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**OVERVIEW OF COMMENTS RECEIVED ON DRAFT
'GUIDELINE ON THE ASSESSMENT OF GENOTOXIC CONSTITUENTS IN HERBAL
SUBSTANCES/ PREPARATIONS'
(EMEA/HMPC/107079/2007)**

Table 1: Organisations that commented on the draft guideline as released for consultation until 3 March 2008

Name of organisation or individual	Country
AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé)	France
AESGP Association of Natural Medicine in Europe e.V.	Belgium Germany
Maharishi Technology Corporation BV	The Netherlands
Ayurvedic Trade Association	UK

Table 2: Discussion of comments

SPECIFIC COMMENTS ON TEXT		
4. MAIN GUIDELINE TEST		
Paragraph no.	Comment and Rationale	Outcome
4.1 Testing strategy Lines 120-145	<p><u>“Step1 : the Ames test” / “scenario 1”</u></p> <p>A “pragmatic” approach is proposed: an Ames test should be performed with the herbal substance/ preparation. In case of a negative result, no further genotoxicity testing is required. However, a few lines above, it is acknowledged that “the Ames test [...] cannot cover all genotoxic endpoints and thus a significant sphere of genotoxic potential, e.g. in relation to chromosomal damage, remains untested”. We agree with this statement. Therefore, we consider that the approach proposed is not acceptable and cannot be justified even by the need of pragmatism.</p> <p>To illustrate our position, here are some examples for which an isolated Ames test would have failed to detect a genotoxic potential:</p> <ul style="list-style-type: none"> – Taxol is an active compound extracted from the bark of the Pacific yew tree (<i>Taxus brevifolia</i>). It has been shown to be clastogenic <i>in vitro</i> (chromosome aberrations in human lymphocytes) and <i>in vivo</i> (micronucleus test in mice), but was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay (see http://www.fda.gov/cder/foi/label/2000/20262S36LBL.PDF) <p>Vincristine is an active compound extracted from the Madagascar periwinkle (<i>Catharanthus roseus</i>). It is a microtubule poison, member of the vinca alkaloid class of chemotherapy drugs. Vincristine was not mutagenic in the Ames test (Bakshi et al, 1985 ; Sakamoto et al, 1985 – CCRIS on Toxnet). Other authors have reported positive results for sister chromatid exchanges <i>in vitro</i>, chromosome aberrations and micronucleus test (GENE-TOX on Toxnet).</p>	<p>Not agreed. Herbal medicinal preparations constitute a special class of medicinal agents, as can be seen from the Community legislation, and consequently a certain measure of pragmatism is deemed necessary. Although potentially some genotoxic agents cannot be detected reliably by the Ames test, a majority of problem cases are probably detectable and should be studied further. Transparent pragmatism, as it is now expressed in the guideline, involves measures, which aim to resolve most pressing genotoxic possibilities with the resources available currently, but which leave some potential, albeit probably rare, problems not screened.</p>

<p>4.1 Testing strategy</p> <p>Lines 147 – 162</p>	<p><u>“Step1 : the Ames test” / “scenario 2” and “scenario 3”</u></p> <p>Scenario 2 concerns equivocal results and scenario 3 concerns positive results. However, the decision will be similar in both cases. Therefore, these scenarios should be merged. Consequently, the resulting scenario should include positive results as well as results remaining equivocal after having excluded false positives and repeated tests.</p>	<p>Not agreed. It is clearer to keep these two possibilities separate, because they deal with somewhat different responses and weight-of-evidence argumentation may lead to different consequences.</p>
<p>4.1 Testing strategy</p> <p>Lines 164 – 171</p>	<p><u>“Step1 : the Ames test” / “Step 1a: a well characterized and assessed genotoxic substance is identified to be responsible for genotoxic activity”</u></p> <p>It should be first demonstrated that the substance is responsible for the whole genotoxic effects observed. In particular, the potential interactions (inhibition, potentiation) between this substance and other herbal compounds found in the tested extract should be taken into account. This could be done by testing the extract enriched with the substance thought to be responsible for the genotoxic effect.</p>	<p>The guideline leaves the specific measures to the responsibility of the applicant to demonstrate that a well characterized and assessed genotoxic substance is responsible for genotoxic activity. When enough experience on how this specific step works in practise has been accumulated, it is possible to come back for possible amendments and other changes.</p>
<p>4.1 Testing strategy</p> <p>Lines 207 – 220</p>	<p><u>“Step 3: mouse micronucleus test or other in vivo genotoxicity tests”</u></p> <p>European and OECD guidelines indicate that the micronucleus test should be performed in rodents, <i>i.e.</i> mice or rats. Therefore, “mouse”/“mice” should be replaced by “rodent”/“rodents”.</p> <p>No additional <i>in vivo</i> test is recommended if a negative result is obtained in the micronucleus test. This approach would not have allowed the detection of known carcinogens such as dimethylnitrosamine and dimethylhydrazine. Therefore, we suggest to fulfil the recommendations of the “note for guidance on genotoxicity: specific aspects of regulatory genotoxicity tests for pharmaceuticals” (CPMP/ICH/141/95 – ICH S2A). This document notably states that “for a compound that induces a biologically relevant positive result in one or more <i>in vitro</i> tests [...], a further <i>in vivo</i> test in addition to the <i>in vivo</i> cytogenetic assay, using a tissue other than the bone marrow/ peripheral blood, may provide further useful information”.</p>	<p>Accepted: “mouse”/“mice” is replaced by “rodent”/“rodents”.</p> <p>Not agreed. However, it is possible that some genotoxicants would be missed with the current guideline recommendations. Again, the HMPC has adopted ‘transparent pragmatism’ in this case.</p>

GENERAL COMMENTS		
Paragraph no.	Comment and Rationale	Outcome
	The title is to our view a bit misleading as it seems to focus only on isolated constituents and their genotoxic effects, although the general intent of the guideline is clearly on herbal substances and preparations. In addition, the title does not reflect the interpretation part of the guidance. Therefore, we would like to propose “Guideline on genotoxicity testing of herbal substances / preparations and interpretations of results” as a possible alternative title.	Partially agreed. The main purpose is indeed to test herbal substances and preparations, although also constituents in this respect may be of significance to the outcome of testing.
	The guideline needs to take into account appropriate existing data/literature informing on genotoxicity aspects which would exempt an herbal medicinal product (HMP) from testing. This needs to be clearly stated in the guideline.	This is clearly stated in the non-clinical guideline and the current guideline (see already the beginning of executive summary).
	We would be keen to have further information on how to address alternate outcomes of positive test results, for instance what is the process for dealing with a well-known genotoxicant that does not have an internationally acknowledged risk assessment?	HMPC is equally keen to see the argumentation of the applicants in this respect. In the guideline, a lot of latitude has been allowed to the applicants in terms of scientific reasoning and justification in the assessment of the product.

SPECIFIC COMMENTS ON TEXT		
EXECUTIVE SUMMARY		
Paragraph no.	Comment and Rationale	Outcome

	<p>The statement made in lines 40-42 (“<i>the complete lack of some specific non-clinical studies (e.g. genotoxicity studies) may also prevent a safety concern because important questions relating to product safety would remain unanswered</i>”) seems exaggerated. For example, this would not apply to plants which have been used as food for centuries and whose safety was proven over time even though specific non-clinical studies may not have been performed. This is true <i>a fortiori</i> if the form/equivalent dose is close to the one used for food consumption. Non-clinical studies may be available for a limited number of herbals, and therefore according to the present statement this would translate into ‘safety concern’ thereby completely negating their extended safe use. We propose that this statement be reworded.</p>	<p>Not agreed. Genotoxicity is a special sphere of toxicity, which requires a different approach.</p>
2. SCOPE		
	<p>To our view, the reference to <i>Angelica archangelica</i> is an inappropriate example. In the case of Angelica, case reports on photocarcinogenic effects caused by high-dose isolated furocoumarins in the established long-term PUVA therapy have been extrapolated to an <i>angelica</i> extract. <i>Aristolochia clematidis</i> which has well-described genotoxic and carcinogenic effects would be a more suitable example than furocoumarins – for which no specific intrinsic genotoxicity has been shown without UVA radiation.</p>	<p>Not agreed. Angelica is a good example to demonstrate a wide variety of problems with herbal medicinal substances and preparations regarding potential genotoxicity and its assessment.</p>
LEGAL BASIS		

	<p><u>Option for test batteries</u> On line 94, we can read that “<i>the basic requirement is to assess genotoxicity initially in a bacterial reverse mutation test using a test battery of different bacterial strains and metabolic activation.</i>” A large number of studies have shown that plants containing high concentrations of flavonoids, e.g. quercetin or other epoxide-formation inducers, invariably lead to positive results in bacterial reverse mutation tests. These plants however do not indicate genotoxicity <i>in vitro</i> in mammalian cells or <i>in vivo</i> and therefore can be rated as false- positive results. Therefore, the performance of such tests on plants known to contain epoxide-formation inducers will have no real use.</p> <p>Therefore, we would like to suggest that, in exceptional cases and on basis of a thorough justification, the necessity to first perform a reverse mutation test be waived for certain herbal medicinal products known to contain substances prone to lead to false positive, and to be able to start directly with the mammalian cell test systems (i.e. figure 1, step 2: mouse lymphoma assay).</p>	<p>Not agreed. The Ames test is a wide screen of potential mutagenicity and it is not restricted to only mutagenic flavonoids known to be negative in higher level testing. Even if there are flavonoids in the preparation, there may be other types of genotoxicants, in which case a further study and confirmation is required.</p> <p>Agreed in the sense, that this approach is possible even now on the basis of reasoning and justification of the applicant. MLA serves as a confirmation of a positive response in the Ames test, or, if MLA assay is negative, it demonstrates that the response in the Ames test is a prokaryotic phenomenon.</p>
	<p><u>Pharmacovigilance</u> The last paragraph states that “<i>it is also important to stress that pharmacovigilance is incapable of detecting genotoxicity and pharmacovigilance observations or documented long-standing use cannot be used as evidence for absence of genotoxic risks</i>”.</p> <p>From our point of view, such an absolute statement is not in accordance with the reality. There are well-known examples in which genotoxicity has been detected during long-standing use. For this reason, we suggest rephrasing the sentence as follows: “<i>...pharmacovigilance observations or documented long-standing use cannot be used <u>alone</u> as evidence for absence of genotoxic risks</i>”</p>	<p>Not agreed. HMPC would be interested to learn about those "well-known examples in which genotoxicity has been detected during long-standing use."</p>

SPECIFIC COMMENTS ON TEXT		
4. MAIN GUIDELINE TEST		
Paragraph no.	Comment and Rationale	Outcome
Testing strategy	<p>However, we suggest that ‘a sound literature search’ be performed first, i.e. before getting into the testing steps <i>per se</i>. In case sufficient data can be found in the scientific literature which substantiates the absence of safety concern, no further testing should be needed. Knowledge about extended use in similar conditions (i.e. same plant part, comparable intake) as food product without demonstrated harm (e.g. linseed) could be provided as well and would argue in favour of waiving the testing requirements.</p> <p>The bibliographical search should also include information on the presence of constituents (e.g. quercetin) whose presence is known to lead to false positive results in Ames test and therefore, alone, not indicative of genotoxic risk in humans. In such cases, it should be possible to by-pass step 1 and start directly with step 2. Having to carry out Ames test indiscriminately does not seem appropriate in light of resources which would need to be invested in order to obtain results which would be anyway known. We suggest the changes could be included in figure 1A.</p>	<p>This is indeed the spirit of legislation concerning well-established and traditional herbal medicinal preparations and it is stated in the guideline. It is also clear (and stated in the guideline) that knowledge concerning food should be taken into consideration in all phases of risk assessment.</p> <p>This is possible already now, if a reasonable justification could be developed.</p> <p>Not agreed. The Ames test is a basic genotoxicity test, with a long experience and a huge data base, so the Ames test should be the starting point for the evaluation of genotoxicity testing of herbal preparations. There is also a certain difference in targets and outcomes between the Ames test and the mouse lymphoma assay.</p>
Step 1: The Ames test	<p>As explained above, false positive can be expected in plants containing flavonoids or epoxide formation inducers, the Ames test should be made optional in such case. Not carrying it out would nevertheless need to be briefly explained.</p>	<p>See above.</p>

<p>Step 2: Mouse lymphoma assay or other mammalian cell assay</p>	<p>Under the conditions described above, it should be acceptable to start the testing at step 2.</p> <p>It is recommended that other options, like the mammalian cell HPRT assay, be listed as an acceptable alternative to the mouse lymphoma assay.</p> <p>As is also indicated in the proposal, the validity of the mouse lymphoma test to predict chromosomal damage is questionable and other tests serve better in this regard, e.g. the micronucleus test. The micronucleus test should therefore not only be considered in ‘special circumstances’ but to primarily evaluate this endpoint.</p>	<p>see above</p> <p>The possibility to use other test systems is stated in the guideline.</p> <p>In principle agreed. However, because of a pragmatic approach, the possibility of using the in vitro micronucleus test has been left to be considered in special circumstances.</p>
<p>Step 3: Mouse micronucleus test or other in vivo genotoxicity tests</p>	<p>We believe that the selection of appropriate <i>in vivo</i> tests must remain flexible, such that an appropriate <i>in vivo</i> test is selected based on expert judgment, taking into consideration not least the <i>in vitro</i> profile and endpoint of consideration.</p>	<p>Expert judgement has been stressed in several places in the guideline. It applies also here.</p>
<p>Step 4: Risk assessment considerations</p>		
	<p>There are a number of examples of classes of chemicals that induce genotoxicity by indirect mechanisms that have thresholds as discussed in the recent ICH genotoxicity guidelines and in lines 264-271 of this EMEA document. Thus, it is not accurate to state that current regulatory practice for pharmaceuticals assumes no threshold so we would suggest deleting line 224-226.</p>	<p>Not agreed. There is an intensive discussion about this matter, but adoption of clear-cut regulatory practices in this respect should await for clear signals from SWP and CHMP.</p>

<p>For the application of this scheme the following precondition is given (line 248): <i>“If a herbal preparation contains an identifiable genotoxic compound, the TTC approach could be applied”</i>.</p> <p>The strict application of this scheme would lead to paradox effects: Step 1a states that a well-characterised and assessed genotoxic substance has to be identified to be responsible for the genotoxic activity. Line 248 states that if such a substance is identified, the TTC approach could be applied. Given the fact that a high proportion of all herbal extracts contain e.g. quercetin, as a genotoxic compound, in concentrations above 1.5 µg, this could lead to a prohibition of all these herbal extracts by regulatory authorities!</p>	<p>Not agreed. Although the guideline suggests that TTC 'could be applied', it is not possible to recommend any single risk assessment scheme.</p>
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	<p>Findings on a large number of compounds present in herbal extracts have led to the assumption that the respective preparations have genotoxic properties. However, this is not relevant for their use in humans at doses present in herbal medicinal products or within the matrix of a certain herbal medicinal product.</p> <p>In addition, this paragraph, line 250, states that genotoxic constituents in herbal preparations are not impurities. The TTC approach was created to assess such impurities, which have per definition no therapeutic benefit. As for any other medicine, a therapeutic risk benefit ratio is to be taken into account.</p> <p>Therefore, the application of the TTC approach does not appear to be an adequate tool for assessing the genotoxicity of herbal extracts. Therefore this approach should not be required routinely but only in well-defined circumstances, e.g. in case of well-characterised carcinogenic substances, such as aristolochic acid.</p> <p>Moreover where risk assessment by the TTC applies, a TTC value of 1.5 µg/day (of a genotoxic impurity) is considered to be associated with an acceptable risk (excess cancer risk of <1 in 100,000 over a lifetime). It should be noted here that this is derived from a 10-fold lower TTC (0.15 µg/day) for chemicals with structural alerts that raise concern for potential genotoxicity. However, in the context of pharmaceuticals, a higher TTC value would be justified.</p>	<p>Not agreed. A risk assessment approach developed for impurities can be applied for other purposes ('chemicals are chemicals'). Therapeutic risk benefit ratio is another matter.</p> <p>As pointed out in the guideline, no single risk assessment approach can be suggested. The applicant has a lot of latitude to reason and justify the approach adopted.</p> <p>Again, it is clearly stated that higher levels are acceptable if the applicant gives reasonable justification.</p>
<p>OTHER COMMENTS</p>		
	<p>The background for such document and relation with the Sept. 2006 guideline (Doc EMEA/HMPC/32116/2005) is not clearly stated. It is recommended that the main points from the Concept paper (EMEA/HMPC/413271/Oct 2006) be added to the Introduction of this document to explain that this guideline was drafted in order to provide greater detail to the practical application of the Sept. 2006 guideline.</p>	<p>This is stated in the Legal basis.</p>

	Rather than listing the bacterial strains in the document, it is recommended to refer to the OECD guidelines which provide the full list of acceptable strains.	Agreed. The list of bacterial strains is deleted.
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GENERAL COMMENTS		
	The proposed guideline on the assessment of genotoxic constituents in herbal substances and herbal preparations may, if applied the way it is proposed, constitute a very high burden for the sector of traditional herbal medicinal products.	HMPC cannot take this as a valid argument.
	The guideline is based on the assumption that generally the absence of a hazard cannot be demonstrated by long-term experience, thus every single plant is suspicious until proven non-genotoxic with adequate experimental data. However, the requirement for genotoxicity testing should not be based on hypothetical considerations, but be restricted to cases where there is a reason for concern, either through observations related to the use of the preparation, or, in some cases, through data related to relevant constituents of the herbal substance, herbal preparation or combinations therefrom.	Not agreed. Genotoxicity is a special sphere of toxicity, which requires a different approach.
	Even in cases where the decision is taken to undergo genotoxicity testing, the described sequence may not be adequate. As mentioned in the guideline, phytochemicals such as quercetin, but also many flavonoids give false-positive results in the AMES test. In cases where the necessity to proceed to step 2 can already be expected from the content of flavonoids, the mandatory testing in step 1 may be considered useless. Regulatory options should be introduced for such cases, allowing to start testing at a later step of the testing sequence.	Not agreed. The Ames test is not useless even in the case of flavonoids and other Ames-positive, but MLA-negative substances, because it is a sensitive and quite comprehensive indicator of reactive metabolite formation.
	It is suggested that results obtained with a combination preparation should be acceptable without the need of testing the individual combination partners. It is also suggested that in cases where there are already existing results for the individual components of a preparation, no further testing should be required for the combination.	This is a valid question, but the HMPC is of the opinion that currently there is not much information and research on this subject to provide a scientifically justifiable recommendation.

SPECIFIC COMMENTS ON TEXT		
Paragraph no.	Comment and Rationale	Outcome
	<p><i>Executive Summary (Lines 36-47):</i> <i>In cases where a safety concern is recognized or suspected, non-clinical investigations may be needed. The complete lack of some specific non-clinical studies (e.g. genotoxicity studies) may also present a safety concern because important questions related to product safety would remain unanswered.</i></p> <p>For traditional herbal medicinal products safety, i.e. acute or chronic toxicity, is derived from the long-standing use as defined by the corresponding directive. According to this draft guideline, the safety derived from long-standing use does not involve genotoxicity. Although it is correct that pharmacovigilance procedures will mostly not be able to discover genotoxic risks related to the use of traditional herbal medicinal products, experience still shows that in cases where this risk was in fact present, corresponding observations in the population have often been indicative. As a consequence, the above mentioned suspicion is exclusively based on the hypothesis that the absence of genotoxicity cannot be derived from traditional experience. This would place all herbs under general suspicion.</p>	<p>Not agreed. Genotoxicity is a special sphere of toxicity, which requires a different approach. The HMPC would be interested in learning about cases where genotoxicity risk had been 'indicated' by 'observations in the population'.</p>

	<p><i>1. Introduction</i> <i>HMPs are made of natural substances that may be part of regular, environmental exposure, i.e., the contribution of the substance to the overall exposure needs to be considered (Lines 52-54).</i></p> <p>It would appear that making reference to food in the paragraph “Exposure considerations, Lines 288-294) would also make sense in this place. The reference to environmental exposure alone might appear too close to environmental toxicity, whereas in most cases the exposure would rather compete with dietary sources. E.g., most traditional herbs used in Ayurveda have a dual use as food and medicinal product. In this context we also refer to the paragraph “Legal Basis”, where it is clearly stated lines 103-108 that „<i>the contribution of the HMPs to the general exposure may not be relevant.</i>“ Experience shows that this is in fact true for many traditional herbal medicinal products. In such cases the necessity for additional genotoxicity testing can not be expected to contribute to the safety of the consumer.</p>	<p>Agreed. 'Dietary and..' added.</p>
	<p><i>The complete composition is very difficult to unravel, so it may be argued that there are always many unknown constituents and thus there may be “hidden” dangers (Lines 59-60).</i></p> <p>We find the notion of “hidden dangers” rather unfortunate. Genotoxicity either exists for a given preparation, or it does not exist. A lack of knowledge regarding the phytochemical composition of an herb or an herbal preparation is not decisive for the question whether toxicity exists or not. As the total phytochemical composition of an herb can never be fully known, and newly developed analytical techniques will always uncover hitherto unknown components, lack of knowledge occurs even with well-known herbs. This partial knowledge would not change the fact that the herb is toxic or non-toxic. Lack of knowledge of the phytochemical composition can therefore not be used as an argument for the necessity to perform genotoxicity studies.</p>	<p>Not agreed. Matrix effects, chemical interactions, transformations upon absorption etc are factors contributing to the problems of risk assessment in complex mixtures, resulting in potential "hidden dangers" (not only for genotoxicity).</p>

	<p><i>Because HMPs <u>shown to be genotoxic</u> [emphasis added] are natural substances to which people may also be exposed via food and other environmental sources, several pertinent questions have to be presented. What is the individual burden to an individual, on top of natural exposure, by using HMPs? Is there a level of exposure that can be regarded as acceptable? (Lines 70-75)</i></p> <p>This paragraph explicitly refers to HMPs <u>shown</u> to be genotoxic. It is undoubtable that herbs or herbal preparations shown to be genotoxic should be examined more closely, and this guideline gives a suitable approach to the topic. However, this paragraph does not refer to the real subject of the guideline, i.e. the genotoxicity assessment of herbs which have as yet <u>not been shown to be genotoxic</u>.</p>	<p>Naturally the same reasoning, concerning exposures via food and other environmental sources, applies in both cases.</p>
	<p><i>2. Scope</i> <i>... recent experience in the hazard and risk assessment of some specific preparations such as genotoxicity risks associated with furocoumarins in Angelica archangelica L. [...] have been taken into account. (Lines 80-83)</i></p> <p>The example of <i>Angelica</i> which is used to underline the necessity of genotoxicity testing is in this place unsuitable. The discussion on <i>Angelica</i> preparations was merely a hypothetical issue as exemplified in the psoralen-UV_A-irradiation therapy. In fact, the furocoumarins of <i>Angelica</i> are not expected to make a relevant contribution to the overall exposure to furocoumarins from food, which can reach levels of 14 mg/day without any known toxicological consequence. The example should therefore be deleted.</p>	<p>Not agreed. The <i>Angelica</i> case was very illustrative in that it demonstrated many and variable difficulties in risk assessment of herbal constituents, which are also present (abundantly) in food and in ordinary pharmaceuticals.</p>

	<p><i>3. Legal basis</i> <i>It is also important to stress that pharmacovigilance is incapable of detecting genotoxicity and pharmacovigilance observations or documented long-standing use cannot be used as evidence for absence of genotoxic risks. (Lines 110-112)</i></p> <p>It is undoubted that pharmacovigilance will not be able to detect toxicological risks from herbal preparations predominantly used as food or in traditional medicine. However, this would not allow drawing the opposite conclusion that these herbs are in fact potentially dangerous only because no danger is known from traditional use.</p>	<p>Whether the HMPC draws this opposite conclusion, is conjectural, but much of the current thinking in drug safety field is based on the premise that drugs are dangerous unless otherwise demonstrated.</p>
	<p><i>4.1 Testing strategy – Scenario 1</i> <i>A negative [AMES-] test fulfils the genotoxicity testing requirements for including a herbal substance or preparation in the Community list of herbal substances, preparations, and combinations thereof in traditional herbal medicinal products. (Lines 143-145)</i></p> <p>The Community entries of traditional herbal medicinal products are highly welcome, as they will help to avoid multiple testing of the same preparation. Still, this paragraph points to open questions which might need clarification.</p> <p>We suggest that the testing of single herbs should be sufficient to show the safety of a combination. Vice versa, we suggest that in the case of combination products the results obtained with the combination should be sufficient to demonstrate safety, and that there should be no further requirement for the testing of the individual combination partners. The likelihood that a combination product is genotoxic when its single constituents are not seems small. Vice versa, the testing of the individual combination partners would not add to the evidence of safety of the combination, when the combination itself has been shown to be safe.</p>	<p>Not agreed (as such). The comment deals with the extrapolation of a test result from a single herb to combinations and vice versa. The comment neglects potential matrix and interaction effects. The guideline points to a need to gather further experiences before the extent of extrapolation can be defined.</p>

<p><i>Step 1a</i> <i>If a well-known genotoxicant is identified and quantified in the preparation and if an internationally acknowledged risk assessment on this well-known genotoxicant (e.g. quercetin) is available, it may be used as a basis of the genotoxicity risk assessment of the HMPs. In this case the most important factor is to determine the potential exposure scenario in the light of the assessed toxicity risk to humans. The concentration of the identified genotoxicant in the preparation should be measured as a pre-condition for risk-assessment, as outlined in step 4. (Lines 166-171)</i></p> <p>Quercetin does not appear to be a good example. It is a genotoxicant only <i>in vitro</i>, but not <i>in vivo</i>. The text can be interpreted in the sense that quercetin is internationally acknowledged as a genotoxicant, which is not the case.</p> <p>The crucial point in the description of step 1a is obviously the reference to step 4 in the text and in the flow chart. Step 4, which will be discussed below, refers to the risk assessment methods such as the TTC concept. As it is phrased now, the only possible interpretation is that in case of a positive AMES-test where quercetin as a known <i>in vitro</i>-genotoxicant (but not <i>in vivo</i>) has been detected, the TTC-concept has automatically to be applied. However, limiting quercetin to 1.5 µg/day would strongly affect the composition and efficacy of herbal medicinal products, especially as quercetin genotoxicity is known not to be relevant <i>in vivo</i>.</p> <p>We suggest a clarification:</p> <p>In cases where there are negative results of step 2 and/or step 3 testings, the risk assessment according to step 4 should not be necessary.</p> <p>In cases where the only recognized cause of step 1-genotoxicity is a flavonoid such as quercetin, the process should not lead to a risk assessment procedure according to step 4.</p>	<p>This is exactly the point and such a genotoxicant may be a justified reason not to advance the testing scheme.</p> <p>As is evident from the guideline, the application of TTC is optional and even its specific application can be modified if adequately justified. As pointed out in the guideline, no single risk assessment approach can be suggested. The applicant has a lot of latitude to reason and justify the approach adopted.</p> <p>Not agreed. Explicit and transparent risk assessment is always necessary, as emphasised in several places in the guideline.</p>
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	<p><i>Step 2 (Lines 177-205)</i></p> <p>Typically, AMES tests with herbs containing flavonoids regularly lead to false-positive results in the AMES test. If an herb contains flavonoids and a positive result from the AMES-test must be expected, step 1 cannot be expected to give any useable or relevant result. Thus, it would be considered useless to go through step 1 first. It is suggested that in such cases the testing may be started at a later point of the testing sequence.</p>	<p>Not agreed. The Ames test is a basic genotoxicity test, with a long experience and a huge data base, so the Ames test should be the starting point for the evaluation of genotoxicity testing of herbal preparations. There is also a certain difference in targets and outcomes between the Ames test and the mouse lymphoma assay.</p>
	<p><i>Step 4: Risk assessment by the Threshold of Toxicological Concern (TTC)</i></p> <p><i>... Although genotoxic constituents in herbal preparations are not impurities... (Line 250-251)</i></p> <p>We consider this a very important statement. The TTC concept was originally developed for unavoidable impurities emerging from the synthesis of an active chemical substance, and thus for compounds with – by definition – no benefit. In contrast, the isolated constituents from herbs which are assumed to be representative for the total extract belong to the matrix which defines the clinical efficacy.</p> <p>In addition, the choice of the potentially hazardous constituent should reflect considerations regarding the composition of the fraction containing the genotoxicant in question. Typically, the various phytochemical constituents of a given class (e.g., furocoumarins) are not all genotoxic. The quantification of the total fraction, calculated as the supposed genotoxicant, will automatically lead to an overly high risk expectation not reflecting the true risk, especially when the genotoxicant in question is only a minor constituent of the fraction and/or of the herbal matrix.</p> <p>We suggest that in such cases the calculation should be made for the identified genotoxicant, but not for the fraction of parented compounds as a total.</p>	<p>The applicant has a possibility to make a justified risk assessment, which takes all the mentioned points into consideration.</p>

In the absence of data needed for the application of one of the established risk assessment methods, implementation of a generally applicable approach as defined by the TTC concept is proposed. (Lines 252-254)

We do not think the use of the TTC concept is a suitable way to assess the safety of HMPs. The TTC concept is a risk-management method, but cannot be considered a risk assessment method. Irrespective of the nature of the compound it limits the presence of a potential (and unavoidable) genotoxicant to 1.5 µg in the daily dose, which is considered irrelevant for human health. Thus, the TTC concept does not reflect a substance-specific individual threshold.

Again, it must be emphasized that if the TTC were to be applied despite of the fact that this method has to be considered unsuitable for herbal preparations, the calculation should only be made for the genotoxicant, but not for the total fraction of parented constituents, especially if these constituents have not been shown to have relevant genotoxic effects. If the calculation is made with the total fraction, the TTC concept leads to completely random results.

We suggest that for the application of the TTC concept the observation of genotoxicity *in vivo* should be a precondition. As of now, even a positive AMES-test with the presence of quercetin as an *in vitro*-genotoxicant would lead to the application of the TTC concept, and thus to potentially unfounded limitations.

Overall, the risk assessment part of the guideline contains more suggestions and options than absolute requirements. Because the HMPC has not had thus far too much experience in risk assessment of genotoxicity of herbal medicinal products, a lot of latitude in argumentation and justification has been allowed to the applicant.

	<p><i>From this threshold value, a permitted level in the active substance can be calculated based on the expected daily dose. Higher limits may be justified under certain conditions such as short-term exposure periods. The same approach might be considered for genotoxic constituents in herbal substances/preparations, if sufficiently justified by the applicant. Also, higher limits may be applied when the applicant submits additional data and a toxicologically plausible argumentation for the required justification. (Lines 256-261)</i></p> <p>We suggest a clarification in which cases this rule would be applicable. A reference to this point is made in step 1, but then further testing with negative results should clearly overrule the requirement for the assessment by the TTC approach. In cases where a negative step 2 or step 3 results exists, the TTC approach, which is entirely theoretical, should not apply.</p>	<p>Not agreed. The TTC approach is partially theoretical, but it nevertheless links potential hazard to the actual concentrations and doses of components in herbal medicinal products. This is also a very important aspect of safety assessment, which has not thus far been very prominent in herbal medicinal product field.</p>
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4.2 Specific considerations

Problems with complex mixtures (Lines 300-309)

The guideline gives a strategy to exclude genotoxicity of herbal preparations based on a step-wise approach. Basically, there is no difference between single herb preparations and combinations: even in the case of a single herb preparation the active is a complex matrix and thus a “combination” by itself. Negative results from genotoxicity testing are therefore obviously accepted as the demonstration of a lack of a risk from a complex mixture. Herbal combinations should not be treated differently: if the combination as such does not show a genotoxic effect, there should be no need to further break down the preparations to their single herbs. As the patient will use the herbal combination and not the single herb (or even isolated constituents), it is the (negative) test results of the former that should count, not a hypothetical consideration.

The problem of extrapolation of test results from one single herb to combinations (and vice versa) as well as from one type of the same preparation to another will require more research and experience.

Extrapolation from combination to a single herb may be allowable if adequately justified by the applicant.

GENERAL COMMENTS		
Paragraph no.	Comment and Rationale	Outcome
	<p>This guideline puts all herbs under suspicion of having a possible genotoxic effect unless proven otherwise, as it specifically denies that long-term experience is relevant in assessing genotoxic risk. Therefore it requires a test procedure for possible genotoxicity for all herbs.</p> <p>This approach is not reasonable as it is well know that genotoxic effects are exceedingly rare among herbs, especially among herbs which are otherwise unproblematic toxicologically.</p>	Not agreed. These are just very general statements, which may not even be true.
	<p>Requiring genotoxicity tests on all herbs or herbal preparations puts a great pressure on companies attempting to register preparations under the Traditional Herbal Medical Products guideline (2004/24/EC). It is well know that the only relatively simple test for genotoxicological effects, the AMES test, very often produces false positive results. This would make other in-vitro or in vivo tests necessary, which are extremely cost intensive. (In addition, preclinical genotoxicological results are often irrelevant clinically). Considering that the companies interested in THMD registrations are mostly small to medium-size corporations with limited means, the requirement for genotoxicity testing could make THMD registrations difficult or even impossible for many products, without bringing significant benefits in terms of drug safety.</p>	Safety testing is conducted for the benefit of the consumer, not to the producer. All tests have their strengths and weaknesses, but their results are regarded beneficial for the consumer safety.

GENERAL COMMENTS		
Paragraph no.	Comment and Rationale	Outcome
	We understand that there are hardly any or no proven cases of <i>in vivo</i> genotoxicity from herbal substances or preparations derived from whole herbs or standard aqueous or alcoholic extracts. Certainly none is presented in this consultation. Without any such data these detailed proposals seem to be premature.	Not agreed. Genotoxicity is a special sphere of toxicity, which requires a different approach.
	The proposals seem to be based on circumstantial evidence. It seems unlikely that any European regulator of herbal medicines would accept the argument that because a single chemical entity has certain therapeutic effects therefore a whole herbal substance with the same concentration of the chemical entity must have the same effect. Likewise it seems to be very premature to suppose that because a single chemical entity has an <i>in vitro</i> genotoxic effect on microorganisms or even rats that a whole herbal substance with the single chemical entity at the same concentration will have an <i>in vivo</i> genotoxic in humans.	Not agreed. There may be circumstances in which genotoxic effect of a single chemical is not expressed in complex matrices or that an effect detectable <i>in vitro</i> does not show up <i>in vivo</i> . However, the answer has to come from scientific studies and tests.
	It is essential to firstly show that one or more whole herbal substance has a significant probability of a genotoxic effect in humans or mammals at realistic levels of consumption. Two or three such cases would probably provide useful data on which a practical assessment of risk and its management can be based. Until then these proposals seems like the tail is attempting to wag the dog.	Not agreed. Genotoxicity is a special sphere of toxicity, which requires a different approach.
	If any EU Member State had the responsibility for both assessing the risk of genotoxicity from herbal substances and mitigating the risk there is little doubt that they would not start spending their tax revenues on testing large numbers of herbal substances in common use unless there was clear evidence of real risk. This brings into question the appropriateness of the authorities requiring the industry/public to do the testing at this stage of our knowledge.	Not agreed. Genotoxicity risk is not the same as, e.g. hepatotoxicity risk and it requires a different approach.

	It would have been very helpful if more information had been supplied about what is actually known about this matter as background to this consultation paper. Without it we have to conclude that more knowledge is needed before any real risk can be established and a practical regime for managing the risk can be evaluated.	Agreed; we need further information and research on better defining risks associated with genotoxicity. However, even before that we need to protect consumers from exposures to potentially genotoxic herbs and this has to be executed through rational risk assessment according to the best science we have currently available.
Line 59 and 300-309	In the context of the Traditional Herbal Medicines Directive the whole herb or whole preparation is considered to be the active substance. Any practical test of herbal preparations for genotoxicity would have to be able to evaluate the whole preparation however many herbs it contains.	The guideline has been developed to deal with also such eventualities.
Line 246-261	Safety levels of chemical impurities in pharmacological manufacturing seem to be of very little relevance to individual components of complex herbal substances to which the human race has been exposed at various levels for a very long time.	Not agreed. TTC is a generic tool to be used with various kinds of chemical substances at low concentrations. Although it was originally developed for carcinogenic impurities, it is currently applied, at least tentatively, to variable mixtures and preparations.