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5 **Reflection paper on microbiological aspects of herbal**
6 **medicinal products and traditional herbal medicinal**
7 **products**
8 **Draft**

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10 Comments should be provided using this [template](#). The completed comments form should be sent to hmpc.secretariat@ema.europa.eu

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35 **1. Introduction**

36 Directive 2001/83/EC as amended and Directive 2001/82/EC as amended provide definitions for herbal
37 substances, herbal preparations, and herbal medicinal products (HMPs)¹. The basic legislation applies
38 to both HMPs for human and veterinary use². An additional simplified registration procedure has been
39 established for traditional herbal medicinal products (THMPs) for human use under Directive
40 2004/24/EC. The principles of this reflection paper apply equally to such THMPs.

41 According to these definitions a herbal medicinal product is any medicinal product, exclusively
42 containing as active ingredients one or more herbal substances or one or more herbal preparations, or
43 one or more such herbal substances in combination with one or more such herbal preparations.

44 THMPs may also contain vitamins and minerals, provided that the action of the vitamins and minerals
45 is ancillary to that of the active herbal ingredient(s).

46 HMPs have a number of characteristics that differentiate them from medicinal products containing
47 chemically defined active substances. Specific guidelines have therefore been established for HMPs
48 which cover particular aspects that general guidelines do not. Herbal substances and herbal
49 preparations are complex mixtures of natural constituents and, potentially, also contaminants, with a
50 natural variability. Being of natural origin herbal substances generally have a higher microbial content
51 compared to chemical drug substances. In this reflection paper consideration is given as to how the
52 microbial contamination of herbal substances, herbal preparations, and HMPs can be limited by
53 preventative measures and by applying decontamination processes. The need for testing and
54 regulatory documentation is also discussed.

55 Sterile dosage forms and methods of sterilisation are not covered by this paper.

1 The term "herbal substance" should be considered as equivalent to the term "herbal drug" as defined in the European Pharmacopoeia, and the term "herbal preparation" should be considered as equivalent to the term "herbal drug preparation" as defined in the European Pharmacopoeia.

2 Directive 2001/83/EC as amended and Directive 2001/82/EC as amended.

56 2. Discussion

57 The active ingredients of HMPs are herbal substances and/or herbal preparations derived from herbal
58 substances. Being of natural origin, the active ingredients in HMPs tend to have higher microbial
59 contamination (bioburden) than chemically defined active substances and the microbial population
60 present may differ qualitatively and quantitatively. Therefore, particular attention should be paid to the
61 microbiological quality of HMPs and specific guidance should be provided. The Ph. Eur. recognises the
62 need to allow wider acceptance criteria for the microbial quality HMPs depending on the nature of the
63 product and method of preparation e.g. herbal teas.

64 Herbal substances/preparations may be contaminated with numerous species of bacteria and fungi
65 (yeasts and moulds). Viruses are not usually considered to be a concern with herbal
66 substances/preparations. The content of live bacteria, fungi and their spores should be determined and
67 limited in herbal substances/preparations and HMPs.

68 Pathogenic micro-organisms

69 Some bacterial species are pathogenic (e.g. *Salmonella* spp., *Shigella* spp., some pathovars of
70 *Escherichia coli*; *Listeria monocytogenes* and some clostridial species) and therefore pose a risk of
71 inducing infectious diseases or other unwanted effects in patients taking the HMP. Such micro-
72 organisms should not be present in the HMP.

73 Spores

74 Endospores are bacterial spores formed by certain Gram-positive bacteria e.g. *Bacillus* and *Clostridium*
75 species. Spores are formed when bacteria are exposed to unfavourable environmental conditions
76 (heat, drought, irradiation or depletion of nutrients). Generally, a higher number of spores are found in
77 dry herbal substances compared to fresh herbal substances, especially when inappropriate drying
78 procedures are used. Bacterial spores are highly resistant to various environments (desiccation,
79 freezing, dry heating, vapour, elevated pressure, UV radiation and various chemicals including
80 extraction solvents such as ethanol). Bacterial spores have the potential to be reactivated into the
81 vegetative state as bacteria when favourable environmental conditions are present again. Nutrients
82 and elevated temperatures are used during the incubation phase of testing of total aerobic microbial
83 count (TAMC; Ph. Eur. 2.6.12, 2.6.13, 2.6.31) of a product and thus spores of certain bacterial species
84 (mostly aerobic from *Bacillus* spp.) are detected together with the bacteria by these quantitative *in*
85 *vitro* methods.

86 Fungi, and particularly moulds, also produce spores (conidia). However, they are generally not as
87 resistant as bacterial spores to unfavourable environmental conditions.

88 Physicochemical characteristics

89 From a quality point of view, some micro-organisms can alter the physicochemical characteristics of
90 the product which may lead to detrimental changes to the product's quality. Constituents of the plant
91 material may be metabolised by the micro-organism, leading to chemically changed substances. It is
92 undesirable to have chemical degradation of constituents of the plant material, (especially constituents
93 with known therapeutic activity), active markers and chemical preservatives (added to e.g. a liquid
94 aqueous extract or liquid dosage form). Any potential reduction or change in the therapeutic activity of
95 the product must be evaluated.

96 Micro-organisms may also lead to sensory changes (appearance, smell, or taste) and to changes in pH
97 of the HMP, due to metabolic substances formed by the micro-organism. If the pH changes significantly
98 in a HMP containing a chemically ionisable preservative and the efficacy of that preservative is pH
99 dependent (e.g. benzoic acid and sorbic acid), then the efficacy of the preservative may be diminished.
100 Such risks should be considered.

101 **Mycotoxins**

102 During mycelial growth on substrates, some moulds produce mycotoxins. These substances are
103 secondary metabolites with lipophilic (e.g. aflatoxins and ochratoxin A) or hydrophilic (e.g. fumonisins)
104 properties. Mycotoxins can be formed during plant growth (cultivation or wild growth) or during
105 storage of the herbal substance/preparation or HMP.

106 The most important mycotoxins are highly toxic and carcinogenic aflatoxins. Aflatoxin B1 is considered
107 to be the most toxic mycotoxin.

108 In principle, aflatoxins are only formed by specific fungal species, which favour certain plants, plant
109 parts and growing conditions. For example, formation of aflatoxins may be initiated only after exposure
110 of the plant to unfavourable environmental conditions (e.g. drought or flooding). The geographical
111 origin may have a marked impact on the extent of aflatoxin formation. Aflatoxin forming moulds prefer
112 elevated temperatures and humid conditions, so herbal substances originating from plants grown in
113 (sub)tropical climates may show significantly higher levels of aflatoxins than those grown in cooler,
114 drier climates. Formation of aflatoxins is also dependent on the pH of the material.

115 The main producer organisms for aflatoxins are *Aspergillus flavus* and *Aspergillus parasiticus*. Generally
116 all plant parts are at risk of contamination by aflatoxins. However seeds, fruits, roots, and rhizomes
117 present a greater risk as they contain the best combination of nutrients for growth of the fungi.
118 Furthermore, as *A. flavus* and *A. parasiticus* are soil borne this presents an added risk for roots and
119 rhizomes. The presence of water is essential for both growth of micro-organisms and formation of
120 aflatoxins; therefore the content of water is a critical parameter and testing of loss on drying or water
121 content is crucial for dried herbal substances, preparations and HMPs.

122 Some plant materials (e.g. liquorice root) may be contaminated by ochratoxin A. This toxin is produced
123 by *Aspergillus ochraceus*, *Penicillium verrucosum* and some other species of *Aspergillus* and
124 *Penicillium*. Ochratoxin A is nephrotoxic and carcinogenic.

125 Aflatoxins and ochratoxin A are heat stable and soluble in hydro-alcoholic solvents. There is therefore a
126 potential risk of carry-over of aflatoxins and ochratoxin A from the herbal substance to the herbal
127 preparation or HMP which could lead to the presence of higher concentrations of aflatoxins in the
128 herbal preparation or HMP. This risk should be fully evaluated by validation of the extraction process of
129 a herbal preparation.

130 **2.1. Minimizing microbial contamination by prevention**

131 Microbial contamination originates from primary and secondary contamination. Primary contamination
132 is the naturally occurring microbial flora of the plant to be harvested. Secondary contamination is
133 caused by handling of the plant material (human intervention, equipment, buildings, air ventilation
134 systems, and contamination during transportation). Minimising contamination with micro-organisms
135 and microbial toxins should be ensured ideally by monitoring and limiting both primary and secondary
136 contamination, i.e. by prevention rather than by use of decontamination methods.

137 The herbal substance should be manufactured in compliance with good agricultural and collection
138 practice (GACP) and, from the starting material onwards, the herbal preparation should be

139 manufactured in compliance with good manufacturing practice (GMP). Some herbal substances/herbal
140 preparations (e.g. certain essential oils) exhibit a certain degree of inherent antimicrobial activity. This
141 should not be used to justify a lack of compliance with GACP and GMP.

142 **2.1.1. Herbal substances**

143 For cultivated plants, the growing conditions should be chosen in order to avoid unnecessary microbial
144 contamination. If manure is used as a fertiliser, the manure should be carefully composted before use.
145 In view of the fact that many micro-organisms are host specific human faeces must not be used as
146 fertiliser and direct use of sewage must also be avoided.

147 Where justified, fungicides can be used during cultivation of the plant in order to reduce fungal growth.
148 For both cultivated and wild plants, the time of harvest should be chosen so that the presence of
149 external water on the plants is limited, i.e. by avoiding harvesting during or immediately after rainfall
150 or heavy morning/evening dew. Growing the plants in green houses provides some opportunity to
151 control airborne and animal contamination which may help to reduce microbial contamination.

152 After harvest, unless frozen, herbal substances intended for fresh use, should be processed
153 immediately. If the herbal substance is to be dried before use, the drying process (method and time)
154 should be described. Drying should be as fast and uniform as possible, as this step is the most critical
155 for the growth of moulds and bacteria and formation of mycotoxins. Insufficient drying which leads to
156 increased levels of microbial contamination should not be resolved primarily by applying
157 decontamination methods to the product.

158 Any use of cleaning (dusting off or washing), cutting, freezing and storage of the herbal substance may
159 have a positive or negative impact on the final level of microbial contamination. If the herbal substance
160 is cleaned by washing with water, the quality of the water should be considered as a possible risk for
161 microbial contamination.

162 The packaging material and storage conditions for the herbal substance should be chosen in order to
163 prevent microbial growth and secondary contamination. Storage at low temperatures may lead to
164 formation of condensed water, which may pose a contamination risk.

165 **2.1.2. Herbal preparations**

166 The principles of fast, efficient and homogenous processing during manufacture for the herbal
167 substance should also be applied to herbal preparations. Relevant steps and in-process controls include
168 extraction temperatures and times, in particular for aqueous extractions, vacuum evaporation of
169 extracts, distillation of essential oils and holding times. The manufacturing method should be validated
170 and appropriate IPCs should be set. Expressed juices and herbal extracts prepared with water or with
171 low concentrations of alcohol are at particular risk of microbial contamination. The addition of
172 preservatives to extracts and expressed juices may be considered as an option. The choice and
173 concentration of the preservative should be fully justified, in accordance with current guidelines, which
174 should include evidence of preservative efficacy.

175 In addition to microbial contamination arising from the herbal substance itself, microbial contamination
176 arising from water, organic extraction solvents, excipients for standardisation or technological purposes
177 should also be considered.

178 The packaging material and storage conditions for the herbal preparation should be chosen in order to
179 prevent microbial growth and secondary contamination.

180 **2.1.3. Herbal medicinal products**

181 The principles for addressing microbial contamination in herbal substances and herbal preparations
182 also apply to manufacture, transportation and storage of the HMP.

183 Microbial contamination of excipients used to produce the chosen dosage form should be controlled and
184 monitored.

185 The limits for microbiological purity of the finished product will depend on the dosage form and
186 administration route, cf. section 2.3.

187 **2.2. Methods for reduction of microbial contamination**

188 As described in the sections above, microbiological quality of HMPs is the result of the quality of the
189 materials used and the manufacturing process. According to GMP criteria, good quality cannot be
190 controlled at the end of the process but should be built-in and should include the quality of the starting
191 material.

192 Minimisation of microbial content of herbal materials during cultivation, harvesting, storage and
193 processing is essential because the possibility of reducing the microbial bioburden in herbal materials
194 by means of post-processing treatments is very limited. This is due to the fact that herbal materials
195 are prone to deterioration by many of the treatments available; but, in addition, the potential for
196 harmful residues to remain needs to be addressed fully.

197 This issue is highlighted in the Ph. Eur. monograph "Herbal drugs", which under the section on
198 production states: "if a decontamination treatment has been used, it is necessary to demonstrate that
199 the constituents of the plant are not affected and that no harmful residues remain."

200 Despite its effectiveness in bioburden reduction (including endospores) the use of ethylene oxide for
201 the decontamination of herbal substances has been prohibited in the European Union since 31
202 December 1989 by Directive 89/365/EEC due to the formation of toxic by-products, such as ethylene
203 chlorohydrin and ethylene glycol.

204 **2.2.1. Justification for applying a decontamination process**

205 Complete elimination of micro-organisms from a given herbal substance, preparation or HMP, by
206 sterilisation methods, is not necessary, provided that pathogenic micro-organisms are excluded,
207 microbial contamination is limited to an acceptable level and any microbial growth can be controlled
208 during storage until the end of shelf-life.

209 Information on microbiological quality of a product should be provided to justify the need for the
210 decontamination treatment and to establish a procedure to reduce microbial contamination. A risk
211 assessment should be performed based on the microbial population and the initial level of
212 contamination taking account of the recommended acceptance criteria for non-sterile pharmaceutical
213 products: total aerobic microbial count (TAMC) and total combined yeasts/moulds count (TYMC), as
214 defined in the Ph. Eur.

215 The use of a decontamination process should be selected and fully justified on the basis of the type
216 and composition of the herbal material, its intended use and route of administration. Important
217 considerations are the initial microbial bioburden and the desired maximum final microbial
218 contamination level and should take account of the subsequent steps in the manufacturing process and
219 factors likely to influence microbial growth such as the water activity and the proposed shelf-life and
220 storage conditions.

221 A decontamination treatment should not be used simply as a precautionary measure and
222 decontamination treatments should not be used where the herbal substances/preparations/HMPs have
223 microbial contents unfit for human or animal consumption. The presence of pathogenic bacteria must
224 be avoided or these bacteria must be completely killed or removed. Micro-organisms capable of
225 producing toxins, such as *Clostridium botulinum* or fungi, are harmless provided conditions prevent
226 their growth; however once the toxins are produced they are very difficult to eliminate. Therefore the
227 possible presence of microbial metabolites needs to be carefully considered since the majority of
228 microbial decontamination methods lead to reduction of viable microorganisms (TAMC and TYMC) but
229 do not reduce the levels of mycotoxins or endotoxins. Furthermore, only some decontamination
230 methods reduce the number of spores.

231 The quality of a decontaminated herbal substance/preparation/HMP can be greatly influenced by
232 storage and shipping conditions due to the growth of bacteria surviving the process and chemical
233 reactions such as oxidation and biochemical modifications of the chemical constituents of the herbal
234 material.

235 If a decontamination method is used, it should be demonstrated that the chemical profile of the
236 product has not been affected by the process. If any change in the chemical profile occurs this should
237 be addressed and fully justified. The impact on safety and efficacy aspects of the herbal
238 substance/preparation/HMP should be considered and degradation products should be qualified
239 toxicologically, as appropriate.

240 **2.2.2. Choice of decontamination method**

241 A number of different methods are available which may be used to reduce microbial contamination of
242 the herbal substance, the herbal preparation or during manufacture of the finished product. Where
243 used, they should be performed as early as possible in order to maintain microbial quality at an
244 appropriate level throughout the entire manufacturing process and to minimise further microbial
245 growth during and after manufacture of the product.

246 Any treatment should be chosen to be as gentle as possible in order to avoid unwanted changes
247 (chemical and physical) in the quality of the product. The choice of method and establishment of
248 process parameters (times, temperatures, pressures, concentrations, dose etc.) should be based on
249 development and validation data.

250 ***The extraction process itself***

251 In many cases, the manufacturing process itself may provide a degree of microbial decontamination to
252 a certain extent. For example, extraction of the raw material with an alcoholic solution may represent a
253 microbial-reducing method. However, only higher alcohol concentrations (60 to 80%) have marked
254 decontamination effects because, at lower concentrations of alcohol, the presence of water potentially
255 facilitates the growth of the micro-organisms.

256 No obvious differences in microbial decontamination have been shown between the use of ethanol and
257 methanol. Vegetative cells, particularly those of Gram-negative species, are very sensitive to heat and
258 alcoholic solutions. The residual microbial contamination from such extraction processes is represented
259 mainly by bacterial endospores, which are resistant to e.g. ethanol. Hydroalcoholic extraction with
260 heating usually yields products with relatively low TAMC ($<10^4$ CFU/ml).

261 Production of a dry extract normally involves cautious evaporation of the organic solvent in a vacuum-
262 evaporator. In most cases, the resulting aqueous soft extract is mixed with suitable excipients and
263 then further evaporated to dryness using suitable equipment (e.g. spray drier or belt drier): the total

264 microbial level may be increased after alcohol evaporation, as the aqueous soft extract is an ideal
265 medium for microbial growth.

266 Extraction with boiling water reduces the TAMC and TYMC as shown by several studies on the effects of
267 the use of boiling water to prepare herbal teas. Experiments with artificial contamination by non-
268 sporulating (*E. coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Klebsiella pneumoniae* and
269 *Enterobacter cloacae*) and spore-forming microbial species (*Bacillus cereus*) demonstrated that the
270 non-sporulating bacteria were fully eliminated while the sporulating organisms survived extraction with
271 boiling water almost completely. However, as water is ideal for the growth of micro-organisms, the
272 storage period should be less than 24 h at the temperature of a refrigerator (2-8°C) to prevent
273 microbial growth.

274 Extraction with supercritical carbon dioxide reduces the TAMC and TYMC by combining the effect of the
275 solvent with the effect of high pressure which both reduce the level the micro-organisms.

276 Distillation of essential oils usually leads to very low microbial contamination because of the process
277 itself (high temperature and phase change) and, additionally, due to the often intrinsic antimicrobial
278 properties of the essential oils.

279 ***Treatment with ethanol***

280 Data are available on the bactericidal effects of ethanol as a function of concentration and contact
281 time. Ethanol is bactericidal in aqueous mixtures at concentrations between 60-95% V/V but is
282 ineffective against bacterial spores.

283 In view of the fact that extraction with ethanol helps to reduce the microbial contamination, repeated
284 treatments with ethanol followed by evaporation may be performed to minimise the microbial content.
285 However, use of ethanol may cause chemical changes in the composition of extracts and such changes
286 should be evaluated.

287 ***Heat treatment: dry or steam***

288 In order to minimise microbial contamination, short heat treatment (ultra high heat (UHT)) or
289 pasteurisation may be performed before drying, if necessary.

290 However, such treatments are not usually suitable for extracts with high contents of resinous
291 substances, highly viscous extracts (dry residue more than 50%) or extracts with thermolabile or
292 volatile constituents.

293 The use of heating as a microbial decontamination method is based on the assumption that the death
294 of micro-organisms is log-linear with time. However, the use of this method may be limited by the
295 highest temperature that can be used, particularly when thermolabile and volatile constituents are
296 present in the herbal material.

297 Drying at high temperatures for a few minutes, such as in tumble dryers used for industrial production,
298 generally reduces the microbial bioburden. Drying at lower temperatures in static dryers for a longer
299 time, may have a lesser impact on some chemical constituents, but does not sufficiently reduce the
300 viable count as much as in tumble dryers and has no effect on spores. The spores of Gram-positive
301 bacteria are highly heat resistant and temperatures required to kill them may induce physicochemical,
302 chemical and sensory changes to the product.

303 Water vapour treatment at 65°C may destroy certain undesirable micro-organisms (e.g. *Salmonellae*,
304 *E. coli* and *Pseudomonas aeruginosa*). However, residual moisture should be removed and carefully
305 controlled after the treatment in order to avoid subsequent microbial growth.

306 **Fumigation**

307 Fumigation of herbal substances to control pests and plant diseases may also reduce microbial
308 contamination. It is recommended that the use of fumigant products is limited as far as possible and
309 should only be used when a genuine need is identified. Fumigation should be carried out at the earliest
310 possible stage and the choice of fumigant, concentration and conditions of use (temperature, humidity,
311 exposure time) should be carefully assessed to minimise residues in the herbal material. Potential
312 carry-over of residues to the herbal preparation and HMP should be addressed fully and controls
313 applied where necessary. Aspects of fumigation of herbal substances are discussed in the *Reflection*
314 *paper on the use of fumigants* (EMA/HMPC/125562/2006) and in the *Questions & answers on quality*
315 *of herbal medicinal products/traditional herbal medicinal products* (EMA/HMPC/41500/2010, as
316 revised).

317 **Irradiation**

318 Irradiation is restricted or not permitted in a number of European Member States and, when allowed, it
319 should only be used when there is a reasonable need and no other methods can be applied.

320 Irradiation should be carried out under specified conditions and the safety of irradiated products should
321 be fully evaluated.

322 Three different types of ionising radiation are used; gamma rays, X rays and electrons.

323 The effectiveness of the treatment is dependent on several factors including the composition of the
324 substrate, the number and types of micro-organisms and the dose applied. The lethal dose of radiation
325 varies depending on the type of radiation and the type of micro-organisms. In general, vegetative
326 forms of bacteria are more sensitive to ionizing radiation than fungi are. The number of spores may
327 also be reduced by X ray and gamma irradiation.

328 **Freeze drying**

329 Freeze-drying is reported to decrease microbial contamination, but there is little information on the
330 effect of this technique. Moreover, the sensitivity of micro-organisms may differ considerably to this
331 method and conditions capable of reducing microbial contamination should be evaluated. On the other
332 hand, the use of cryo-protectants as part of the process may allow for the survival of micro-organisms
333 and their subsequent recovery and proliferation after reconstitution. This should be addressed during
334 method validation.

335 **High pressure processing**

336 High pressure processing (HPP), also known as high hydrostatic pressure processing (HHPP) and
337 ultrahigh pressure (UHP), is a method of processing, where the material is subjected to elevated
338 pressures, up to 1000 MPa (145,000 psi), applied with a pressure-transmitting medium (water or other
339 liquids as appropriate). The process inactivates/kills micro-organisms, reduces the need for
340 preservatives and eliminates post-process contamination, while retaining organoleptic properties such
341 as freshness, flavour and colour of the plant material. Moreover HPP can also be used to inactivate (or
342 to activate) enzymes.

343 Although the mechanism of inactivation by HPP is not well understood, it is considered that the
344 compression process induces cell membrane rupture and macromolecular transformations, e.g. protein
345 denaturation.

346 HPP can be applied to solids, liquids and to packaged products as high pressure acts instantaneously
347 and uniformly without a gradient of effectiveness from surface to centre, regardless of shape, size, and
348 composition. Pressure, temperature and exposure time can be adjusted for optimal results, and the
349 process may be carried out at ambient, cooling or freezing temperatures, with exposure times ranging
350 from a millisecond to over 20 minutes.

351 The sensitivity of micro-organisms to HPP is variable and influenced by several factors, therefore the
352 processing conditions (holding time of the pressure, temperature of pressure processing, composition
353 of the medium) have to be carefully selected for the individual herbal material to be treated.
354 Conditions and specifications should be validated. Any impact of pH modifications applied during the
355 HPP process on the composition of the product should be evaluated.

356 To improve the efficacy HPP can be combined with heat and/or low pH as well as pressure cycling
357 treatments for inactivation and control of outgrowth of spores. Ultrasound, alternative currents, high-
358 voltage electric pulses and antimicrobial agents may also be used.

359 HPP may only damage the microbial cells, thus the sub-lethally injured cells may recover and multiply
360 when they find suitable conditions during subsequent processing and storage. This phenomenon may
361 lead to an over-estimation of microbial reduction because the counts determined immediately after
362 HPP will be lower than those reached after the recovery of injured cells.

363 ***Instant controlled pressure drop***

364 In recent years a new technology, Instant controlled pressure – drop (DIC for Détente Instantanée
365 Contrôlée) has been developed (and patented) as a decontamination process, particularly for heat-
366 sensitive solids and powders. It is based on short time heating of the material and an instantaneous
367 pressure drop towards vacuum, which causes an abrupt cooling by evaporation of part of the water of
368 the treated material. The micro-organism cells (both spores and vegetative forms) explode as a
369 consequence of a thermo-mechanical effect. Heating of the material may be achieved by saturated or
370 superheated steam injection (STEAM-DIC) or compressed air but other media can be used such as
371 carbon dioxide, when a dissolution effect is expected to be achieved (e.g. extraction of non-volatile
372 constituents). The higher the amount of the steam or gas injected and the shorter the pressure drop
373 time, the more efficient the mechanical effect is. When the process is repeated several times it is
374 possible to lower the heating temperature to achieve the desired microbial contamination reduction,
375 thus preserving thermolabile constituents (Multi-cycle DIC).

376 A possible negative impact of this method is the loss of volatile constituents through auto-vaporisation.
377 However, this feature offers a potential for use of DIC technology in the extraction of essential oils
378 from aromatic plants.

379 ***Treatment with alkaline or acidic substances***

380 Treatment with alkaline or acidic chemical substances is known to reduce microbial contamination,
381 including spores. However, such treatments are not usually applicable to herbal substances or herbal
382 preparations, as alkaline and acidic compounds may lead to significant chemical alterations of the
383 constituents of the herbal substance/preparation. Residues of any toxic substance applied should also
384 be avoided.

385 ***Preservation***

386 Addition of a preservative is not considered to be a decontamination method. However, addition of
387 preservatives to prevent microbial growth on storage and to cover the entire shelf-life should be

388 considered when the unpreserved product supports microbial growth. Preservatives must not be used
389 to replace GACP and GMP or to disguise products with initial high levels of microbial contamination.

390 ***New, alternative methods***

391 The list of methods outlined above is not exhaustive and other methods may be applied. Manufacturers
392 and regulatory agencies have a responsibility to ensure appropriate microbiological quality of herbal
393 substances/preparations/HMPs and where necessary, appropriate decontamination methods could be
394 employed to reduce microbial contamination.

395 **2.2.3. Herbal substances**

396 Methods for reducing microbial contamination of herbal substances are not only dependent on the
397 above mentioned specific factors, but also on the subsequent use of the herbal substance. When the
398 herbal substance is intended for further processing it might be sufficient to dry or freeze the plant
399 material to prevent microbial growth and spoilage until further processing takes place.

400 Fumigation may be appropriate for herbal substances but it should be limited when other approaches
401 are possible and should be applied at the earliest possible stage, taking into consideration all relevant
402 aspects, precautions and prohibitions.

403 The use of steam is, in general, not advisable to reduce the microbial contamination of plant material,
404 unless, following the process, the material is dried immediately as any residual water may affect the
405 subsequent processing stages.

406 Irradiation should be limited to exceptional circumstances, when no other method is feasible. Attention
407 should be paid to herbal substances imported from Third Countries, which may have been irradiated
408 but this is not declared or adequately documented. A suitable test to detect possible irradiation should
409 be established for herbal substances at risk.

410 HPP can be used for eliminating bacteria of concern and to ensure microbiological safety. This process
411 is used in the food area for fruit juices and fruit and is suitable for the treatment of herbal substances.
412 Heat sensitive products with a high acid content are particularly good candidates for the application of
413 this technology.

414 **2.2.4. Herbal preparations**

415 The extraction process itself may contribute to microbial contamination reduction notably when high
416 concentrations of ethanol are used. However it should be noted that extraction with cold water may
417 result in large increases in microbial levels such as in case of maceration.

418 Fumigation is not a suitable treatment for herbal preparations and irradiation of herbal preparations is
419 not advisable.

420 Preservatives may be added to herbal preparations in order to prevent microbial growth but not to
421 lower microbial contamination.

422 Heat treatments (e.g. UHT on soft extracts) or HPP may be suitable; specific conditions have to be
423 selected and validated to allow assessment of the impact on the composition of the preparation.
424 Possible changes should be investigated and justified.

425 **2.2.5. Herbal medicinal products**

426 Microbial quality of HMPs is determined by the quality of starting materials, hygiene conditions and the
427 manufacturing process. Therefore, following application of GACP and GMP criteria the need for
428 microbial decontamination of the finished product should be minimal.

429 The Ph. Eur. recognises the need to allow wider acceptance criteria for the microbial quality HMPs
430 depending on the nature of the product and method of preparation, as discussed below.

431 In the specific case of herbal teas for example, relatively high TAMC and TYMC are accepted taking
432 account of the method of preparation with boiling water (brewing). However, consideration should be
433 given to the fact that herbal teas inappropriately prepared, using only hot instead of boiling water, may
434 result in preparations with inadequate microbial quality.

435 HMPs sensitive to heat (e.g. emulsions and suspensions) may be treated with HPP without affecting
436 their physico-chemical properties.

437 Irradiation of HMPs is not advisable.

438 Addition of preservatives should be minimised, but may be considered for medicinal products, which
439 could potentially support the growth of micro-organisms, if unpreserved, and when packaged in
440 multidose containers. Antimicrobial preservative effectiveness should be demonstrated according to Ph.
441 Eur. 5.1.3, during development, scale-up, at the end of shelf-life and in-use of the product (e.g., in
442 stability testing), and chemical testing of preservative content (ID and assay) is the attribute normally
443 included in the specification.

444 **2.3. Testing of the herbal substance, herbal preparation, and herbal** 445 **medicinal product**

446 Microbiological contamination is evaluated by the microbial count. Microbial count is determined by a
447 microbiological plate-count technique with enumeration of colony forming units (CFU) per ml or g of
448 herbal material.

449 **Microbial counts: Analytical methods**

450 Usually the assessment of microbiological quality of herbal substance, preparation and HMP is
451 performed in accordance with the reference methods given in three general chapters of the Ph. Eur.
452 i.e. 2.6.12 "Microbiological examination of non-sterile products: Microbial enumeration tests",
453 2.6.13 "Microbiological examination of non-sterile products: Test for specified micro-organisms" and
454 2.6.31 "Microbiological examination of herbal medicinal products for oral use and extracts used in their
455 preparation".

456 The tests described in Ph. Eur. 2.6.12 allow quantitative enumeration of mesophilic bacteria and fungi
457 that may grow under aerobic conditions. Ph. Eur. 2.6.31 describes tests for the specified micro-
458 organisms *E. coli*, bile-tolerant gram-negative bacteria and *Salmonella*. Specified micro-organisms
459 listed in Ph. Eur. 2.6.13 include the same micro-organisms as in 2.6.31, with the addition of
460 *P. aeruginosa*, *S. aureus*, *Clostridia*, and *Candida albicans*.

461 As conventional microbiological methods are slow (results are not available before an incubation period
462 of 5-14 days), an additional chapter has been published in the Ph. Eur. for information in order to
463 facilitate the use of alternative methods (5.1.6. "Alternative methods for control of microbiological
464 quality"): some of these methods have shown potential for real-time or near-real-time results with the
465 possibility of earlier corrective action. For each method, the basic principle is stated and the benefits
466 and disadvantages of the method are then discussed. Chapter 5.1.6 may be used in the process of

467 choosing a microbiological method as a supplement or as an alternative to conventional microbiological
468 approaches and to give guidance on the process of validating the chosen method.

469 **Microbial counts: Acceptance criteria**

470 Microbial limit testing is seen as an attribute of both GMP, and quality assurance.

471 Chapter 5.1.8 "Microbiological quality of Herbal medicinal products for oral use and Extracts used in
472 their preparation" of the Ph. Eur. provides general acceptance criteria for a non exhaustive list of
473 specified micro-organisms and maximum acceptable counts (expressed as TAMC and TYMC). However
474 testing for other micro-organisms may be necessary or less-stringent criteria may be applied on the
475 basis of a risk-assessment which takes into due consideration the nature of the starting materials, the
476 qualitative and quantitative characterisation of the microbial contamination, the manufacturing process
477 and the intended use of the HMP or extract.

478 Finished HMPs are grouped into three categories A, B and C, taking into account the manufacturing
479 method, the intended use and, in the case of herbal teas, the method of preparation by the patient.

480 Extracts for oral use should fulfil the acceptance criteria for category C when it is demonstrated that
481 the method of processing would not reduce the level of micro-organisms sufficiently to reach the
482 criteria of category B.

483 More-stringent acceptance criteria may be required for extracts that are to be incorporated into
484 pharmaceutical preparations to be administered by other routes of administration as reported in Ph.
485 Eur. chapter 5.1.4 "Microbiological quality of non-sterile pharmaceutical preparations". This chapter
486 includes special Ph. Eur. provisions for other dosage forms containing raw materials of natural origin
487 (e.g. herbal) for which antimicrobial pre-treatment is not feasible and for which TAMC of the raw
488 material exceeding 10^3 CFU/g or CFU/ml may be accepted.

489 The absence of specific bacteria of concern should be tested (e.g. *S. aureus*, *E. coli*, *Salmonella*
490 *enterica subsp. P. aeruginosa*). The source of the herbal material should be taken into account when
491 considering the inclusion of other possible pathogens (e.g. *Shigella*, *Campylobacter* and *Listeria*
492 species) in addition to those specified in the Ph. Eur.

493 Acceptance criteria for herbal substances and herbal preparations other than extracts are not currently
494 given in Ph. Eur. Limits for TAMC, TYMC and specified micro-organisms should be established on a
495 case-by-case basis.

496 Further indications on interpretation and risk-assessment as well as guidance on the parameters to be
497 taken into account in setting these limits by the applicant are given in the document "Questions &
498 Answers on quality of herbal medicinal products/traditional herbal medicinal products"
499 (EMA/HMPC/41500/2010 current revision) and in the "Guideline on specifications: test procedures and
500 acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional
501 herbal medicinal products" (EMA/CPMP/QWP/2820/00 Rev. 2).

502 **Mycotoxins**

503 The potential for mycotoxin contamination should be fully evaluated, even when microbial
504 decontamination treatments have been carried out. For aflatoxins, the Ph. Eur. has included a method
505 2.8.18 for determination of Aflatoxin B1 in herbal substances and sets limits for herbal substances,
506 unless otherwise indicated in the monograph, at NMT 2 µg/kg. The Ph. Eur. method of analysis 2.8.18
507 states that the Competent Authority may also require compliance with a limit for the sum of aflatoxins
508 (B1, B2, G1 and G2) of NMT 4 µg/kg.

509 For ochratoxin A, the procedure is described in Ph. Eur. 2.8.22 and acceptance criteria are given in
510 specific monographs.

511 Since mycotoxin contamination is expected to be non-homogenous and contamination may not spread
512 to all parts of the plant, only some parts of a stored herbal material batch may contain mycotoxins
513 (e.g. spot contamination by fungi). This issue must be carefully evaluated and an appropriate sampling
514 regime should be established to determine the risk of mycotoxin contamination³.

515 **Loss on drying, water content or water activity**

516 Testing for loss on drying, water content or water activity on the herbal substance/preparation is useful
517 for the risk assessment of potential microbial growth. Such testing cannot replace a test on TAMC and
518 TYMC, but it can support a justification for skip testing of the herbal substance/preparation/finished
519 product.

520 Hygroscopic herbal substances/preparations are more prone to support microbiological growth.
521 Therefore the acceptance criteria for water content should be assessed in the light of the effects of
522 moisture absorption. A loss on drying test may be adequate, however, this may not be reliable for
523 some extracts (e.g. milk thistle) and in such cases the water content determination is preferred (Ph.
524 Eur. 2.5.12).

525 For essential-oil containing plants a test that is specific for water is required.

526 The Ph. Eur. describes a test for "Water in essential oils" (2.8.5.), a method "Determination of water
527 by distillation (2.2.13)" which may be used for herbal drugs and a method "Water - semi-micro
528 determination" (2.5.12) useful for the extracts.

529 Water activity (a_w) is a measure of the energy status of the water in a system and it is one of the most
530 critical factors in determining if and how fast a micro-organism will grow. Since water activity, and not
531 water content, determines the lower limit of available water for microbial growth, the control of a_w is a
532 valuable tool for controlling microbial growth and a test to determine a_w may be useful in predicting the
533 potential for an increase in microbial contamination during storage.

534 It is generally recognised that in products with a_w below 0.60 moulds and yeasts do not proliferate.
535 The lowest a_w at which the vast majority of bacteria and moulds will grow is about 0.85 and 0.70,
536 respectively, whilst dried herbal materials stored under normal conditions have a lower a_w (usually
537 0.50-0.60). Halophilic (salt-loving) bacteria will grow at an a_w as low as 0.75, but they pose no known
538 threat to public health. With the exception of *S. aureus*, the minimum a_w level for growth of pathogenic
539 bacteria known to cause food borne infections or intoxications is ≥ 0.93 . *S. aureus* can proliferate in
540 products with an a_w as low as 0.86. Production of *S. aureus* enterotoxins may, however, require a
541 higher a_w .

542 **Ethanol**

543 Methods to determine the ethanol content in liquid pharmaceutical preparations such as extracts and
544 tinctures are given in Ph. Eur. chapter 2.9.10 "Ethanol content". Reduced (or omission of)
545 microbiological testing of the herbal preparation in presence of suitable ethanol concentration must be
546 justified.

³ Ph. Eur. General Chapter 2.8.20 describes a sampling plan for herbal drugs "Herbal drugs: sampling and sample preparation"

547 **Preservatives**

548 For HMPs needing an antimicrobial preservative, e.g. oral liquids, acceptance criteria for preservative
549 content must be stated, based on the levels necessary to maintain the product's microbiological quality
550 throughout storage and use. The lowest specified concentration of antimicrobial preservative should be
551 demonstrated to be effective in controlling microorganisms by using the Ph. Eur chapter 5.1.3.
552 "Efficacy of antimicrobial preservation". A similar approach could be used for preserved herbal
553 preparations.

554 Release and stability testing for the identification and assay of antimicrobial preservative content
555 should normally be performed. Under certain circumstances, in-process testing may suffice in lieu of
556 release testing. When antimicrobial preservative content testing is performed as an in-process test, the
557 acceptance criteria should remain part of the specification.

558 **Residues of fumigants**

559 The potential for residues of fumigation agents in herbal substances and herbal preparations should be
560 fully evaluated. For HMPs it is not necessary to test residues of fumigants when they are controlled in
561 the herbal substance/preparation.

562 Where necessary, suitable validated methods should be used to control potential residues and the
563 acceptance criteria should be justified.

564 **Residues of "irradiation"**

565 The potential for residues of irradiation in herbal substances and herbal preparations should be fully
566 evaluated and tested when there is reason, or a concern that irradiation has been performed. Where
567 necessary suitable validated methods should be used to control potential residues and the acceptance
568 criteria should be justified.

569 **Testing frequencies - Release and stability testing**

570 Microbial counts should be determined using pharmacopoeial procedures or other validated procedures
571 and at a sampling frequency and/or time point in the manufacture which is justified by data and
572 experience ("Guideline on quality of herbal medicinal products/traditional herbal medicinal products
573 EMA/CPMP/QWP/2819/00).

574 Further guidance on routine and reduced microbiological testing as well as testing for mycotoxins and
575 during stability studies is given in the document "Questions & Answers on quality of herbal medicinal
576 products/traditional herbal medicinal products" (EMA/HMPC/41500/2010 current revision) and in
577 "Quality of medicines questions and answers: Part 1 ([Active Substance - Starting materials of herbal
578 origin](#))".

579 **2.3.1. Herbal substances**

580 In general, routine testing is applicable for herbal substances. Limits and acceptance criteria should be
581 established and justified through a risk assessment taking into account the specific microbial
582 contamination, information from validation studies on the capability of subsequent steps of the
583 manufacturing process to decrease the microbial count and the intended use. Possible contamination
584 by mycotoxins should be also considered.

585 **2.3.2. Herbal preparations**

586 Excluding or reducing tests for microbial contamination in herbal preparations such as extracts or
587 tinctures depending on the ethanol content must be justified by scientific evidence. The frequency of
588 testing of herbal preparations should be justified by the applicant e.g. based on the validation of the
589 manufacturing process and of the holding time of the bulk product. Possible contamination by
590 mycotoxins should be also considered.

591 **2.3.3. Herbal medicinal products**

592 HMPs must be tested for microbiological quality. Skip testing may be applied in circumstances where
593 components are tested before manufacture and validation studies have demonstrated no significant
594 risk of microbial contamination during the manufacturing process. Possible contamination by
595 mycotoxins should be also considered.

596 Decision tree #8 reported in the "ICH Topic Q 6A Specifications: Test Procedures and Acceptance
597 Criteria for New Drug Substances and New Drug Products: Chemical Substances" (CPMP/ICH/367/96)
598 provides additional guidance on the use of microbial limits testing for non-sterile medicinal products.

599 **3. Conclusion**

600 Being of natural origin, herbal substances generally have higher contents of micro-organisms when
601 compared to chemically defined drug substances. This presents particular challenges as the micro-
602 organisms may be carried over to the herbal preparation and herbal medicinal product. In addition,
603 spores and toxic mycotoxins generated by the micro-organisms may also be present and these are
604 more difficult to eliminate, once they are present in the herbal material.

605 Satisfactory quality of HMPs with respect to microbiological and mycotoxin contamination cannot
606 merely be controlled by final testing; it should be built-in the entire process, from starting material to
607 finished product. Minimizing and testing/monitoring of microbial contamination and mycotoxins in
608 herbal substances, herbal preparations and herbal medicinal products must be based on a case-by-
609 case risk assessment.

610 A number of critical points need to be considered and taken into account. These include the source of
611 the herbal substance, knowledge about the micro-organisms, the manufacturing processes and any
612 decontamination procedure used, microbiological purity of excipients, the protective capacity of the
613 packaging material chosen, the dosage form, administration route, posology and patient population
614 groups. Most importantly, preventive measures are preferred rather than interventions for decreasing
615 the contamination.

616 Compliance with GACP and GMP throughout the entire manufacturing process from herbal substance to
617 the finished product is crucial in order to ensure acceptable microbiological quality of the HMP. The
618 HMP should not support microbial growth; drying processes and final contents of water are critical
619 parameters in this respect.

620 If a decontamination process is to be applied to the herbal material, usually the herbal substance or
621 herbal preparation, the need for such use should be fully justified and the decontamination method
622 should be selected with care. The initial and desired final maximum level of micro-organisms should be
623 taken into account and it should be demonstrated that the decontamination process does not alter the
624 chemical composition of the herbal material or leave residues of toxic components in the product.

625 The specifications of the herbal substance, herbal preparation and HMP should include tests for TAMC
626 and TYMC and absence of certain specified micro-organisms, unless otherwise justified. Limits and

627 analytical methods are given in Ph. Eur. for extracts and for HMPs, although alternative validated
628 methods also can be applied. Testing of loss on drying/water content and mycotoxins should also be
629 considered in the risk assessment. However, these parameters cannot replace testing of the microbial
630 contamination itself.

631 **4. Definitions**

632 **Acceptance criteria:** Numerical limits, ranges, or other suitable measures for acceptance of the
633 results of analytical procedures.

634 **Constituents with known therapeutic activity:** are chemically defined substances or groups of
635 substances, which are generally accepted to contribute substantially to the therapeutic activity of a
636 herbal substance, a herbal preparation or a herbal medicinal product.

637 **Degradation product:** Any impurity resulting from a chemical change in the composition of the active
638 substance brought about during manufacture and/or storage of the active substance/ medicinal
639 product by the effect of, e.g., light, temperature, pH, water, or by reaction with an excipient and/or
640 the immediate container closure system. Due to the particular nature of herbals, for herbal
641 substances/herbal preparations/herbal medicinal products in general only toxicologically relevant
642 degradation products must be specified.

643 **Extraction solvents:** are solvents, which are used for the extraction process.

644 **Herbal medicinal products:** any medicinal product, exclusively containing as active substances one
645 or more herbal substances or one or more herbal preparations, or one or more such herbal substances
646 in combination with one or more such herbal preparations.

647 **Herbal preparations:** are obtained by subjecting herbal substances to treatments such as extraction,
648 distillation, expression, fractionation, purification, concentration or fermentation. These include
649 comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and
650 processed exudates.

651 **Herbal substances:** all mainly whole, fragmented or cut plants, plant parts, algae, fungi, lichen in an
652 unprocessed, usually dried form but sometimes fresh. Certain exudates that have not been subjected
653 to a specific treatment are also considered to be herbal substances. Herbal substances are precisely
654 defined by the plant part used and the botanical name according to the binomial system (genus,
655 species, variety and author).

656 **Herbal teas:** consist exclusively of one or more herbal substance(s) intended for oral aqueous
657 preparations by means of decoction, infusion or maceration. The preparation is prepared immediately
658 before use. Herbal teas are usually supplied in bulk form or in sachets.

659 **Impurity:** (1) Any component of the herbal substance, which is not the entity defined as the herbal
660 substance. (2) Any component of the herbal preparation/herbal medicinal product that is not the entity
661 defined as the herbal substance/preparation or an excipient in the herbal preparation/herbal medicinal
662 product.

663 **Markers:** are chemically defined constituents or groups of constituents of a herbal substance, a herbal
664 preparation or a herbal medicinal product which are of interest for control purposes independent of
665 whether they have any therapeutic activity. Markers serve to calculate the quantity of herbal
666 substance(s) or herbal preparation(s) in the herbal medicinal product if the marker has been
667 quantitatively determined in the herbal substance or herbal preparation.

668 There are two categories of markers:

669 *Active markers* are constituents or groups of constituents which are generally accepted to contribute to
670 the therapeutic activity.

671 *Analytical markers* are constituents or groups of constituents that serve for analytical purposes.

672 **Solvent:** An inorganic or an organic liquid used for the preparation of solutions or suspensions in the
673 manufacture of a herbal preparation or the manufacture of a herbal medicinal product.

674 **Specification:** A list of tests, references to analytical procedures, and appropriate acceptance criteria,
675 which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of
676 criteria to which a herbal substance/preparation or herbal medicinal product should conform to be
677 considered acceptable for its intended use. "Conformance to specifications" means that the herbal
678 substance/preparation and/or herbal medicinal product, when tested according to the listed analytical
679 procedures, will meet the listed acceptance criteria. Specifications are binding quality standards that
680 are agreed to between the appropriate governmental regulatory agency and the applicant.

681 **Specific test:** A test which is considered to be applicable to a particular herbal substance/preparation
682 or a particular herbal medicinal product depending on their specific properties and/or intended use.

683 **TAMC:** Total aerobic microbial count.

684 **Traditional herbal medicinal products:** are medicinal products for human use, that fulfil the
685 conditions laid down in article 16a(1) of Directive 2001/83/EC, as amended.

686 **TYMC:** Total combined yeasts and moulds count.

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