two concentrations (100 and 200 µM) in MN frequency  L5178Y tk +/- mouse lymphoma: Equivocal Concentration-dependent and statistically significant increase in MN frequency at top two concentrations (314 and 376 µM)	2 Limited information reported overall. Lack of key experimental details (e.g. exact concentrations used in study with TK6 cells; lack of positive control cell proliferation data, MN scoring criteria etc.)	High/  Limited - for the study with TK6 cells  Low - for the study with L5178Y cells	Sun et al., 2025

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Micronucleus assay - GLP compliance: NR	Human osteosarcoma cell lines U2OS  - positive control: NR	Berberine - Purchased from Sigma Chemical Co. (St. Louis, MO, USA) -CAS: NR -Purity: NR	0, 10, 20, 50 µg/ml for 48h	Positive - significant increase at all concentrations tested. Cytotoxicity did not exceed 60% at 10 and 20 µg/mL; >60% at 50 µg/mL Concentration-dependent increase from 10 µg to 20 µg with a leveling off at 50 µg likely due to excessive toxicity	2 The test is not performed according to OECD TG 487: - use of a non-validated cell line - missing the short-term exposure and +/- S9 - only 1000 cells were scored - lack of positive control	High/Limited	Liu et al., 2009
Mitotic Recombination (Crossing-over, Gene Conversion) - GLP compliance: NR	<i>S. cerevisiae</i> diploid strain XS2316 - Vehicle: Water or saline - Negative control: Untreated cells (vehicle only) - Positive control: 8-MOP + UVA	Berberine chloride -purity: NR	- Nondividing cells: 0, 75, 150, 225, 300 µg/mL (21 h) - Dividing cells: 0, 10, 20, 30, 40, 50, 75, 100 µg/mL (21 h)  Cytotoxicity ≤ 50% up to 50 µg/mL; around 80-90% at the two highest concentrations	Positive Significant increase in crossing-over frequency starting from 20 µg/mL in dividing cells only.	2 This assay is not an OECD recognised assay for genotoxicity testing.	Limited/Limited	Pasqual et al., 1993

DNA damage<sup>b</sup>

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Alkaline comet assay - GLP compliance: NR	Mouse NIH/3 T3 and human Caco-2 cell lines - negative control: DMEM with 1% DMSO - positive control: not used	Berberine - purity: NR	0, 5, 10, 25 and 50 µM for 4h	NIH/3 T3 cells: Equivocal Significant increase of DNA damage (% tail DNA) at all concentrations, concentration-dependent  Caco-2 cells: Equivocal - no increase in % tail intensity - After 24h exposure the apoptotic index of Caco-2 cells was close to 100%.  These results are based on figure 7B from the article.	3  This assay is not an OECD recognised assay for genotoxicity testing. - No positive control - Description of the results does not match the figure  Apoptosis/cytotoxicity measurements after 4h exposure were not provided.	Limited/Low	Secerli et al., 2023
Alkaline comet assay - GLP compliance: NR	Human bladder cancer cell lines T24, 5637 and 253J - Vehicle: DMSO - Negative control: vehicle - Positive control: not used	Berberine chloride - CAS: 633-65-8 - purity: NR	0 and 40 µM for 72h	Equivocal - Significant increase of DNA damage (olive tail moment) in T24 and 5637 cells, but not in the 253J cell line  High levels of apoptosis at the concentration tested (approx. 50-80%)	3  This assay is not an OECD recognised assay for genotoxicity testing. - Only one concentration was tested. - Concomitant high levels of apoptosis - Measurement of DNA damage by olive tail moment only - No positive control	Limited/Low	Xia et al., 2021

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Comet assay - GLP compliance: NR	Hep3B cells - Vehicle: NR - Negative control: NR - Positive control: not used	Coptisine - Catalogue No. SMB00314 - CAS: NR - Purity: NR	0 and 50 µM for 24h  Cell viability (MTT assay) approximately 70% at 50 µM	Inconclusive Significant increase of DNA damage (tail length)	3 These assays are not an OECD recognised assays for genotoxicity testing.  - Only a single concentration was tested. - Concurrent induction of high frequency of apoptotic cells (approximately 90%). - Measurement of DNA damage by tail length only. -No positive control	Limited/Low	Kim et al., 2020
Alkaline comet assay -GLP compliance: NR	Human cancer cell lines (BT549 and MDA-MB-231) - Vehicle: NR - Negative control: NR - Positive control: NR	Berberine - CAS: NR - Purity: NR	0, 20, 40, 80, 160 µmol/L, duration: "overnight".  After 48 hr exposure, cell viability (MTT assay) across the range of concentrations tested varied from 60% to 10%.	Equivocal - Significant concentration-dependent increase of DNA damage (tail length)	3 This assay is not an OECD recognised assay for genotoxicity testing. - No positive control. - Duration of exposure not explicitly stated - Only 30 comets were analysed per experimental point - Cell viability data for "overnight" exposure were not provided -Measurement of DNA damage by tail length only	Limited/Low	Gao et al., 2019

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Alkaline comet assay - GLP compliance: NR	Human keratinocyte cell line HaCaT - Vehicle: DMSO - Negative control: vehicle - Positive control: not used	Berberine chloride - CAS: NR - Purity: NR	0, 40 µM for 48h  After 72 hr exposure to 40 µM cell viability (clonogenic assay) was >50% and approximately 50% apoptotic cells were detected	Equivocal - significant increase of DNA damage (olive tail moment)	3  This assay is not an OECD recognised assay for genotoxicity testing.  - Only one concentration was tested.  - No positive control.  - Measurement of olive tail moment only.  -Cytotoxicity/apoptosis data after 48h exposure were not provided	Limited/Low	Sun et al., 2019
Alkaline comet assay) - GLP compliance: NR	Human ovarian cancer cells (A2780 and HO8910) Normal human ovarian surface epithelial cells (FTE-187) – - Vehicle: DMSO - Negative control: vehicle - Positive control: not used	Berberine chloride - CAS: NR - Purity: NR	0, 10, 20 µM for 48h  Cytotoxicity (MTT assay) ≤20% at both concentrations tested in all cell lines  Apoptotic cells not exceeding 20% at 20 µM in all cell lines	Positive Significant increase of DNA damage (tail moment) at both concentrations tested DNA damage in both cancer cell lines. Not significant increase in FTE-187 normal epithelial cell line	2  These assays are not an OECD recognised assays for genotoxicity testing.  - Measurement of DNA damage by tail moment only  - Concomitant analysis of γ-H2AX and RAD51 foci and 8-OHdG levels  - No positive control	Limited/Limited	Hou et al., 2017

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Alkaline comet assay - GLP compliance: NR	Human cervical adeno carcinoma HeLa S3 cell line - Vehicle: distilled water - Negative control: vehicle - Positive control: NR	Berberine chloride - CAS: NR - Purity: NR	0, 1, 2, 4, 6, 8 µg/mL for 2h or 4h  Survival higher than 60% up to 4 µg/mL; approximately 40% at 8 µg/mL (clonogenic assay) after 4 hr exposure	Equivocal Significant increase of DNA damage (Olive tail moment) as a function of both concentration and time of exposure; additional accumulation of DNA damage during post-treatment times	2 This assay is not an OECD recognised assay for genotoxicity testing. - Measurement of olive tail moment - no positive control - no S9-mix - cytotoxicity may have interfered with the detection of DNA damage at concentrations ≥4 µg/mL	Limited/Low	Jagetia et al., 2015
Comet Assay -GLP compliance: NR	Mouse L929 fibroblast-like cell line - Vehicle: NR - Negative control: NR - Positive control: H <sub>2</sub> O <sub>2</sub>	Berberine -CAS: NR -Purity: NR	0, 2.5, 12.5, 25µg/mL for 24h  No cytotoxicity (CCK-8 assay) observed at tested concentrations.	Positive Significant increase of DNA damage (tail length and % tail DNA) as a function of concentration	2 This assay is not an OECD recognised assay for genotoxicity testing. - no S9-mix  Mild increase of apoptosis rate starting from 0,005 mg/ml; 0.1mg/mL almost twofold increase over control	Limited/Limited	Gu et al., 2015
Alkaline comet assay - GLP compliance: NR	Human hepatoma HepG2 cell line - Vehicle: DMSO - Negative control: vehicle - Positive control: H <sub>2</sub> O <sub>2</sub> or 4-NQO	Berberine - CAS: NR - Purity: NR  Palmatine - CAS: NR - Purity: NR	0, 3.125, 6.25, 12.5, 25, 50 µM for 2h, 6h or 24h.  - no significant cytotoxicity was observed with the lactate dehydrogenase (LDH) assay in the range of concentrations tested	Positive - Berberine: significant increase of DNA damage (% tail DNA) in a concentration- and time-dependent manner starting from the lowest concentration, 6 h exposure  - Palmatine: moderate induction of DNA damage (data not shown)	2 - for the assay with berberine These assays are not an OECD recognised assay for genotoxicity testing  4 - for the assay with palmatine	Limited/Limited	Chen et al., 2013

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Comet assay -GLP compliance: NR	HL-60 human promyelocytic cells (p53-deficient) - Negative control: solvent-treated cells - Positive control: H <sub>2</sub> O <sub>2</sub>	Berberine chloride dihydrate -CAS: NR -purity 98.92%	0, 0.3, 0.6, 1.2 µg/ml for 48h Survival rate > 80% (trypan blue assay) IC50 1.2 µg/mL (cell proliferation assay)	Inconclusive No evidence of DNA damage (%tail DNA)	3 This assay is not an OECD recognised assay for genotoxicity testing.  The concentrations may have been too low to induce detectable levels of DNA damage in the comet assay, as also concluded by the authors.  Apoptosis was observed at 0.6 and 1.2 µg/mL for 48 h.  High cytostatic effect (IC50 1.2 µg/mL by cell proliferation assay); DNA damage might be underestimated (e.g. might not reveal the replication stress-induced DNA breaks).	Limited/Low	Khan et al., 2010
Comet Assay +/- incubation with Fpg and Endo III -GLP compliance: NR	NIH-3T3 murine fibroblasts cell line and EAC Ehrlich ascites carcinoma cell line - Vehicle: DMSO - Negative control: vehicle - Positive control: NR	Berberine chloride -CAS: NR -Purity: NR	(NIH-3T3 cells): 0, 0.27, 1.35, 2.69 µM for 24h IC50 11.43 ± 0.32 µM  (EAC cells): 0, 0.14, 0.27, 0.67 µM for 24h IC50 2.69 ± 0.19 µM	Positive Significant increase of DNA damage (% tail DNA) concentration-dependent No detection of oxidative DNA lesions	2 This assay is not an OECD recognised assay for genotoxicity testing  - No apoptosis detected in the range of concentrations tested  - No positive control	Limited/Limited	Jantova et al., 2006

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Comet Assay -GLP compliance: NR	Human prostate carcinoma cell line DU145 - Negative control: cells without Berberine - Positive control: NR	Berberine chloride -CAS: NR -Purity: NR	0, 25, 50 µmol/L for 72h  Cell viability>80%	Equivocal Significant increase of DNA damage (tail length) at both concentrations	3 This assay is not an OECD recognised assay for genotoxicity testing.  - Approx. 20 and 50% apoptotic cells were measured at 25 and 50 µM berberine, respectively. DNA breaks are likely to be apoptosis driven. (reduction in the presence of a caspase inhibitor)  - Measurement of DNA damage by tail length only  -No positive control	Limited/Low	Mantena et al., 2006
Alkaline comet Assay -GLP compliance: NR	NPC/HK1 cell line (squamous cell carcinoma) - Negative control: PBS - Positive control (H2O2)	Berberine chloride - CAS: NR - Purity: NR	0, 50, 100, 150, 200 µM for 30 min and 1h  Viability (trypan blue exclusion assay) is 70-80% at 50 µM and drops to 60% at 200 µM, 1 h exposure	Equivocal Significant increase in DNA damage (% tail DNA) observed only at 200 µM after 30 min treatment  No statistically significant increase in DNA damage after 1h treatment at all concentration tested.	3 This assay is not an OECD recognised assay for genotoxicity testing.  A large variation in %tail DNA was observed in the comet assay data.	Limited/Low	Szeto et al., 2002
Other assays <sup>b</sup>							

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
γ-H2AX foci assay	Human renal cell carcinoma cell lines (ACHN and A498) - Vehicle: DMSO - Positive control: not used	Berberine - CAS: NR - Purity: NR	0, 20, 50, 100 μM for 24h  Cell viability (MTT assay) approx. 60% at the highest concentration tested (100 μM)	Positive Significant concentration-dependent increase of γ-H2AX relative intensity starting from 20 μM in both cell lines.  Although only one representative immunofluorescence image for each concentration is shown, quantification of γH2AX intensity was performed using ImageJ across more than three independent experiments	2 This assay is not an OECD recognised assay for genotoxicity testing.  - positive control not used  - Missing some methodological details such as definition of foci in terms of size, intensity, and number and clarification of the control groups.  Frequency of apoptotic cells approx. 20 and 5% at the highest concentration tested (100 μM) in ACHN and A498 cells, respectively; <5% at lower concentrations (20 and 50 μM)	Limited/Limited	Zhao et al., 2023
γ-H2A.X foci assay - GLP compliance: NR	Human ovarian cancer cells (A2780 and HO8910) - vehicle: DMSO - negative control: vehicle	Berberine chloride - CAS: NR - Purity: NR	0, 10, 20 μM for 48h  Cytotoxicity (MTT assay) 20% at both concentrations tested in all cell lines  Apoptotic cells not exceeding 20% at 20 μM in all cell lines	Positive Significant increase in the percentage of cells with γ-H2A.X foci (>5) in both cancer cell lines	2 This assay is not an OECD recognised assay for genotoxicity testing	Limited/limited	Hou et al., 2022

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Pulse Field Gel Electrophoresis (PFGE). - GLP compliance: NR	SV40-transformed human fibroblast MRC5sv cell line  Various human cancer cell lines  - Negative control: untreated cells  - Positive control: NR	Berberine chloride -CAS: NR -Purity: NR  Coptisine chloride -CAS: NR -Purity: NR  Palmatine -CAS: NR -Purity: NR	0, 10, 20, 40 µM for 24 h      0, 10, 20, 40 µM for 24 h	Positive  Significant accumulation of broken DNA (DSBs) as a function of concentration with both berberine and coptisine but not with palmatine.	2  This assay is not an OECD recognised assay for genotoxicity testing.  These breaks are associated with DNA replication rather than apoptosis (inhibition by aphidicolin but not by the apoptotic nuclease inhibitor Z-VAD-FMK)  - No positive control	Limited/Limited	Inoue et al., 2021
γ-H2AX foci assay -GLP compliance: NR	MG-63 human osteosarcoma cell line	Berberine -CAS: NR -Purity: > 98%	0, 20, 40, 60, 80 µM for 12h and 24h	Positive  Significant increase concentration and time-dependent of γ-H2AX foci and % of γ-H2AX-positive cells (foci ≥4)	2  This assay is not an OECD recognised assay for genotoxicity testing.  Apoptosis was observed at 12–24 h, reaching 30% at 40 µM and 40% at 80 µM, with no significant increase at 20 µM  The γ-H2AX foci pattern (Fig. 4) suggests DSBs as indicated by discrete rather than diffuse foci	Limited/Limited	Zhu et al., 2014

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
γ-H2AX foci assay -GLP compliance: NR	Murine RM-1 prostate cancer cell line	Berberine chloride -CAS: NR -Purity: NR	0, 10, 20, 50 μM for 24h	Positive Significant increase concentration-dependent of γ-H2AX foci (fluorescence microscope) and % of γ-H2AX positive cells (flow cytometry)  Early apoptotic cells approx. 15%, necrotic cells approx. 8% at the highest concentration tested	2 This assay is not an OECD recognised assay for genotoxicity testing.	Limited/Limited	Wang et al., 2012
γ-H2A.X foci assay -GLP compliance: NR	Human osteosarcoma cell lines U2OS	Berberine -CAS: NR -Purity: NR	0, 10, 20, 50 μg/mL for 48 h	Equivocal Increased number of γ-H2AX positive cells as a function of concentration, as inferred from a single representative immunofluorescence image per concentration.  Concentration-dependent increase of γ-H2AX expression by WB  Apoptotic cells approx. 15% at the highest concentration tested	3 This assay is not an OECD recognised assay for genotoxicity testing.  Quantitative analysis (e.g., mean number of foci per cell or fluorescence intensity from multiple fields) is not provided,  Lack of methodological details	Limited/low	Liu et al., 2009

4138 <sup>a</sup> Bacterial reverse mutation test, Mammalian cell gene mutation test in vitro and Micronucleus test in vitro are considered of high relevance for hazard identification (EFSA, 2023). EFSA. 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, 20(9), 8270E. <https://doi.org/10.2903/sp.efsa.2023.EN-8270>.

4139 <sup>b</sup> Comet assay in vitro and γH2AX assay with lesser relevance can be used as supporting information for hazard identification (EFSA, 2023). EFSA. 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, 20(9), 8270E. <https://doi.org/10.2903/sp.efsa.2023.EN-8270>.

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4142 **Table E2.** Evidence table on in vivo genotoxicity studies on berberine

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
<b>Chromosomal aberration and micronucleus<sup>a</sup></b>							

Type of test	Tested system	Test substance (key info)	Concentration/ treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
Micronucleus assay - GLP compliance : NR	Bone marrow from male B6C3F1 (5 animals/group) - Vehicle: phosphate-buffered saline (solvent) - Negative control: (solvent) - Positive control: (cyclophosphamide)	Berberine chloride - CAS: 633-65-8 - Purity NR	0, 41.125, 82.25, 164.50, 329, 658 mg/kg bw by intraperitoneal injection 3 times at 24h intervals	Inconclusive No increase in the frequency of micronucleated PCEs; no dose-related change in the percentage of PCEs in the bone marrow	2 Route of exposure (i.p.) other than oral.	High/Low	NTP, 2010
<b>Comet assay<sup>a</sup></b>							
Comet assay - GLP compliance : NR	Heart cells from male mice - Vehicle: NR - Negative control: untreated mice - Positive control: not defined	Berberine - CAS: NR - Purity: 95%	0, 7.5, 15, 30, 60, 120 mg/kg bw by gavage	Inconclusive - significant dose-dependent increase in %DNA tail (at doses of 30 mg/kg bw and above), tail length and tail moment (at doses of 15 mg/kg bw and above) but notable experimental flaws	3 Limited information reported overall. Lack of key experimental details (e.g. methodology for heart cell isolation and comet assay, number of cells investigated, treatment schedule etc) - No data on cytotoxicity/histopathology	High/Low	Xu et al., 2014a
Comet assay - GLP compliance : NR	Bone marrow from male mice - Vehicle: NR - Negative control: untreated mice - Positive control: NR	Berberine - CAS: NR - Purity: 95%	0, 7.5, 15, 30, 60, 120 mg/kg bw by gavage	Inconclusive - significant dose-dependent increase in %DNA tail, tail length and tail moment (at doses of 30 mg/kg bw and above) but notable experimental flaws	3 Limited information reported overall. Lack of key experimental details (e.g. methodology for heart cell isolation and comet assay, number of cells investigated, treatment schedule etc.) - No data on cytotoxicity/histopathology	High/Low	Xu et al., 2014b

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<sup>a</sup> Micronucleus test in vitro and comet assay in vivo are considered of high relevance for hazard identification (EFSA, 2023). EFSA. 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, 20(9), 8270E. <https://doi.org/10.2903/sp.efsa.2023.EN-8270>.

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4148 **Table E3.** Evidence table on in vitro genotoxicity studies on berberine-containing plant preparations

Type of test	Tested system	Test substance (key info)	Concentration/treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
<b>Gene mutation<sup>a</sup></b>							
Bacterial reverse mutation assay (pre-incubation method) - GLP compliance: NR	<i>S. typhimurium</i> (TA98 and TA102); +/- S9-mix - Vehicle: DMSO - Negative control: vehicle - Positive control: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), MCC, 2AA	Hot water extracts of <i>C. japonica rhizome</i> (cultivated with a novel hydroponic system) - BBR content: from 18.0% to 22.2% in dry extracts  Two commercial crude drugs of <i>C. japonica rhizome</i> - Berberine content: 12.6 and 20.5%	(-S9-mix): 0, 4.88, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1,250, 2,500 and 5,000 µg/plate  (+S9-mix): 0, 39.1, 78.1, 156, 313, 625, 1,250, 2,500 and 5,000 µg/plate	Negative - in TA102 (with and without S9 mix)  Positive - TA98 (with and without S9 mix) dose-related mutant increase	2  - not all bacterial strains were tested; - limited information on preparation of test solutions, and statistical analysis	High/Limited	Akiyama et al., 2019
Bacterial reverse mutation assay (plate incorporation method)	<i>S. typhimurium</i> (MTCC 1251) - Vehicle: NR - Negative control: no sodium azide - Positive control: sodium azide	Aqueous extract of the root bark of <i>B. aristata</i> - berberine content: NR	NR	Inconclusive	3  No information on: - the tested bacterial strain (it is not among the strains indicated in the OECD TG 471), and although it technically corresponds to TA98 strain, it is less sensitive lacking some auxiliary mutations; - negative control (seems not to be used), - concentrations tested	High/Low	Sood et al., 2019
Bacterial reverse mutation assay (pre-incubation method) - GLP compliance: NR	<i>S. typhimurium</i> (TA98 and TA100), <i>E. coli</i> WPM <i>uvrA</i> /pKM101; +/- S9-mix - Vehicle: NR - Negative (solvent) and Positive Control (sodium azide, 4-nitro- <i>o</i> -	<i>H. canadensis</i> root powder - > 2.0% (-)-β-hydrastine, > 2.5% berberine - all relevant analytical tests performed, as well as the stability study	0, 1,000, 1,500, 2,500, 5,000, 5,000, 7,500, 10,000 µg/plate	Negative - Non-mutagenic in tested strains, with or without S9 mix	2  Deviations from the OECD TG 471: Not all bacterial strains were tested ( <i>S. typhimurium</i> TA1535 and TA1537 (or TA97 or TA97a)	High/Limited	NTP, 2010

Type of test	Tested system	Test substance (key info)	Concentration/ treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
	phenylenediamine, methylmethanesulfonate and 2-aminoanthracene)						
<b>Comet assay<sup>b</sup></b>							
Comet assay - GLP compliance: NR	HeLa cells - Negative control: cell without the treatment - Positive control: not used	<i>H. canadensis</i> root powder ethanolic root extract - content of berberine: NR	0, 380 µg/mL and 570 µg/mL for 24h  IC <sub>50</sub> = 498.67 µg/mL	Inconclusive Results shown as fluorescence images of only two cells, claimed to present comet tails	3 This assay is not an OECD recognised assay for genotoxicity testing. Apoptosis observed at the same doses used for the comet assay	Limited/Low	Saha et al., 2013
<b>Other assays<sup>b</sup></b>							
γ-H2A.X and 53BP1 foci assay - GLP compliance: NR	MRC5sv cells - Negative control: cell without the treatment - Positive control: camptothecin (CPT)	Extract of bark of <i>P. amurense</i>	0, 25, 50, and 100 µg/ml for 24h	Inconclusive Lack of proper characterization of <i>P. amurense</i>	3 - This assay is not an OECD recognised assay for genotoxicity testing. - no characterisation of tested items - concurrent cytotoxicity was not investigated	Limited/Low	Inoue et al., 2021

4149 <sup>a</sup> Bacterial reverse mutation test is considered of high relevance for hazard identification (EFSA, 2023). EFSA. 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, 20(9), 8270E. <https://doi.org/10.2903/sp.efsa.2023.EN-8270>.

4150 <sup>b</sup> Comet assay in vitro and γH2AX assay with lesser relevance can be used as supporting information for hazard identification (EFSA, 2023). EFSA. 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, 20(9), 8270E. <https://doi.org/10.2903/sp.efsa.2023.EN-8270>.

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4153 **Table E4.** Evidence table on in vivo genotoxicity studies on berberine-containing plant preparations

Type of test	Tested system	Test substance (key info)	Concentration/ treatment time	Results	Reliability	Relevance of test (method)/Relevance of the study result	Reference
<b>Chromosomal aberration and micronucleus<sup>a</sup></b>							
Micronucleus assay	Peripheral blood erythrocytes of	<i>H. canadensis</i> root powder	0, 3,121, 6,250, 12,500, 25,000	Inconclusive	2	High/Low	NTP, 2010

- GLP compliance: NR	B6C3F1 (5 animals/sex/group) - Vehicle: phosphate-buffered saline (solvent) - Negative control: untreated animals - Positive control: not used	- > 2.0% (-)- $\beta$ -hydrastine, > 2.5% berberine - all relevant analytical tests performed, as well as the stability study	and 50,000 mg/kg of feed for three months	No increase in the frequency of micronucleated NCEs; no significant exposure-related changes in the percentages of PCEs, lack of evidence of exposure of bone marrow	- no positive control data - MN were scored in NCEs, not PCEs - duration of the exposure
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<sup>a</sup> Micronucleus test in vivo is considered of high relevance for hazard identification (EFSA, 2023). EFSA. 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. *EFSA Supporting Publications*, 20(9), 8270E. <https://doi.org/10.2903/sp.efsa.2023.EN-8270>.

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4157 Appendix F. Randomised controlled trials on reporting adverse events

4158 **Table D4.** Evidence table on randomised controlled trials on berberine reporting adverse events, ordered by intervention dose and duration

Reference Country	Design Participants characteristics N randomised participants	Intervention Duration	Adverse events reported
<b>Rondanelli M., et al., 2023</b> <b>Italy</b>	RCT Overweight men/women with impaired fasting blood glucose (6.1-7.0 mmol/L) INT: 24, PLA: 25	376 mg/d BBR (extracted from <i>B. Aristata</i> , in the form of BBR phospholipids) vs placebo 8 weeks	No intervention-related AEs identified, including blood biochemistry parameters (creatinine and liver function); data not shown.
<b>Rabbani G. H., et al., 1987</b> <b>Bangladesh</b>	RCT Adult men with ETEC ( <i>enterotoxigenic Escherichia coli</i> ) diarrhoea INT: 33, PLA: 30 RCT Adult men with cholera ( <i>Vibrio cholerae</i> ) diarrhoea INT: 30, PLA: 31	BBR sulphate 400mg/d (purity NR) vs placebo 1 day	No intervention-related serious AEs. There were a few cases of transient nausea and abdominal discomfort in the BBR group; data not shown.
<b>Chen C., et al., 2015</b> <b>China</b>	RCT Men/Women with diarrhoea-predominant irritable bowel syndrome INT: 70, PLA: 62	BBR CI 400 mg/d (purity NR) vs placebo 8 weeks	INT: 8 AEs: slightly upset stomach, recovered after about one or two weeks. PLA: No AE.
<b>Moon J.M., et al., 2022</b> <b>USA</b>	RCT crossover design (randomised order of interventions) Healthy men 5	BBR CI 500mg/d (purity ≥99%) vs placebo 6 days, with at least 72h wash out between each intervention	INT: 1 AEs in 1 individual: headache. PLA: 3 AEs in 2 individuals: abnormal heart rhythm (1), stomach cramping (2).
<b>Chan M., et al. 2022</b> <b>China</b>	RCT Men/Women with schizophrenia and metabolic syndrome INT: 58, PLA: 55	BBR CI 600 mg/d (purity NR) vs placebo 12 weeks	Risk Difference PLA vs INT Amenorrhea 0.03 (-0.06, 0.11)      Sweating: -0.04 (-0.16, 0.09) Salivation 0.05 (-0.09, 0.19)      Abdominal pain: -0.01 (-0.09, 0.07) Lim stiffness -0.05 (-0.14, 0.05)      Abdominal bloating: 0.06 (-0.07, 0.18) Forgetfulness 0.06 (-0.1, 0.22)      Constipation: -0.05 (-0.18, 0.07) Attention deficit -0.07 (-0.19, 0.05)      Diarrhoea: -0.02 (-0.14, 0.11) Weight gain -0.05 (-0.14, 0.05)      Vomiting: 0.06 (-0.01, 0.13) Insomnia 0.04 (-0.08, 0.15)      Nausea: -0.01 (-0.1, 0.09) Headache: 0.08 (-0.01, 0.18)      Dizziness: 0.07(-0.4, 0.18) Tachycardia: 0.02 (-0.1,0.14)
<b>Chen et al., 2020</b>	RCT Men/Women with colorectal adenoma	BBR CI 600 mg/d (purity ≥97%) vs placebo	INT: 7 AEs; constipation (6), hepatic dysfunction (1). PLA: 2 AEs; constipation (1), rash (1).

Reference Country	Design Participants characteristics N randomised participants	Intervention Duration	Adverse events reported
<b>China</b>	INT: 553, PLA 555	2 years	
<b>Pu Z., et al., 2021</b>	RCT Men/Women with colorectal adenoma INT: 65, PLA 66	BBR 300-900 mg/d (purity NR) vs placebo 12 weeks	INT: nausea (3), mild stomach pain (4), constipation (1), patient withdrawal due to AEs (3). PLA: patient withdrawal due to AEs (9) (AEs not specified).
<b>China</b>			
<b>Tehrani S. O., et al., 2022</b>	RCT Men/Women with COVID-19 INT: 23, PLA: 23	BBR 900 mg/d (purity NR) vs placebo 2 weeks	"Some people experienced nausea and diarrhoea after using BBR capsules" (data not shown)
<b>Iran</b>			
<b>Li G.H., et al., 2009</b>	RCT Men/Women with seminoma (postoperative) or lymphomas treated with abdominal radiation therapy INT: 18, PLA: 18 Patients with cervical cancer treated with whole pelvic radiation therapy INT: 21, PLA: 21	BBR 900 mg/d (purity NR) vs placebo 5 weeks	No intervention-related AEs identified; data not shown.
<b>China</b>			
<b>Li M., et al., 2021</b>	RCT Men/Women with schizophrenia INT: 35, PLA: 30	BBR CI 900 mg/d (purity NR) vs placebo 8 weeks	INT: 8 AEs; abdominal distention (3), constipation (2), diarrhoea (2), sinus bradycardia (1). No serious adverse events. PLA: 7 AEs (not specified); No serious adverse events.
<b>China</b>			
<b>Xu L., et al., 2020</b>	RCT Men/Women with ulcerative colitis INT: 14, PLA: 4	BBR CI 900 mg/d (>98% purity) vs placebo 3 months	INT: 7 AEs; upper respiratory infection (1), pancreatitis (1), increased transaminases (1), diarrhoea (1), nausea (1). PLA: No AEs.
<b>China</b>			
<b>Pu Z., et al., 2023</b>	RCT Men/Women with schizophrenia INT: 67, PLA: 67	BBR 900 mg/d (purity NR) vs placebo 3 months	INT: 19 AEs; nausea (5), mild stomach pain (7), constipation (2), discontinued intervention due to AEs (not specified) (5). PLA: NR.
<b>China</b>			
<b>Zhao M.M., et al., 2021</b>	RCT crossover design (randomised order of interventions) Healthy male volunteers 15	BBR 1000 mg/d (purity NR) vs placebo 2 days	ECG was monitored after the study and no differences between placebo and BBR groups were observed. No other side effects were observed on the BBR arm.
<b>China</b>			
<b>Rashidi H., et al., 2018</b>	RCT Men/Women with T2DM INT: 42, PLA: 42	BBR 1000 mg/d (purity NR) vs placebo 4 weeks	INT: 2 AEs; feeble (2). No serious AEs. PLA: No serious AEs.
<b>Iran</b>			
<b>Zhao J.V., et al., 2021</b>	RCT Men with hyperlipidaemia INT: 42, PLA: 42	BBR 1000 mg/d (purity NR) vs placebo 12 weeks	INT: 2 AEs; headache (1), diarrhoea (1). No serious AEs. PLA: 3 AEs; headache (1), nausea (1), vomiting (1). No serious AEs.
<b>China</b>			

Reference Country	Design Participants characteristics N randomised participants	Intervention Duration	Adverse events reported
<b>Zhang Y.F., et al., 2008</b>  <b>China</b>	RCT (multicentre) Men/Women with T2DM and dyslipidaemia INT: 59, PLA: 57	BBR 1000 mg/d ( $\geq 97\%$ purity) vs placebo 3 months	INT: 5 AEs; constipation (5). No serious AEs. PLA: 1 AE; constipation (1). No serious AEs.
<b>Kong W., et al., 2004</b>  <b>China</b>	RCT Men/Women with hyperlipidaemia INT: 63, PLA: 28	BBR CI 1000 mg/d (purity NR) vs placebo 3 months	INT: 1 AE; constipation (1). No serious AEs. PLA: NR.
<b>Ming J., et al., 2021</b>  <b>China</b>	RCT Men/Women with hyperglycaemia INT: 49, PLA: 99	BBR 1000 mg/d (purity NR) vs placebo 16 weeks	INT: hypoglycaemia (14), faecal abnormalities (5), abdominal discomfort/digestive tract disease (9), dental and oral disorders (0), others (1), upper respiratory tract infection (0), changes in body weight (0), dizziness (0), trauma/arthropathy (0), abnormal ECG/cardiac dysfunction (3), blood routine/biochemistry/urinalysis (1), haemorrhoids (0), discontinued due to AEs (1). PLA: hypoglycaemia (19), faecal abnormalities (12), abdominal discomfort/digestive tract disease (8), dental and oral disorders (1), others (0), upper respiratory tract infection (1), changes in body weight (1), dizziness (1), trauma/arthropathy (0), abnormal ECG/cardiac dysfunction (2), blood routine/biochemistry/urinalysis (10), haemorrhoids (1), discontinued due to AEs (2).
<b>Derosa G., et al., 2013</b>  <b>Italy</b>	RCT Men/Women with hypercholesterolaemia INT: 71, PLA: 70	BBR 1000 mg/d (purity NR) vs placebo 2 years	INT: transient headache (for 1 day in the run-in period) and transient flatulence (2, for 2 days). No serious AEs. No musculoskeletal system disorders or hepatotoxicity. No changes in transaminases (AST and ALT), g-GT and CPK. PLA: No serious AEs. No musculoskeletal system disorders or hepatotoxicity. No changes in transaminases (AST and ALT), g-GT and CPK.
<b>Liu Y., et al., 2008</b>  <b>China</b>	RCT Men/Women with lung cancer treated with radiotherapy INT: 42, PLA: 43	20 mg/kg bw per day BBR (purity NR) vs placebo + radiotherapy in both groups 6 weeks	AEs (including radiation-induced AEs) INT: radiation esophagitis (25), leukopenia (17), nausea/vomiting (21), diarrhoea (11) PLA: radiation esophagitis (28), leukopenia (22), nausea/vomiting (25), diarrhoea (10)
<b>Zhang J., et al., 2022</b>  <b>China</b>	RCT Men/Women undergoing isolated coronary artery bypass grafting INT: 100, PLA: 100	BBR 1200 mg/d (purity NR) vs placebo + coronary artery bypass grafting in both groups (d7) 14 days	INT: 6 AEs; mild rash (2), mild constipation (4). PLA: No AEs.
<b>Zhang Y., et al., 2020</b>	RCT (multicentre) Men/Women with T2DM INT: 98, PLA: 103	BBR 1200 mg/d (purity NR) vs placebo + gentamicin sulphate 80	INT: 37 AEs; GI (14). No serious AEs. PLA: 45 AEs; GI (4). Serious AEs: 2.

Reference Country	Design Participants characteristics N randomised participants	Intervention Duration	Adverse events reported
<b>China</b>		mg twice daily for 7 days in both groups (run-in) 12 weeks	
<b>An Y. et al., 2014</b> <b>China</b>	RCT Women with infertility, PCOS, undergoing IVF treatment INT: 50, PLA: 50	BBR Cl 1500 mg/d (purity NR) vs placebo 12 weeks	INT: Total GI effects (11), nausea (9), vomiting (4), diarrhoea (6), abdominal pain (7). No severe AEs. PLA: Total GI effects (6), nausea (4), vomiting (1), diarrhoea (1), abdominal pain (2). No severe AEs.
<b>Panigrahi A. et al., 2023</b> <b>India</b>	RCT Men/Women with prediabetes INT: 17, PLA: 17	BBR Cl 1500 mg/d ( $\geq 97\%$ purity, extracted from <i>B. aristata</i> root) vs placebo 12 weeks	INT: 3 AEs; mild nausea or vomiting (3). No serious AEs. PLA: NR.
<b>Perez-Rubio K.G. et al., 2013</b> <b>Mexico</b>	RCT Men/Women newly diagnosed with metabolic syndrome, w/o diabetes and w/o being in treatment INT: 12, PLA: 12	BBR Cl 1500 mg/d (purity NR) vs placebo 12 weeks	INT: No serious AEs. PLA: NR.
<b>Yan H.M. et al., 2015</b> <b>China</b>	RCT Men/Women with NAFLD patients with impaired glucose regulation or T2DM INT: 62, PLA: 62	BBR 1500 mg/d (purity NR) + lifestyle intervention vs lifestyle intervention 16 weeks	INT: Discomfort (1), fatigue (1), itching (1), dyspepsia (13), nausea (4; 9.52%), diarrhoea (11), abdominal pain (1), hunger (2), constipation (6), leukopenia (1), proteinuria (1), discontinued due to AEs (1). No events reported for headache, pain, chest tightness, palpitation, menstruation, hypoglycaemia. PLA: No AEs.
<b>Ruiz-Herrera V.V., et al. 2023</b> <b>Mexico</b>	RCT Men/Women with MetS and HIV infection INT: 22, PLA: 21	BBR 1500 mg/d (purity NR) vs placebo 20 weeks	INT: 2 AEs; diarrhoea (1), abdominal pain (1). Incidence of any AE: 10.5%. No serious AEs. PLA: AEs: 1; diarrhoea (1). Incidence of any AE: 5.8%. No serious AEs.
<b>Zeng X.H., et al., 2003</b> <b>China</b>	RCT Men/Women with CHF treated with ACE inhibitors INT: 79, PLA: 77	BBR Cl 1200 to 2000 mg/d (purity NR) vs placebo 8 weeks	INT: increase in gastrointestinal side effects, consisting of diarrhoea, constipation, vomiting, and abdominal pain (data not shown). PLA: NR.

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Abbreviations: ACE, angiotensin-converting enzyme; AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BBR, berberine; BBR Cl, BBR chloride; CHF, congestive heart failure; CPK, creatine phosphokinase; DHBBR, dihydroBBR; g-GT, gamma-glutamyl transferase; GI, gastrointestinal; INT, intervention; IVF, in vitro fertilisation; MetS, metabolic syndrome; NR, not reported; PCOS, polycystic ovary syndrome; PLA, placebo; RCT, randomised controlled trial; T2DM, type 2 diabetes mellitus.

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4163 Appendix G. Human case reports and case-series on preparations claimed to contain  
 4164 berberine

4165 **Table G1.** Evidence table on case reports on berberine food supplements (n=3)

Reference Country	Sex, age	Form Daily dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors
<b>As the only ingredient in the food supplement</b>									
<b>Cannillo et al., 2013</b>	M, 53	"Berberine" unspecified	NR	Unremarkable (hyperlipidaemia and overweight)	Berberine overdose, hepatic or biliary disease excluded	Fatigue and dyspnoea upon exertion	Cardiac arrhythmia and loss of AV synchronization during stress (ergometric stress test)	Yes (10 days); NA	NA
<b>Italy</b>		NR 6 days		Alcoholism: NR				a latent hypervagotonic state was diagnosed based on ECG-Holter monitoring	
<b>Florek et al., 2018</b> <b>Labadie et al., 2018</b>	M, 54	Berberine HCl extracted from <i>B. aristata</i> (unknown purity)	Aliskiren	Hypertension, prediabetes	No contact with known allergens	Sharply demarcated erythematous scaly patches of the bilateral flanks, groin, bilateral upper inner thighs, buttocks, and axillae. Skin biopsy revealed perivascular oedema and infiltrate with lymphocytes and eosinophils.	Symmetric drug-related intertriginous and flexural exanthema	Yes (2 weeks)	NA
<b>USA</b>		1200 mg/day 2 months							
<b>As part of a multi-ingredient food supplement</b>									
<b>Déléaval et al., 2022</b>	F, 56	Herbal supplements with 250 mg of berberine (other ingredients NR)	Hemp Oil supplements containing cannabidiol and cannabigerol (up to 6 times)	Unremarkable Alcoholism: NR	Normal kidney function, serum magnesium at the lower bound, normal potassium levels	Dizziness, syncope without prodromes in a supine position	Acquired long QT syndrome with <i>torsades de pointes</i>	Yes (normalisation of QT interval after 5 days; full recovery at 3 months FU)	NA
<b>Switzerland</b>									

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NR recommended  
dose).

6 weeks

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Abbreviations: AV, atrioventricular; F, female; FU, follow-up; M, male; NA, not available; NR, not reported.

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**Table G2.** Evidence table on the case reports in which *C. majus* (greater celandine) was the only ingredient in the food supplement (n=21)

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Greving et al., 1998</b>	F, 28	Panchelidon ®	St. John's wort preparation (discontinued 2 months before); chlormadinon e acetate and mestranol (for several yrs)	Recurrent bronchitis, multiple allergies, dysmenorrhoea.  <u>Alcoholism</u> : no.	<u>HV</u> : neg <u>HAI</u> (ANA, SMA, AMA): neg <u>Ig</u> : normal range <u>WD, HC</u> : excluded	<u>Clinical features</u> : Jaundice, itching, fatigue. <u>Biochemistry</u> : Marked increases in AST, ALT, Bil. <u>Imaging</u> : hepatosplenomegaly and two biliary concrements <u>Biopsy</u> : extensive necrosis of parenchymal tissue with partial fibrosis	Drug-induced hepatitis	Yes (1 month)	CIOMS scale: +4, possible
<b>BfArM, 2005 (case 97901299)</b>		60 drops/d for 2 weeks (4 mg alkaloids) 2 caps/d for 2 months (8 mg alkaloids) 6 caps/d for 2 months (24 mg alkaloids)  4.5 mo							
<b>Teschke et al., 2011 (case 04)</b>									
<b>Germany</b>									
<b>Greving et al., 1998</b>	F, 36	Panchelidon ®	L-thyroxine (for 5 yrs), roxithromycin for 10 d (1 month before, for paranasal sinusitis).	Hypothyroidism, allergies, fully recovered HVB.  <u>Alcoholism</u> : NR	<u>HV</u> : neg, except HVB <u>HAI</u> (ANA, SMA, AMA): neg <u>Ig</u> : normal range <u>WD, HC</u> : excluded	<u>Clinical features</u> : Jaundice, abdominal pain, diarrhoea, pale faeces and dark urine. <u>Biochemistry</u> : Marked increases in AST, ALT, Bil. <u>Imaging</u> : hepatomegaly and accessory liver lobe on the right side. <u>Biopsy</u> : liver cell necrosis and activation of Kupfer's cells; signs of cholestasis; no fibrosis.	Drug-induced hepatitis	Yes (NR)	CIOMS scale: -1, excluded
<b>Teschke et al., 2012 (case 03)</b>		2 caps/d (8 mg alkaloids)  4 months							
<b>Germany</b>									
<b>Im et al., 2014</b>	F, 62	Greater celandine solution (not	None	Unremarkable.  <u>Alcoholism</u> : no.	No history of hepatic disease or metabolic disease.	<u>Clinical features</u> : Anorexia, dyspepsia,	Hepatocellular drug-induced hepatitis	Yes (1 month)	CIOMS/RUC AM: 9,

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>South Korea</b>		further described)  400 mL  2 months			<u>HV</u> : neg <u>HAI</u> (ANA, SMA, AMA, ANCA, LKM): neg <u>WD, HC</u> : excluded	nausea, abdominal discomfort, jaundice. <u>Biochemistry</u> : Marked increases in AST, ALT, ALP, GTP, Bil. <u>Imaging</u> : mildly fatty liver. <u>Biopsy</u> : moderate lobular hepatitis with hepatocyte ballooning degeneration and numerous acidophil bodies; mild inflammation of portal area (lymphocytes and eosinophils).			highly probable
<b>Moro et al., 2009</b>	M, 65	Decoction of greater celandine dried leaves	Lansoprazole; clarithromycin and amoxicillin (2 months before)	Mild chronic gastritis, hiatus hernia, esophagitis, minor antral erythematous gastropathy associated to HP infection.  Alcohol: NR	No history of disease associated with liver impairment  <u>HV</u> : neg except anti-HVC (RNA -)	<u>Clinical features</u> : Asthenia, dyspepsia, jaundice, dark urine. <u>Biochemistry</u> : Marked increases in AST, ALT, ALP, GGT, Bil. <u>Imaging</u> : Moderate hepatomegaly. <u>Biopsy</u> : -	Drug-induced hepatitis	Yes (2 months)	CIOMS scale: +7, probable
<b>Teschke et al., 2012 (case 20)</b>		NR  1 month							
<b>Italy</b>									
<b>BfArM, 2005 (case 02001171)</b>	F, 66	Panchelidon®  Up to 2 caps/d (8 mg alkaloids)	NR	Alcohol: NR	<u>HV</u> : neg (no details)  <u>HAI</u> : -	<u>Clinical features</u> : jaundice <u>Biochemistry</u> : Marked increases in AST, ALT, Bil. <u>Imaging</u> : no evidence of a mechanical cause of cholestasis, no	CD-induced cholestatic hepatitis	Yes (NR)	CIOMS scale: +8, probable
<b>Teschke et al., 2011 (case 20)</b>		4.5 months							

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Germany</b>						indication of liver cirrhosis <u>Biopsy</u> : -			
<b>BfArM, 2005 (case 00000278)</b>	M, 65	Panchelidon ®  3 caps/d (12 mg alkaloids)	Diclofenac (intermittent), sitosterols, butizide, raubasine, rescinamine, and reserpine	Alcohol: NR	<u>HV</u> : neg <u>HAI</u> : neg (no details)	<u>Clinical features</u> : jaundice <u>Biochemistry</u> : Marked increases in AST, ALT, Bil; ALP normal <u>Imaging</u> : no evidence of a mechanical cause of cholestasis <u>Biopsy</u> : Hepatitis with cholestasis	CD-induced cholestatic hepatitis	Yes (NR)	CIOMS scale: +8, probable
<b>Teschke et al., 2011 (case 17)</b>		4 weeks							
<b>Germany</b>									
<b>BfArM, 2005 (case 98008527)</b>	F, 61	Panchelidon ®  3 caps/d (12 mg alkaloids)	Faros®(hawt horn)	Alcohol: NR	<u>HV</u> : neg, except HAV and HBV <u>HAI (ANA, AMA, SMA, and LKM)</u> : neg	<u>Clinical features</u> : jaundice, abdominal pain <u>Biochemistry</u> : Marked increases in AST, ALT, Bil. <u>Imaging</u> : no evidence of a mechanical cause of cholestasis <u>Biopsy</u> : lobular epithelial necroses and chronic portal inflammatory infiltration with focal portal fibrosis	Drug-induced cholestatic hepatitis	Yes (NR)	CIOMS scale: +8, probable
<b>Teschke et al., 2011 (case 14)</b>		Several weeks							
<b>Germany</b>									
<b>BfArM, 2005 (case 98007984)</b>	F, 51	Panchelidon ®  1–3 caps/day (4-12 mg alkaloids)	Hyperforat® (St John's wort), OC	Alcohol: NR	<u>HV</u> : neg <u>HAI</u> : ANA 1:40; AMA, SLA, LKM, and cANCA: neg	<u>Clinical features</u> : jaundice, fatigue, loss of appetite <u>Biochemistry</u> : Marked increases in AST, ALT, Bil.	Drug -induced cholestatic hepatitis	Yes (NR)	CIOMS scale: +3, possible
<b>Teschke et al., 2011 (case 13)</b>									

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
Germany		3.5 months				<u>Imaging</u> : no evidence of a mechanical obstruction of the bile ducts <u>Biopsy</u> : hepatitis of the cholestatic type			
<b>BfArM, 2005 (case 98006425)</b>	F, 52	Panchelidon N®  10–30 drops/day (0.7–2 mg alkaloids)	L-thyroxine; Phosetamin; several homeopathic/phytotherapies (Meditonsin H, Venobiase capsules mono, Anabol [Hevert], Hwerbeberol drops [Hevert], Bomaklim drops [Hevert])	Alcohol: NR	<u>HV</u> : neg  <u>HAI (ANA, AMA, LKM, and pANCA)</u> : neg	<u>Clinical features</u> : jaundice <u>Biochemistry</u> : slight increases in AST, ALT, and marked increase in Bil. <u>Imaging</u> : no evidence of a mechanical obstruction of the bile ducts <u>Biopsy</u> : cholestasis without inflammation or liver cell necroses	Cholestatic hepatitis	Yes, but persistent hyperbilirubinemia at discharge (NR)	CIOMS scale: +1, unlikely
<b>Teschke et al., 2011 (case 12)</b>									
Germany		5 months							
<b>BfArM, 2005 (case 98005000)</b>	M, 48	Chol 4000 Tropfen®  NR  Few days	Prosta Fink® (pumpkin seed extract)	Chronic cholecystitis  History of HAV and HBV  Alcohol: no	<u>HV</u> : neg, except HAV and HBV <u>HAI</u> : neg (no details)	<u>Clinical features</u> : jaundice <u>Biochemistry</u> : marked increases in AST, ALT, and Bil. <u>Imaging</u> : blocked cystic duct <u>Biopsy</u> : marked acute to subacute cholestatic lobular hepatitis	Possible drug-induced (cholestatic) hepatitis	Yes (NR)	CIOMS scale: -1, excluded
<b>Teschke et al., 2011 (case 10)</b>									
Germany									
<b>BfArM, 2005 (case 98001447)</b>	F, 49	Cholarist®  3 caps/d	None	Alcohol: no	<u>HV</u> : neg	<u>Clinical features</u> : jaundice, epigastric discomfort	Drug-induced hepatitis	Yes (NR)	CIOMS scale: +8, probable

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Teschke et al., 2011 (case 6)</b>  <b>Germany</b>		(300-450 mg of dry celandine herb extract)  4 weeks			<u>HAI (ANA, AMA, and SMA):</u> neg	<u>Biochemistry:</u> marked increases in AST, ALT, and Bili. <u>Imaging:</u> cholecystolithiasis without evidence of existing mechanical obstruction of bile flow <u>Biopsy:</u> portal hepatitis with hepatic fibrosis			
<b>BfArM, 2005 (case 98000501)</b>  <b>Teschke et al., 2011 (case 5)</b>  <b>Germany</b>	M, 65	Panchelidon N®  2-3 caps/d (8-12 mg alkaloids)  6 weeks	None	Cholecystectomy  Alcohol: NR		<u>Clinical features:</u> jaundice <u>Biochemistry:</u> marked increases in AST, ALT, ALP, GGT and Bili. <u>Imaging:</u> ruled out extrahepatic cause of cholestasis <u>Biopsy:</u> -	Drug-induced (cholestatic) hepatitis	Yes (NR)	CIOMS scale: +10, highly probable
<b>BfArM, 2005 (case 96026841)</b>  <b>Teschke et al., 2011 (case 3)</b>  <b>Germany</b>	F, 55	Panchelidon N®  3 caps/d (12 mg alkaloids)  6 weeks	Diltiazem (for years); Doxycycline x~10 days	Hypertension, tick bite with erythema, migrans.  Alcohol: NR	<u>HV:</u> neg <u>HAI:</u> neg (no details)	<u>Clinical features:</u> jaundice <u>Biochemistry:</u> slight increases in AST, ALT, and marked increase in Bil. <u>Imaging:</u> no indication of a mechanical cause <u>Biopsy:</u> toxic liver injury	Drug-induced (cholestatic) hepatitis	Yes (NR)	CIOMS scale: +9, highly probable
<b>BfArM, 2005 (case 95003849)</b>	F, 31	Panchelidon N®  2 caps/day	Estrogen-containing OC	Prior jaundice with cholangitis few weeks before; Alcohol: NR	<u>HV:</u> neg <u>HAI:</u> neg (no details)	<u>Clinical features:</u> nausea, occasional vomiting, jaundice	drug-induced acute hepatitis	Yes (NR)	CIOMS scale: +3, possible

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Teschke et al., 2011 (case 2)</b>  <b>Germany</b>		(8 mg alkaloids)  3 weeks				<u>Biochemistry</u> : marked increases in AST, ALT, and Bil. <u>Imaging</u> : normal biliary system <u>Biopsy</u> : acute hepatitis consistent with a drug-induced toxic aetiology			
<b>BfArM, 2005 (case 95003848)</b>  <b>Teschke et al., 2011 (case 1)</b>  <b>Germany</b>	M, 31	Panchelidon N®  2 caps/d (8 mg alkaloids)  NR	NR	Alcohol: NR	<u>HV</u> : neg (no details) <u>HAI</u> : neg (no details)	<u>Clinical features</u> : loss of appetite, upper abdominal discomfort, jaundice <u>Biochemistry</u> : marked increases in AST, ALT, and Bil. <u>Imaging</u> : no indication of a mechanical obstruction of bile flow <u>Biopsy</u> : pattern consistent with drug-induced hepatitis	drug-induced hepatitis	Yes ( <i>new episode after 2 months upon unintentional rechallenge with the same CM preparation</i> )	CIOMS scale: +8, probable
<b>BfArM, 2005 (case 02007637)</b>  <b>Teschke et al., 2011 (case 22)</b>  <b>Germany</b>	F, 43	Ardeycholan N®  3 dragées/d (900 mg of dry celandine extract)  18 days	None	Allergies (apples, carrots)  Alcohol: NR	NR	<u>Clinical features</u> : Jaundice <u>Biochemistry</u> : marked increases in AST, ALT, GGT, and Bil. <u>Imaging</u> : NR <u>Biopsy</u> : NR	Drug-induced liver injury with jaundice (icteric hepatitis)	Yes (NR)	CIOMS scale: +3, possible
<b>BfArM, 2005 (case 01000644)</b>	NR, NR	Panchelidon N®  4–6 caps/d	NR	Cholecystectomy  Alcohol: NR	Gallstones ruled out given previous cholecystectomy	<u>Clinical features</u> : Jaundice <u>Biochemistry</u> : marked increases GGT, GOT, GPT, Bil	Drug-induced hepatitis	Yes (NR)	CIOMS scale: +3, possible

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Teschke et al., 2011 (case 19)</b>		(16-24 mg alkaloids)				<u>Imaging</u> : NR <u>Biopsy</u> : hepatitis with early fibrosis			
<b>Germany</b>		2 months (prior long-term use; 1 year break)							
<b>BfArM, 2005 (case 99000607)</b>	M, 62	Chol 4000® 1 tab/day	None	Alcohol: NR	Report states that "other causes of hepatitis were excluded clinically, biochemically, and by liver biopsy."	<u>Clinical features</u> : NR <u>Biochemistry</u> : marked increases AST, ALT, Bil <u>Imaging</u> : NR <u>Biopsy</u> : NR	Drug-induced hepatitis	Yes (NR)	CIOMS scale: -1, excluded
<b>Teschke et al., 2011 (case 16)</b>		(200 mg of dry celandine extract, 4.4 mg alkaloids)							
<b>Germany</b>		3 weeks							
<b>BfArM, 2005 (case 99000061)</b>	F, 74	Chelidophyt® 4 mg/day	Jodthyrox®; Pankreon forte® and a homeopathic treatment (NR)	Hx pancreatitis; s/p thyroid resection with hypoparathyroidism	NR	<u>Clinical features</u> : <u>Biochemistry</u> : marked increases AST, ALT, ALP, Bil <u>Imaging</u> : NR <u>Biopsy</u> : NR	Drug-induced hepatitis	Yes (NR)	CIOMS scale: +5, possible
<b>Teschke et al., 2011 (case 15)</b>		4 weeks							
<b>Germany</b>									
<b>BfArM, 2005 (case 98003489)</b>	F, 49	Panchelidon® (drops) 90 drops/d (6 mg alkaloids)	Triquilar® (EE/levonorgestrel); Normabrain® (piracetam)	Known cholelithiasis. Alcohol: NR	<u>HV</u> : neg (no details) a post-hepatic cause of jaundice was excluded (no details)	<u>Clinical features</u> : jaundice <u>Biochemistry</u> : marked increases AST, ALT, ALP, Bil <u>Imaging</u> : NR <u>Biopsy</u> : NR	Drug-induced hepatitis	Yes (NR)	CIOMS scale: +4, possible
<b>Teschke et al., 2011 (case 09)</b>									

Reference country	Sex, age	Preparation Daily dose Duration	Co- medications	Medical history, Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Germany</b>		6 weeks							
<b>BfArM, 2005 (case 98001607)</b>	F, 60	Chol 4000 Lichtenstein ®	Inhaled: Berodual® (ipratropium/ fenoterol), Salbutamol, Inhacort® (flunisolide)	Asthma Alcohol: NR	VH: neg (no details)	<u>Clinical features</u> : fatigue jaundice <u>Biochemistry</u> : marked increases AST, ALT, ALP, bil <u>Imaging</u> : excluded mechanical obstruction of bile duct <u>Biopsy</u> : yes, but results not reported	drug-induced hepatitis	Yes (NR)	CIOMS scale: +8, probable
<b>Teschke et al., 2011 (case 08)</b>		NR 7 weeks							
<b>Germany</b>									

4169 Abbreviations: AMA, Antimitochondrial antibodies; ANA; antinuclear antibodies, ANCA, Anti-neutrophil cytoplasmic antibodies; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CM,  
4170 Chelidonium Majus; GGT,  $\gamma$ -glutamyl transferase; GTP:  $\gamma$ -glutamyl transpeptidase; ALP, Alkaline Phosphatase; Bil, Total bilirubin; CIOMS, Council for International Organizations  
4171 of Medical Sciences; CT, Computed Tomography; HAI, autoimmune hepatitis; HC, hemochromatosis; HP, Helicobacter pylori; HV viral hepatitis; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV,  
4172 hepatitis C virus; LKM, liver-kidney-microsomal antibodies; ERCP, endoscopic retrograde cholangiopancreatography; NA, not available; NR, not reported; OC, oral contraceptive; pANCA, Perinuclear  
4173 Anti-Neutrophil Cytoplasmic Antibodies; RUCAM, Roussel Uclaf Causality Assessment Method; SMA, smooth muscle antibodies; WD, Wilson's disease.

4174 <sup>(a)</sup> Where reported, the CIOMS score and resulting causality grading is extracted from the reviews by Teschke et al, 2011, 2012, except for the case reported by Im et al 2015 where it is extracted  
4175 from the original paper. CIOMS causality grading,  $\leq 0$ : Excluded; 1-2: Unlikely; 3-5: Possible; 6-8: Probable;  $\geq 9$ : Highly probable. Teschke, R., Schwarzenboeck, A., & Hennermann, K. H. (2008).  
4176 Causality assessment in hepatotoxicity by drugs and dietary supplements. *British Journal of Clinical Pharmacology*, 66(6), 758-766. <https://doi.org/10.1111/j.1365-2125.2008.03264.x>.  
4177

4178 **Table G3.** Evidence table on case reports in which *Chelidonium majus* (greater celandine) was part of multi-ingredient preparations (n=24)

Reference Country	Sex, age	Preparation Dose Duration	Co-medication	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Saez-Gonzales et al., 2016 Spain</b>	M, 37	Iberogast® Preparation containing 9 plant extracts including greater celandine  NR  NR	None	No history of liver disease.  Alcohol: no	Viral serology (HAV, HBV, HCV, HEV, cytomegalovirus and Epstein-Barr) neg.; autoimmunity profile: neg; Copper metabolism, iron metabolism, alpha 1-antitrypsin, and thyroid function: neg.	<u>Clinical features:</u> painless jaundice, asthenia, and anorexia; hepatic encephalopathy; acute liver injury requiring liver transplantation. <u>Biochemistry:</u> marked increase in ALT, AST, GGT, Bil. <u>Imaging:</u> no liver abnormality detected, no gallstone or obstruction of the bile duct, normal liver blood flow <u>Biopsy:</u> mixed hepatocellular-cholestatic pattern of liver injury; coagulative necrosis, with hepatocellular necroinflammation and signs of cholestasis	Drug-induced liver injury	NR	CIOMS scale: +7, probable
<b>Gerhardt et al., 2019 Germany</b>	F, 56	Iberogast® Preparation containing 9 plant extracts including greater celandine  NR  NR	Metamizole for several months (chronic pain syndrome), valsartan (hypertension)	NR	NR	<u>First exposure:</u> <u>Clinical features:</u> jaundice <u>Biochemistry:</u> marked increase in ALT, AST, GGT, Bil.  <u>Re-exposure 2 months after</u> <u>Clinical features:</u> jaundice, , hepatic encephalopathy, acute	Drug-induced liver injury	<u>First exposure:</u> yes (NR)  <u>Re-exposure:</u> The patient died following the liver transplantation	CIOMS scale: +9, highly probable

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Hardeman et al., 2008</b>	F, 58	Curcumar <sup>®</sup>	Vitamin supplements (not specified)	History of blood transfusions when delivering; gastric banding for obesity 4 yr before.	<u>HV</u> : neg <u>HAI</u> (ANA, SMA, AMA, LKM): neg <u>IgG</u> : within normal range <u>WD, HC, alpha-1-antitrypsin deficiency</u> : excluded	liver injury requiring liver transplantation. <u>Biochemistry</u> : marked increase in ALT, AST, GGT, Bil.	Drug-induced hepatitis, ascribed to CM	Yes (14 d)	CIOMS scale: +5, possible
<b>Teschke et al., 2012(case 18)</b> <b>Belgium</b>		50 mg of greater celandine 50 mg of gentian 100 mg of curcuma root  NR  6 weeks		History of alcoholism: no.		<u>Clinical features</u> : Jaundice, dark urine and pale stools. <u>Biochemistry</u> : Marked elevations ALP, AST, ALT, Bil <u>Imaging</u> : mass near the porta hepatis, causing bile duct obstruction. <u>Biopsy</u> : necrosis of liver cells, inflammatory infiltration of neutrophils and lymphocytes, presence of gal pigment in the residual hepatocytes Biopsy of the mass: benign reactive adenopathy			
<b>Rifai et al. (2006)</b>	M, 58	Aristochol CC <sup>®</sup> , an oral preparation containing greater celandine	None	NR	<u>HAI</u> : neg Infectious, metabolic, or genetic causes of acute hepatitis were excluded.	<u>Clinical features</u> : jaundice, pruritus, pale stools, dark urine, fatigue <u>Biochemistry</u> : Marked elevations ALT, Bil <u>Imaging</u> : hepatomegaly <u>Biopsy</u> : lobular hepatitis with severe cholestasis	Drug-induced cholestatic hepatitis	1 month	CIOMS scale: +10, probable
<b>Teschke et al., 2012 (case 17)</b> <b>Germany</b>		NR  3 weeks							

Reference Country	Sex, age	Preparation Dose Duration	Co-medication	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>Stickel et al., 2003</b>	F, 39	Preparation containing Greater Celandine (Gallemolan ©, Cesra Arzneimittel, Baden - Baden, Germany)	Sulfamethoxazole 10 days before (Painful cystitis)	Painful cystitis, dental surgery to treat a jaw abscess.	Hepatitis serology negative; normal Cu and Fe	<u>Clinical features:</u> Jaundice, fatigue, dark urine <u>Biochemistry:</u> Marked increases in AST, ALT, GGT, bilirubin <u>Imaging:</u> normal <u>Biopsy:</u> -	Drug-induced cholestatic hepatitis	7 wks	CIOMS scale: +4, possible
<b>Teschke et al., 2012 (case 15)</b>		NR 1mo	Penicillin (dental surgery)	History of alcoholism: No	ANA 1:80; liver-kidney-microsomal antibodies (LKM) and antimitochondrial antibodies (AMA) negative			Cholestatic hepatitis recurred after involuntary re-exposition	
<b>Stickel et al., 2003</b>	M, 69	Drug containing Greater Celandine e (Cholagogum © F Nattermann, Cologne, Germany)	Aspirin (occasional)	Uncomplicated cholecystectomy, hip implant.	Hepatitis serology negative	<u>Clinical features:</u> Jaundice, weakness, abdominal pain in the right upper quadrant and nausea, dark urine. <u>Biochemistry:</u> Marked increases in AST, ALT, ALP, GGT, bilirubin <u>Imaging:</u> normal <u>Biopsy:</u> Marked portal and periportal mononuclear infiltration with some eosinophils; Intralobular changes with inflammatory infiltrates, hepatocyte degeneration, acidophilic bodies and cholestasis	Drug-induced cholestatic hepatitis	Yes (NR)	CIOMS scale: +7, probable
<b>Teschke et al., 2012 (case 16)</b>		80 capsules of this preparation for 5 weeks; each capsule contains 4 mg chelidonin		History of alcoholism: no					
<b>BfArM, 2005 (case 98005833)</b>	F, 77	Aristochol (granules) 2 sachets/d	Ginkgo; Flupirtine	History of cholelithiasis	<u>HV:</u> neg (no details) <u>HAI:</u> neg (no details)	<u>Clinical features:</u> jaundice, itching	Drug induced hepatitis.	NR (NR)	CIOMS scale: +5, possible

Reference Country	Sex, age	Preparation Dose Duration	Co-medication	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
Teschke et al., 2011 (case 11) Germany		2 mo		Alcohol: NR	hepatotropic viruses neg, except for an elevated complement-binding reaction (CBR) titer for adenoviruses. no indication of primary biliary cirrhosis	Biochemistry: marked increases in AST, ALT, and Bili. Imaging: no evidence of a mechanical obstruction of the bile ducts Biopsy: drug-induced toxic liver cell damage			
BfArM, 2005 (case 01004774) Teschke et al., 2011 (case 18) Germany	F, 67	Neurochol® 1 cap/day 3.5 weeks	Magnesium Verla® tablets [magnesium L-hydrogen aspartate 5 mmol/tablet], Verla-Lipon 200® [200 mg α-lipoic acid]	Intestinal bleeding due to sigmoid diverticulitis and two laparoscopy procedures of the abdominal cavity Alcohol: NR	HV: no data ANA titer 1:320 Thromboplastin time < 40 %	Clinical features: Hepatitis followed by death due to toxic liver failure Biochemistry: marked increases in AST, ALT, and Bil Imaging: - Biopsy: -	Drug-induced autoimmune hepatitis leading to acute liver failure and death	-	CIOMS scale: 0, excluded
BfArM, 2005 (case 02001832) Teschke et al., 2011 (case 21) Germany	F, 35	Cholagogum F Nattermann® and Spasma gallosanol® "Rather high" doses (NR) 1 month	None	Cholecystectomy (3 months before start of CM treatment); past malaria (resolved 2 years before) Alcohol: "elevated consumption in the past" (no qty)	NR	Clinical features: jaundice Biochemistry: marked increases GGT, ALP, AST, LDH, Bil Imaging: - Biopsy: -	Drug-induced liver injury, assessed as probably caused by celandine	Yes (NR)	CIOMS scale: +4, possible

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
<b>BfArM, 2005 (case 98001603)</b>	F, NR	Neurochol C®	NR	NR	Exclusion of post-hepatic cholestasis (no details)	<u>Clinical features:</u> <u>Biochemistry:</u> marked increases in AST, ALT <u>Imaging:</u> no evidence of mechanical cholestasis <u>Biopsy:</u> -	Hepatitis with a cholestatic component		CIOMS scale: +3, possible
<b>Teschke et al., 2011 (case 07) Germany</b>		1–2 dragées/day 5 weeks		Alcohol: NR					
<b>Valente et al., 2010 Italy</b>	F, NR	Mixture of herbal substances, among which Greater Celandine, <i>Lycopodium serratum</i> and arsenic NR NR	NR	Cholelithiasis (gallbladder stones) noted on imaging Alcohol: NR	Any aetiology of liver dysfunction was excluded; lack of improvement after ERCP with sphincterotomy and subsequent laparoscopic cholecystectomy argued against persistent obstruction due to gallbladder stones	<u>Clinical features:</u> asthenia, nausea, jaundice <u>Biochemistry:</u> elevated liver enzymes (unspecified), no biochemical improvement after ERCP and cholecystectomy <u>Imaging:</u> Ultrasound and MRCP showed gallbladder stones <u>Biopsy:</u> Liver histology compatible with hepatotoxic injury	Herbal-induced liver injury with mixed cholestatic/hepatocellular pattern	Yes (normal liver tests after 2 months on ursodeoxycholic acid)	NA <sup>b</sup>
<b>Crijns et al., 2002</b>	F, 42	"Steigal" herbal preparation with <i>Chelidonium majus</i> (herba) and <i>Curcuma longa</i> (rhizoma) NR	NR	'Skin complaints' Alcohol: No	Viral serology (HAV, HBV, HCV, CMV, EBV) neg.; autoimmune antibodies neg.; IgG/IgA/IgM not elevated; Wilson disease excluded (normal ceruloplasmin, serum/urine copper; no Kayser-Fleischer rings). Paracetamol level 2.6 mg/L excluded	<u>Clinical features:</u> fever, upper-abdominal pain, headache, eye tenderness, myalgia, fatigue, jaundice, dark urine and pale stools. <u>Biochemistry:</u> elevated GGT, ALP, AST, ALT, LDH, TBil <u>Imaging:</u> normal liver and bile ducts. <u>Biopsy:</u> severe acute hepatitis with portal and lobular inflammation	Acute hepatitis attributed to <i>Chelidonium majus</i> (based on temporal relationship, dechallenge, and exclusion of alternatives)	Yes (clinical recovery after withdrawal near-normal at ~3 weeks; complete normalization by ~2 months)	CIOMS scale: +8, Probable
<b>Teschke et al., 2012 (case 14)</b>									
<b>The Netherlands</b>		Duration: 2 months							

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
					intoxication. No ascites.	(lymphocytes, some eosinophils), bile duct injury, zone-3 hepatocyte ballooning, cholestasis.			
<b>Tarantino et al., 2009</b>	F, 22	Herbal preparation, containing Lycopodium serratum and Greater Celandine	Occasional use of paracetamol to treat dysmenorrhoea	Obesity (BMI 32)	Viral serology (hepatitis, CMV, EBV and other viral and bacterial infections) neg.; neg. autoimmune parameters (ANA, AMA, SMA, ANCA, LKM); serum copper and caeruloplasmin, α-fetoprotein, α-1 antitrypsin and iron deposits were in the normal range.	<u>Clinical features:</u> jaundice, pruritus, right upper quadrant pain, epigastric tenderness, fever of low grade, nausea, vomiting, dark urine and pale stools. <u>Biochemistry:</u> elevated ALP, AST, ALP <u>Imaging:</u> ultrasound showed microcalculi in the gallbladder; MRC showed dilatation of the choledocus with some evidence of microstones in the gallbladder <u>Biopsy:</u> NR	Drug-induced liver injury (initial diagnosis was acute cholecystitis)	Yes (1 month after cessation)	CIOMS scale: 0, excluded
<b>Teschke et al., 2012 (case 21)</b>		NR		History of alcoholism: no					
<b>Italy</b>		NR							
<b>Whiting et al., 2002</b>	F, 23	Herbal remedy containing Greater Celandine, buchus leaf, <i>Uva ursi</i> , juniper, parsley piert, choline bitartrate and dandelion	NR	No history of liver or biliary tract disease.	Viral serology (HAV, HBV, HCV) neg.; SMA and AMA neg.; normal alpha-1-antitrypsin; iron and copper profiles consistent with acute-phase response; normal upper-abdominal ultrasound/CT	<u>Clinical features:</u> jaundice, pruritus, fatigue <u>Biochemistry:</u> elevated ALT, AST, GGT, ALP, TBil <u>Imaging:</u> Ultrasound showed normal liver <u>Biopsy:</u> severe lobular inflammation and Zone-3 (centrilobular) damage, with focal bridging necrosis and	Acute hepatitis	Yes (normalization of labs at 25 weeks; treated with prednisone)	NA
<b>Australia</b>				History of alcoholism: no					

Reference Country	Sex, age	Preparation Dose Duration	Co-medication	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
		NR				eosinophils, but no bile duct injury and no fibrosis.			
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 8)</b> <b>Germany</b>	F, 37	Neurochol C® 12 weeks	NR	Atopic eczema NR	Viral serology (HAV, HBV, HEV, cytomegalovirus and Epstein-Barr) neg.; autoimmune hepatitis excluded	<u>Clinical features:</u> jaundice, nausea, <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP, TBil <u>Imaging:</u> normal liver parenchyma and bile ducts <u>Biopsy:</u> refused by the patient	Cholestatic hepatitis	Yes (after 5 months)	CIOMS scale: +11, highly probable
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 4)</b> <b>Germany</b>	F, 67	GC extract NR 9 months	None	Constipation NR	Positive ANA	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP <u>Imaging:</u> normal liver parenchyma and bile ducts <u>Biopsy:</u> moderate drug induced hepatitis, periportal and intralobular single cell necrosis, septal fibrosis	Drug-induced liver injury	Yes (after 6 months)	CIOMS scale: +5, possible
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 5)</b> <b>Germany</b>	F, 46	GC extract NR 4 months	Iodine, oestradiol, levonorgestrel	Gallbladder stones NR	NR	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, TBil <u>Imaging:</u> NR <u>Biopsy:</u> NR	Drug-induced liver injury	Yes (after 2 months)	CIOMS scale: +4, possible
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 6)</b>	F, 54	GC extract NR Duration: 1 month	Iodine	Gallbladder stones NR	Positive SMA at 1:40	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP, TBil <u>Imaging:</u> NR	Drug-induced liver injury	Yes (after 5 months)	CIOMS scale: +5, possible

Reference Country	Sex, age	Preparation Dose Duration	Co-medication	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
Germany						Biopsy: Severe drug-induced hepatitis, bridging necrosis, severe septal fibrosis and incipient cirrhosis			
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 7)</b> Germany	F, 46	GC extract NR Duration: 6 months	Valerian, pancreatic enzymes and loperamide	Right-sided abdominal pain  Alcohol: moderate intake	Positive ANA	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP, TBil <u>Imaging:</u> NR <u>Biopsy:</u> Moderate drug-induced hepatitis, periportal and intralobular single cell necrosis, septal fibrosis	Drug-induced liver injury	Yes (after 2 months)	CIOMS scale: +4, possible
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 9)</b> Germany	F, 65	GC extract NR Duration: 3 months	None	Dyspepsia  NR	Positive ANA	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP <u>Imaging:</u> NR <u>Biopsy:</u> Moderate drug induced hepatitis, low grade single cell necrosis	Drug-induced liver injury	Yes (after 3 months)	CIOMS scale: +6, probable
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 10)</b> Germany	F, 40	GC extract NR Duration: 3 months	Thyroxine, silymarin, acetylsalicylic acid, zinc, hycrocromone, amitriptyline	Gallbladder stones  NR	Positive ANA	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP, TBil <u>Imaging:</u> NR <u>Biopsy:</u> NR	Drug-induced liver injury	Yes (after 3 months)	CIOMS scale: 0, excluded
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 11)</b>	F, 66	GC extract NR Duration: 4 months	Thyroxine, atenolol, nifedipine, triamterene	Dyspepsia  NR	Positive ANA; Positive HCV	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP <u>Imaging:</u> NR <u>Biopsy:</u> Moderate drug-induced	Drug-induced liver injury	Yes (after 2 months)	CIOMS scale: +3, possible

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors <sup>a</sup>
Germany			hydrochloro-thiazide			hepatitis, periportal and intralobular single cell necrosis, septal fibrosis			
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 12)</b> <b>Germany</b>	F, 40	GC extract NR Duration: 2 months	Magnesium and oestradiol	Dyspepsia NR	Positive ANA	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP, TBil <u>Imaging:</u> NR <u>Biopsy:</u> Severe drug-induced hepatitis, severe periportal and intralobular liver cell necrosis, periportal and septal fibrosis	Drug-induced liver injury	Yes (after 2 months)	CIOMS scale: +4, possible
<b>Benninger et al., 1999</b> <b>Teschke et al., 2012 (case 13)</b> <b>Germany</b>	F, 41	GC extract NR Duration: 3 months	Oestradiol	Dyspepsia NR	Positive ANA	<u>Clinical features:</u> NR <u>Biochemistry:</u> elevated AST, ALT, GGT, ALP, TBil <u>Imaging:</u> NR <u>Biopsy:</u> Severe drug-induced hepatitis, low-grade single cell necrosis	Drug-induced liver injury	Yes (after 3 months)	CIOMS scale: +4, possible

4179 Abbreviations : ALP, Alkaline Phosphatase; ALT, alanine aminotransferase; AMA, Antimitochondrial antibodies; ANA, antinuclear antibodies; ANCA, Anti-neutrophil cytoplasmic antibodies; AST, aspartate  
4180 aminotransferase; TBil, Total bilirubin; CIOMS, Council for International Organizations of Medical Sciences; CT, Computed Tomography; GGT, Gamma-glutamyl transferase; GC, greater celandine; ERCP,  
4181 endoscopic retrograde cholangiopancreatography; HAI, autoimmune hepatitis; HC, hemochromatosis; HP, *Helicobacter pylori*; HV, viral hepatitis; HCV, hepatitis C virus; LKM, liver-kidney-microsomal  
4182 antibodies; MRC, magnetic resonance cholangio-pancreatography; NA, not available; RUCAM, Roussel Uclaf Causality Assessment Method; SMA, smooth muscle antibodies; WD, Wilson's disease.

4183 <sup>a</sup> Where reported, the CIOMS score and resulting causality grading is extracted from the reviews by Teschke et al, 2011, 2012, except for the cases reported by Sáez-González et al. (2016) and Gerhardt  
4184 et al. (2019) where it is extracted from the original papers. CIOMS causality grading, ≤ 0: Excluded; 1-2: Unlikely; 3-5: Possible; 6-8: Probable; ≥ 9: Highly probable. Teschke, R., Schwarzenboeck, A.,  
4185 & Hennermann, K. H. (2008). Causality assessment in hepatotoxicity by drugs and dietary supplements. *British Journal of Clinical Pharmacology*, 66(6), 758-766. <https://doi.org/10.1111/j.1365-2125.2008.03264.x>.

4186 <sup>b</sup> The authors reported a score of sixteen on "the clinical diagnostic scale for hepatotoxic adverse drug reaction", corresponding to a 'probable' diagnosis of herbal hepatotoxicity. However, the reference  
4187 of the scale was not reported.  
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4189 **Table G4.** Evidence table on case reports on food supplements containing *Hydrastis Canadensis* (goldenseal) (n=3)

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors
<b>As the only ingredient in the food supplement (n=2)</b>									
<b>Bhowmick et al. (2007)</b>	F, 11	Goldenseal 1000 – 1500 mg/day 2 weeks	None	None	Normal thyroid function (free T4, TSH) and random cortisol	Lethargy, polyuria, polydipsia, and a weight loss of approximately 20 lbs over a period of 3 weeks. <u>Clinical features:</u> Vomiting and depressed mental status, dehydration (>10%), with poor skin turgor, capillary refill >4 seconds, sunken eyes. <u>Biochemistry:</u> markedly elevated serum glucose, sodium, potassium, low CO <sub>2</sub> , elevated BUN, markedly elevated anion gap; type 1 diabetes immune markers positive (elevated GAD antibody, positive islet cell antibody). <u>Imaging:</u> NR <u>Biopsy:</u> NR	Onset type I diabetes mellitus with diabetic ketoacidosis. Severe hypernatremia attributed to Goldenseal supplement	Yes (hypernatremia resolved after 3 days)  Type I diabetes mellitus treated with insulin. New episode of diabetic ketoacidosis without hypernatremia 11 months later.	NA
<b>USA</b>									
<b>Patel et al.(2015)</b>	F, 60	Goldenseal (root powder) NR 4 months	NR	Paranoid schizophrenia and diabetes mellitus.  History of alcoholism: no	No history of liver disease; Viral hepatitis serology, antinuclear antibody, anti-mitochondrial antibody, anti-smooth muscle antibody, liver/kidney microsome antibodies, ceruloplasmin level, and iron	<u>Clinical features:</u> jaundice, delusional behaviour. <u>Biochemistry:</u> markedly elevated TBil and DBil, elevated AST and ALT, markedly elevated ALP, low albumin. <u>Imaging:</u> CT and liver ultrasound showed hepatomegaly without signs of cholecystitis or extra hepatic obstruction. <u>Biopsy:</u> A liver biopsy showed cholestasis with canalicular and intraductular bile plugs.	Drug-induced cholestatic hepatitis.	Yes (NR)	NA
<b>USA</b>									

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors
					panel were normal.				
<b>As part of a multi-ingredient food supplement (n=1)</b>									
<b>Weissman et al. (2020)</b>	F, 53	Immune factors© supplement with Echinacea (purpurea and angustifolia), Goldenseal and extracts of Shiitake, Maitake, and Reishi mushrooms.	None	One episode of pancreatitis a decade earlier, after taking a single dose of Immune factors© supplement.  History of alcoholism: no	IgG4 and ANA negative	<u>Clinical features</u> : diffuse abdominal pain radiating to the back <u>Biochemistry</u> : serum lipase >2500U/L <u>Imaging</u> : CT scan showed pancreatic inflammation and peri-pancreatic oedema; MRI/MRCP showed no gallstones, no choledocholithiasis, no pancreatic divisum	Drug-induced acute pancreatitis	Yes (3 days)	Naranjo scale score <sup>a</sup> : 9 (definite adverse drug reaction)
<b>USA</b>		'Several doses'  2 days (prior to presentation)							

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CT, computed tomography; DBil, direct bilirubin; F, female; GAD, glutamic acid decarboxylase; M, male; NA, not available; NR, not reported; TBil, total bilirubin.

<sup>a</sup> The Naranjo assessment scale is a tool used to determine the likelihood of an adverse drug reaction based on a cumulative score on 10 questions. A score of <1 is doubtful, 1 - 4 possible, 5 - 8 probable, >9 definitive. Naranjo et al. (1981). A method for estimating the probability of adverse drug reactions. *Clinical Pharmacology & Therapeutics*, 30: 239-245. <https://doi.org/10.1038/clpt.1981.154>

4196 **Table G5.** Evidence table on case reports in which *Tinospora Sinensis* was the only ingredient in the food supplement (n=2)

Reference Country	Sex, age	Preparation Dose Duration	Co-medications	Medical history Alcoholism	Exclusion of other underlying causes	Signs and symptoms	Diagnosis by the authors	Recovery (time)	Causality scale attributed by the authors
<b>Wu et al., (2013)</b> <b>Taiwan</b>	2 males (case 1, case 2) 54, 64	<i>Tinospora sinensis</i> (no further information)  NR  case 1: 18 days case 2: 3 months	cases 1 and 2: NR	cases 1 and 2: NR	cases 1 and 2: NR	cases 1 and 2: Jaundice, elevation of liver enzymes and bilirubin, leucocytosis, monocytosis, coagulopathy, duodenal ulcer with bleeding case 1: eosinophilia	cases 1 and 2: Acute Hepatitis	cases 1 and 2: Yes (NR)	NA

4197 Abbreviations: M, male; NA, not available; NR, not reported.

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4201 **Annexes**

4202 **List of Annexes**

4203 Annex A – Characterisation of the protoberberines and other alkaloids present in the plant species  
4204 (and relevant plant parts thereof) included in the mandate

4205 Annex B – Report on *H. canadensis* benchmark dose modelling (BMD)

4206 Annex C – Results of quantitative structure-activity relationship analysis of protoberberines and other  
4207 alkaloids regarding genotoxicity endpoints

4208 Annex D – Analysis of occurrence data received through the call for data

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2AA	2-aminoanthracene
4-NQO	4-Nitroquinoline-1-oxide
8-MOP	8-Methoxypsoralen
8-OHdG	8-hydroxy-2'-deoxyguanosine
ACE	Angiotensin-Converting Enzyme
ACHN	Human carcinoma cell line
ADME	Absorption, distribution, metabolism and excretion
ADP-ribose	Adenosine diphosphate ribose
AE	Adverse event
AF-2	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
AFB1	Aflatoxin B1
Alb	Albumin
ALI	Acute liver injury
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMA	Antimitochondrial antibodies
ANA	Antinuclear antibodies
ANCA	Anti-neutrophil cytoplasmic antibodies
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
AOP	Adverse outcome pathway
AST	Aspartate aminotransferase
AUC	Area under the concentration–time curve
AV	Atrioventricular
B6C3F1	Mice strain
BBR	Berberine
BCRP	Breast cancer resistance protein
BfArM	German Federal Institute for Drugs and Medical Devices
Bil	Bilirubin
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limit
BMDL10	BMDL for 10% response
BMI	Body mass index
BT549	Human breast cancer cell line
BUN	Blood urea nitrogen
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (German Federal Office of Consumer Protection and Food Safety)
bw	Body weight
C57BL	Mice strain
CAESAR	QSAR model for Ames mutagenicity
cANCA	cytoplasmic Antineutrophil Cytoplasmic Antibodies
CAS	Chemical Abstracts Service
CBR	complement-binding reaction
CCK-8	Cell Counting Kit-8
CD-1	Charles River mice strain
CHF	Congestive heart failure
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
Cmax	Maximum concentration
CM	Chelidonium majus

CMC-Na	Sodium carboxymethyl cellulose
CMV	Cytomegalovirus
CPK	Creatine phosphokinase
CPT	Camptothecin
CrCl	Creatinine clearance
CsA	Ciclosporine A
CT	Computed tomography
CVD	Cardiovascular disease
CYP	Cytochrome P450
DBil	Direct bilirubin
DDY	Deutschland, Denken and Yoken
DEN	Diethylnitrosamine
DER	Drug extract ratio
DHBBR	Dihydroberberine
DMEM	Dulbecco's Modified Eagle Medium
DMN	Dimethylnitrosamine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSB	Double-strand break
DU145	Human prostate carcinoma cell line
DW	Dry weight
EAC	Ehrlich ascites carcinoma
EBV	Epstein-Barr virus
EC	European Commission
ECG	Electrocardiogram
ECG-Holter	Holter Electrocardiogram (ECG) Monitoring
EHPM	European Federation of Associations of Health Product Manufacturers
EMA	European Medicines Agency
EME1	Endonuclease partner of MUS81
ERCP	Endoscopic retrograde cholangiopancreatography
ETEC	Enterotoxigenic Escherichia coli
EU	European Union
F	Females
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FTE-187	Human fallopian tube epithelial cell line
GAD	Glutamic acid decarboxylase
GC	Greater celandine
GD	Gestational day
GGT, g-GT	Gamma-glutamyl transferase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GNDP	Global New Products Database
GNPD	Global New Products Database
GOT	Aspartate aminotransferase
GTP	Gamma-glutamyl transpeptidase
H2A	Histone H2A
H2AX	Histone H2AX
H2O2	Hydrogen peroxide
HaCaT	Human keratinocyte cell line
HAI	Autoimmune hepatitis
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HC	Hemochromatosis

HCl	Hydrochloride
HCT	Hematocrit
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HeLa	Human cervical cancer epithelial cell line
Hep3B	Human hepatocellular carcinoma cell line
HepG2	Human hepatocellular carcinoma cell line
HEV	Hepatitis E virus
HILI	Herb-induced liver injury
HIV	Human immunodeficiency virus
HK1	Human nasopharyngeal carcinoma cell line
HL-60	Human promyelocytic leukemia cell line
HMPC	Committee on Herbal Medicinal Products (EMA)
HO8910	Human ovarian cancer cell line
HP	Helicobacter pylori
HPRT	Hypoxanthine-guanine phosphoribosyl transferase
HR	Homologous recombination
i.p.	Intraperitoneal
i.v.	Intravenous
IARC	International Agency for Research on Cancer
IC50	Half maximal inhibitory concentration
ICR	Mice strain
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgG4	Immunoglobulin G4
IgM	Immunoglobulin M
ImageJ	Image analysis software
INR	International normalised ratio
INT	Intervention
IPGTT	Intraperitoneal glucose tolerance test
ISS	Istituto Superiore di Sanità
iTV	Indicative toxicity value
IVF	In vitro fertilization
KM	Kunming mice (strain)
kNN	k-nearest neighbors
L5178Y	Mouse lymphoma cell line
LB	Lower bound
LC/MS	Liquid chromatography coupled with mass spectrometry
LD50	Median lethal dose
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LKM	Liver–kidney microsomal antibodies
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
M	Males
MATE1	Multidrug and toxin extrusion protein 1 (transporter)
MCC	mitomycin C
MDA-MB-231	Human breast cancer cell line
MetS	Metabolic syndrome
MG-63	Human osteosarcoma cell line
mKM	Modified Karber method
MN	Micronuclei
MoA	Mode/mechanism of action
MRC	Magnetic resonance cholangio-pancreatography

MRC5sv	Human fibroblast cell line
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
MTCC	Microbial Type Culture Collection
MTT	Cell metabolic activity/viability colorimetric assay
MUS81	Structure-specific endonuclease (with EME1)
N	Number
NA	Not applicable
NAFLD	Non-alcoholic fatty liver disease
NCEs	Normochromatic erythrocytes
ND	Not determined / not detected (context-dependent)
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NHEJ	Non-homologous end joining
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NOQ	4-Nitroquinoline-1-oxide
NPC	Nasopharyngeal carcinoma (cell line)
NQ	Detected but not quantified
NR	Not reported
NTP	National Toxicology Program
OAT	Organic anion transporter
OECD	Organisation for Economic Co-operation and Development
OGTT	Oral glucose tolerance test
OHAT	Office of Health Assessment and Translation
P1B1	Transport protein family member
pANCA	Perinuclear Anti-Neutrophil Cytoplasmic Antibodies
PARP	Poly(ADP-ribose) polymerase
PBPK	Physiologically based pharmacokinetic
PBS	Phosphate-buffered saline
PCEs	Polychromatic erythrocytes
PCOS	Polycystic ovary syndrome
PFGE	Pulsed-field gel electrophoresis
P-gp	P-glycoprotein
pKM101	<i>Escherichia coli</i> strain
PLA	Placebo
POWO	Plants of the World Online
PPT	Partial thromboplastin time
PQ37	<i>Escherichia coli</i> strain
PT	Prothrombin time
PTT	Partial thromboplastin time
PubMed	Bibliographic database
QSAR	Quantitative structure–activity relationship
QT	QT interval (ECG)
RAD51	Homologous recombination repair marker
RBC	Red blood cell
RCT	Randomised controlled trial
REF	Reference (placeholder in document)
RM-1	Murine prostate cancer cell line
RNA	Ribonucleic acid
RoB	Risk of bias
ROS	Reactive oxygen species
RUCAM	Roussel Uclaf Causality Assessment Method
SARpy	QSAR/modeling tool for structural alerts
SAs	Structural alerts
SC	Scientific Committee

SD	Sprague-Dawley (rat strain)
SDRIFE	Symmetrical drug-related intertriginous and flexural exanthema
SISTE	Società italiana di scienze applicate alle piante officinali e ai prodotti per la salute
SMA	Smooth muscle antibodies
sQ	Sub-question
SR	Systematic review
SSBs	Single-strand breaks
SULT	Sulfotransferase
T2DM	Type 2 diabetes mellitus
T4	Thyroxine
TA100	Salmonella typhimurium TA100 strain
TA102	Salmonella typhimurium TA102 strain
TA1535	Salmonella typhimurium TA1535 strain
TA1537	Salmonella typhimurium TA1537 strain
TA97	Salmonella typhimurium TA97 strain
TA97a	Salmonella typhimurium TA97a strain
TA98	Salmonella typhimurium TA98 strain
TBil	Total bilirubin
TG	Triglycerides
TG408	OECD guideline (Repeated dose 90-day oral toxicity study in rodents)
TGR	Transgenic rodent mutation assay
TK6	Human lymphoblastoid cell line
TOPO	Topoisomerase
ToxRead	Software for structural alerts/read-across
TP	Total protein
TSH	Thyroid-Stimulating Hormone
U2OS	Human osteosarcoma cell line
UaDP	Up-and-down procedure
UB	Upper bound
UDP	Uridine diphosphate
UGT	UDP-glucuronosyltransferase
UniMi	Università degli Studi di Milano
US, USA	United States of America
UVA	Ultraviolet A
VEGA	In silico QSAR platform (VEGA hub)
VH	Viral hepatitis
VLDL	Very low-density lipoprotein
WB	Western blot
WG	Working Group
WoE	Weight of evidence
WP2	<i>Escherichia coli</i> strain
XRCC1	DNA repair protein
XS2316	<i>S. cerevisiae</i> diploid strain
XV-185-14c	<i>S. cerevisiae</i> haploid strain
Z-VAD-FMK	Nuclease inhibitor