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DRAFT

GUIDELINE ON THE ASSESSMENT OF GENOTOXIC CONSTITUENTS IN HERBAL SUBSTANCES/PREPARATIONS

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GUIDELINE ON THE ASSESSMENT OF GENOTOXIC CONSTITUENTS IN HERBAL SUBSTANCES/PREPARATIONS

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EXECUTIVE SUMMARY

For many herbal substances/preparations, contained in well-established or traditional herbal medicinal products (HMPs), an adequate safety profile may be confirmed by their documented history of medicinal use. However, in cases where a safety concern is recognised 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 relating to product safety would remain unanswered.

This guideline describes a general framework and practical approaches on how to assess or to test the potential genotoxicity of herbal substances/preparations and how to interpret the results.

The stepwise approach described below represents a pragmatic approach to address both scientific aspects of genotoxicity testing and the special needs of HMPs within the current regulatory framework applicable to these products.

1. INTRODUCTION

Herbal medicinal products (HMPs) present a number of characteristics that clearly differentiate them from other medicinal products. Examples of important differences may include:

- 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.
- HMPs contain as active substance(s) complex mixtures with a large number of constituents that are present in sometimes highly variable amounts.
- The composition of a defined preparation may vary as a function of harvesting time, geographical origin, mode of preparation etc.
- 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.

In many other respects, HMPs are similar to other medicinal products for human use that contain synthetic active substances:

- The same basic legislation determines their legal position (1).
- Many HMPs have been used for long time by a sizable portion of the population.
- Clinical experience, despite its shortcomings, may point to their relative safety, at least with respect to the most apparent adverse reactions, but as with other medicinal products, signals of adverse effects arise only occasionally.

Because HMPs shown to be genotoxic are natural substances to which people may be exposed also via food and other environmental sources, several pertinent questions have to be presented. What is the burden to an individual, on top of natural exposure, by using HMPs? Is there a level of exposure that can be regarded as acceptable? Are there scientifically valid procedures for determining this acceptable exposure? Are there circumstances in which the current methodology for genotoxicity testing is not appropriate for herbal substances/preparations?

2. SCOPE

This guideline describes a general framework and practical approaches on how to test the potential genotoxicity of herbal substances/preparations and how to interpret the results. In the development of this guideline, recent experiences in the hazard and risk assessment of some specific preparations such as genotoxicity risks associated with furocoumarins in *Angelica archangelica* L. containing preparations (2) or herbal preparations containing asarone, methyleugenol and safrole (3, 4, 5) have been taken into account.

3. LEGAL BASIS

Guidelines for genotoxicity testing of pharmaceuticals have been established by OECD, ICH and EMEA committees. Testing of medicinal products involves a battery of genotoxicity tests, in which pro- and eukaryotic systems in *in vitro* and *in vivo* experimental setups with and without metabolic

activation are employed (6, 7, 8, 9, 10). A specific CHMP/SWP guidance (11) addresses the situation of well-established ("old") substances where complete data may not be available in all cases. In the HMPC 'Guideline on non-clinical documentation for herbal medicinal products in applications for marketing authorisation (bibliographical and mixed applications) and in applications for simplified registration' (12) a step-wise procedure for assessing genotoxicity of HMPs was established. 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. If positive results cannot be clearly attributed to specific constituents with a well-established safety-profile for example quercetin additional *in vitro*, e.g. mouse lymphoma cell assay, and, if necessary, *in vivo* studies were proposed.
For clarification, it is of importance to explain why the regular testing procedure for synthetic medicinal products needs to be adapted to the specific situation of such HMPs that have a well-established or traditional use. First of all, the stepwise approach presented in this guideline takes into account the fact that HMPs are mixtures of natural substances for which some background exposure through food and other environmental factors can be expected. In those cases the exposure to these constituents can *a priori* not be avoided or the contribution of the HMPs to the general exposure may be not relevant. Secondly, HMPs are indicated for the use in relatively minor health complains for short durations, i.e. the use is mostly sporadic and/or intermittent. Thus the exposure, *vis-a-vis* the natural background exposure to dietary constituents, probably remains in most cases relatively low.

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.

4. MAIN GUIDELINE TEXT

4.1 Testing strategy

The stepwise testing process described below is also presented in the form of a decision tree (Figure 1) which should be read in conjunction with the text.

It is recognised that a single test, i.e. the Ames test, in the first step cannot cover all genotoxic endpoints and thus a significant sphere of genotoxic potential, e.g. in relation to chromosomal damage, remain untested. However, on the other hand, *in vitro* bacterial reverse mutation test systems are likely to cover the majority of "critical" endpoints, i.e. DNA-reactive herbal substances. The stepwise approach described below represents a pragmatic approach to address both scientific aspects of genotoxicity testing and the special needs of HMPs within the current regulatory framework applicable for these products.

Step 1: The Ames test
In general, the Ames test should be performed and interpreted in conformity with existing OECD and EU guidelines (see section 'References'). Briefly, a set of different Salmonella typhimurium strains (e.g. TA1537, TA1535, TA98, TA100, TA 102 or E.coli WP2 uvrA) with various mutations present in a certain amino acid synthesising gene is incubated in the presence of the studied substance/preparation and metabolic activation system (usually rat liver S9 mix containing induced drug-metabolising enzymes). Chemical-induced mutations which restore the functional capability of the bacteria to synthesise an essential amino acid ('revertants') are counted. The purpose of this test is to reveal the mutagenic potential of a substance in a prokaryote organism and whether the reactive metabolite is a product of metabolic activation by mammalian enzymes.

Scenario 1: Negative test result
If the test were considered to have been performed according to the ICH guidelines (6, 7) and the result is unequivocally negative, no further genotoxicity testing is required on the basis of HMPC non-clinical guideline (12). A negative test result fulfils the genotoxicity testing requirements for

including a herbal substance or preparation in the Community list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products.

Scenario 2: Equivocal test result

Genotoxicity result, which is very weak or not consistent regarding the usual positive response in the test, deserves special considerations. The first option is to repeat the test to reveal whether the test outcome is the same as in the original experiment. In all cases, a proper assessment involves a survey of at least the following considerations: Is the response dose-dependent or does it exhibit unusual or irregular features with regard to concentration? Are there indications that the preparation affects the growth of test organisms, thus preventing the detection of genotoxic constituents? The final assessment should be conducted via a thorough and transparent consideration of the test outcome in the light of test material and test conditions.

Scenario 3: Positive test result

If the test outcome is judged clearly positive, the next step is dependent on whether some known genotoxic compounds are present or not in the herbal substance or preparation.

Need of proceeding to step 2 is dependent on the assessment of the result, taking all information about the substance or preparation into consideration.

Step 1a: A well-characterized and assessed genotoxic substance is identified to be responsible for genotoxic activity

If a well-known genotoxicant is identified and quantified in the preparation and if there 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.

Step 1b: Genotoxic response cannot be attributed to any specific constituents

If there is no knowledge about the active principle(s), the herbal substance or preparation has to be studied in a step 2 test.

Step 2: Mouse lymphoma assay or other mammalian cell assay

In general, the mouse lymphoma assay should be performed and interpreted in conformity with existing OECD and EU guidelines (see section 'References'). Briefly, L5178Y mouse lymphoma cells in culture are exposed to a compound or preparation under study and gene mutations in thymidine kinase gene are detected. A purpose is primarily to confirm or refute the positive finding in the Ames test, i.e. the ability of a substance to induce gene mutations ("large colonies") in a mammalian cell line. Additionally, mouse lymphoma assay might give information on the ability of a herbal substance or preparation to cause chromosomal damage ("small colonies").

If other mammalian cell assays such as the CHO, CHO-AS52 and V79 lines of Chinese hamster cells, or TK6 human lymphoblastoid cells are employed for genotoxicity tests, their use has to be justified.

If the test result is negative, no further testing is required. Still the positive test result in the Ames test has to be fully addressed in the assessment report.

If the test result is positive for chromosomal damage ("small colonies") the relevance of the finding should be thoroughly assessed as it is known that the mouse lymphoma assay can give biologically irrelevant findings, e.g. in relation to conditions of high cytotoxicity (13).

If the test result is unequivocally positive and considered relevant either in gene mutation or chromosomal damage, it is advisable to proceed to step 3.

In some special circumstances, e.g. when an herbal preparation is known to contain a compound or compounds, or their close analogues, with chromosomal damaging properties, it may be advisable to perform the *in vitro* micronucleus test in mammalian cells in culture [see the OECD (draft) guideline (14)].

If the test result is unequivocally positive, it is advisable to proceed to step 3.

Step 3: Mouse micronucleus test or other *in vivo* genotoxicity tests

In general, the mouse micronucleus test should be performed and interpreted in conformity with the existing OECD and EU guidelines (see section 'References'). Briefly, mice are treated with a compound or preparation under study in an appropriate vehicle and via appropriate route of administration, and micronuclei in bone marrow or peripheral blood cells are counted. The purpose of the micronucleus assay is to identify agents that cause structural and numerical chromosome changes in *in vivo* condition, i.e. a living mammal.

If other mammalian *in vivo* tests are employed for genotoxicity tests, their use and comparability has to be justified.

If the test result is negative, no further testing is required. Still the positive test results of Step 1 and 2 tests have to be fully addressed in the expert report supporting the marketing authorisation/registration application.

Step 4: Risk assessment considerations

Toxicological background

Current regulatory practice concerning pharmaceuticals assumes that genotoxic compounds have the potential to damage DNA at any level of exposure and thus there is no discernible threshold and any level of exposure carries a risk. However, it has been increasingly recognised that there may be practical thresholds and that linear extrapolation from high *in vitro* or animal concentrations to low human exposures is scientifically questionable. It is equally difficult to experimentally prove both the existence of threshold for the genotoxicity and the linearity of genotoxic response at extremely low exposures. For these reasons, it may be prudent to adopt approaches, which involve a concept of a level of exposure that carries an acceptable risk.

As already stated above, pharmacovigilance and long-standing use cannot be used as evidence for absence of genotoxic risks

It is not possible to recommend a single specific approach to perform risk assessment. The standard uncertainty (safety) factor approach, which is a common practice in toxicology, is probably unsuitable for genotoxicity (and carcinogenicity) in the majority of cases. The margin of exposure approach for the risk assessment of genotoxic and carcinogenic compounds (comparison on the animal experimental dose-response curve divided by the estimated intake by humans), which is recommended by the EFSA Scientific Committee on Food (15), is probably not applicable for HMPs, because this approach is based on available carcinogenicity data, which is usually lacking in case of HMPs. If such data are available, the EFSA Committee is of the opinion that a compound with a calculated margin of exposure of 10,000 or higher would be of low health risk.

Risk assessment by the Threshold of Toxicological Concern (TTC)

Risk assessment schemes have originally been developed for identified single chemicals or well-characterized mixtures of chemicals. If an herbal preparation contains an identifiable genotoxic compound, the TTC approach could be applied. Recently, the CHMP has published a guideline on genotoxic impurities in pharmaceutical preparations (16). Although genotoxic constituents in herbal preparations are not impurities, this guideline offers an example of an approach which may be useful for the assessment of herbal preparations. In the absence of data usually needed for the application of one of the established risk assessment methods, implementation of a generally applicable approach as defined by the TTC is proposed (17, 18). A TTC value of 1.5 µg/day intake of a genotoxic impurity is considered to be associated with an acceptable risk (excess cancer risk of <1 in 100,000 over a lifetime) for most pharmaceuticals. 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.

Genotoxic substances with threshold

If a genotoxic substance is a compound with a demonstrated threshold mechanism, permissible exposure levels without appreciable risk of genotoxicity can be established according to the usual procedure employing the No Observable Effects Level (NOEL) from the most relevant (animal) study applying uncertainty factors, if available. Examples of mechanisms of genotoxicity that may be demonstrated to lead to non-linear or threshold dose-response relationships include interaction with the spindle apparatus of cell division leading to aneuploidy, topoisomerase inhibition, inhibition of DNA synthesis, overloading of defence mechanisms, metabolic overload and physiological perturbations (e.g. induction of erythropoesis, hyper- or hypothermia).

The identification and quantification of the genotoxic constituent

Herbal preparations being complex mixtures with partially unidentified components, it is quite possible that the compound(s) responsible for genotoxicity is(are) still not identified at the end of the testing protocol. There are no established ways to perform risk assessment of genotoxicity due to unidentified substances in herbal preparations. The usual procedure for toxicity testing and risk assessment of mixtures consists in isolation and identification of various principal constituents and testing of the isolated compounds individually. This is a recommended option for clearly genotoxic HMPs, because this approach would provide relevant and reliable information for risk assessment. However, because isolation and identification may require long times and extended efforts, the initial risk assessment should be performed on the basis of the above testing strategy. On the basis of these results and a careful consideration of benefits and risks a marketing authorisation with the obligation to complete some additional tests may be considered. A risk from administration of an HMP might be accepted if its contribution to the overall exposure through food is considered to be small (see also paragraph below 'Exposure considerations').

Exposure considerations

Because many herbal substances and preparations are derived from plants which are also used as food, it is apparent that exposure to various herbal constituents can also occur via diet. It is clear that amounts and ratios of these constituents vary enormously, depending on individual and population dietary preferences. For a proper risk assessment, dietary exposures should be assessed and quantified, as far as possible, and comparative assessment of exposures via diet and herbal substances and preparations consumption should be performed. In many cases it may be advisable to contact dietary health risk assessing bodies for information and/or discussion of risk assessment considerations.

4.2 Specific considerations related to herbal medicinal products

Problems with complex mixtures

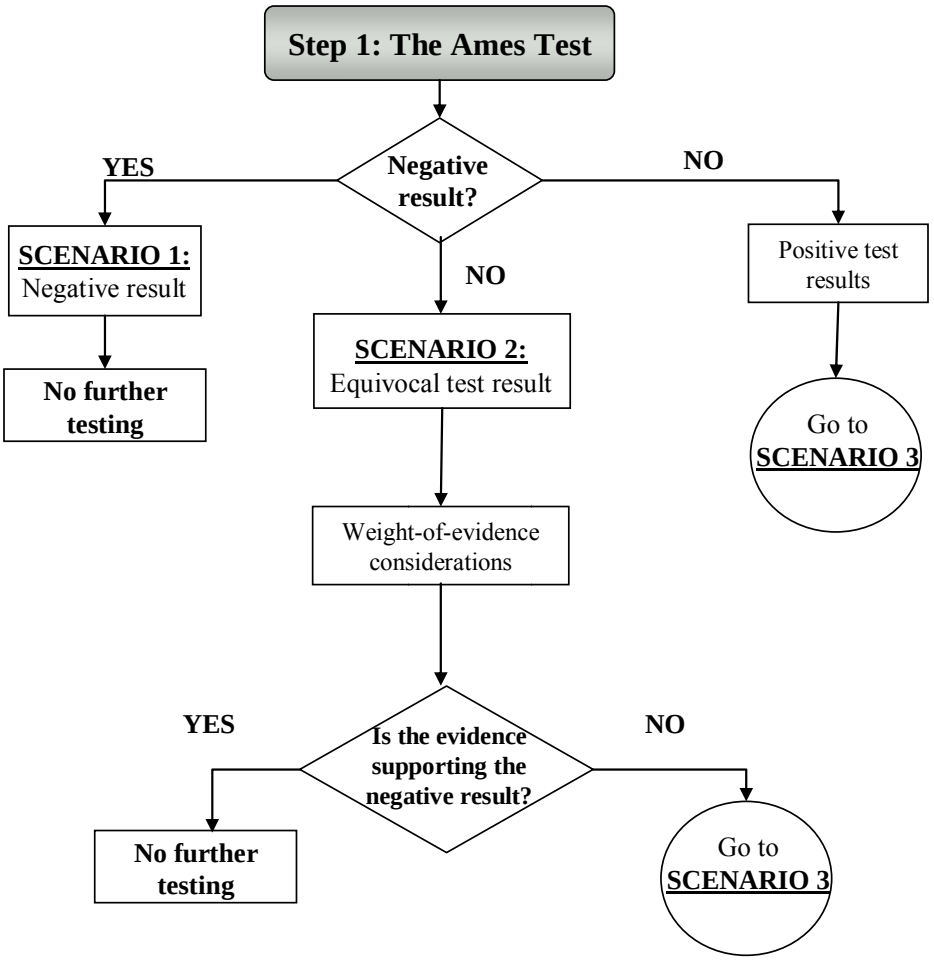
In the interpretation of the test, the fact that HMPs are complex mixtures may pose technical difficulties for their reliable genotoxicity assessment. An analogous precedent in some respects is industrial and environmental mixtures and pollutants, which are challenging to test in *in vitro* and *in vivo* systems. However, experience with these complex mixtures may aid in devising approaches to test HMPs. For example, complex mixtures may contain compounds, which affect, enhance or inhibit the growth of bacteria. They may contain radical scavengers, which trap reactive intermediates produced by the S9 mix enzymes. It is difficult to give unequivocal rules for genotoxicity testing of complex mixtures. Rather, the test interpreter has to present reasonable and transparent argumentation, which led to the proposed test result interpretation.

Interpretation of the test result for related preparations

Herbal preparations display some variability between batches due to their complex nature and a question arises whether additional testing might be needed. If variability between batches is within accepted quality specifications, there is no need to perform additional tests unless there is cause for concern with respect to genotoxicity.

Another consideration needs to address preparations, which contain basically the same herbal substance, but have been prepared by another extraction technique or using a different extraction solvent. For those situations it advised to adopt a case-by-case approach, in which a thorough and transparent assessment is made taking into consideration all the different factors, which might affect the test result. Such an extrapolation beyond closely related preparations such as extracts prepared with ethanol/water mixtures of different concentration, might become possible when more studies on different preparations of the same herbal substance have been submitted and assessed.

Figure 1. A decision tree on the assessment of genotoxicity of herbal preparations.



328 **Figure 1. A decision tree on the assessment of genotoxicity of herbal preparations. (cont.)**
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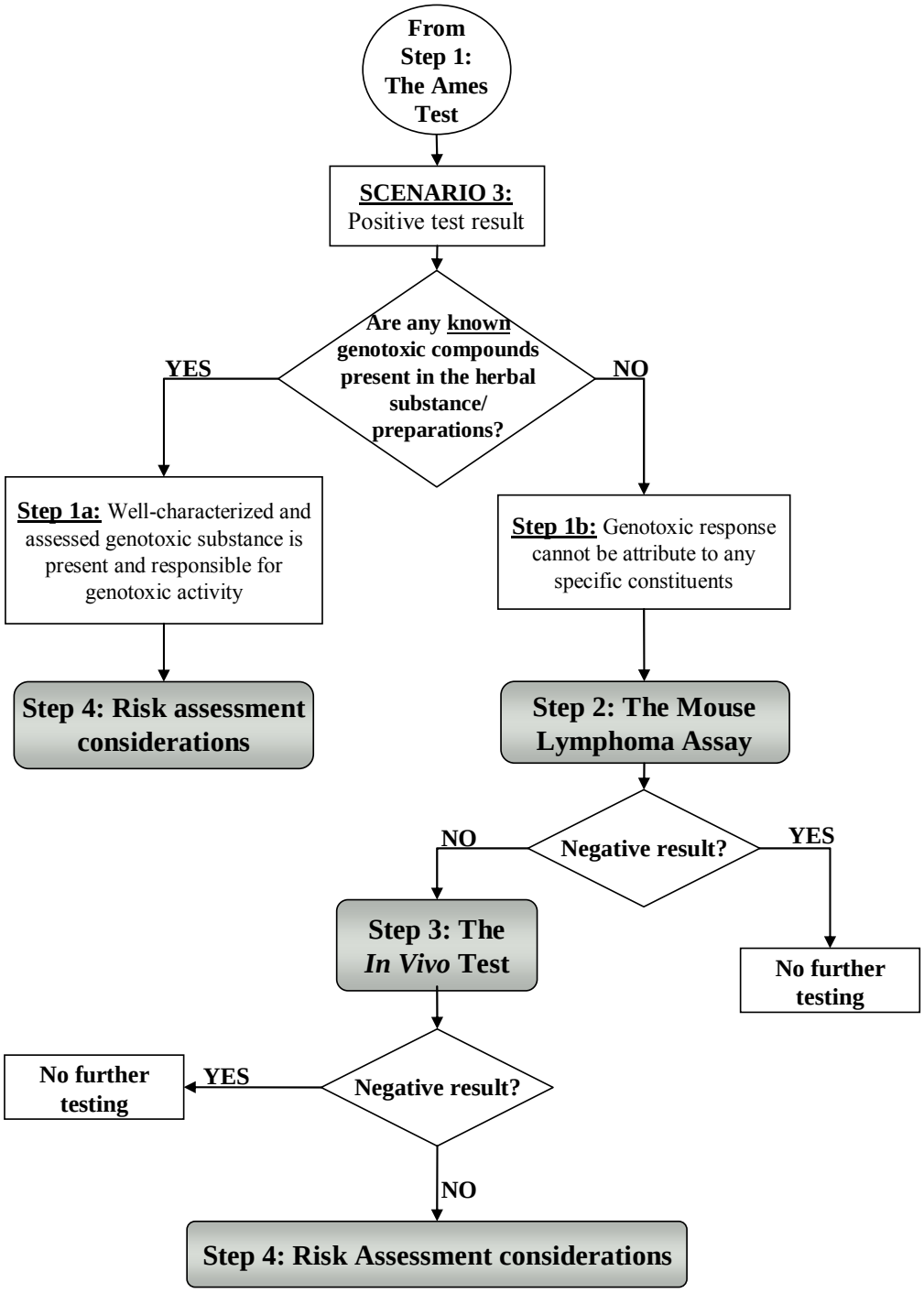

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5. DEFINITIONS

For definitions reference is made to the relevant guidelines on pre-clinical and clinical safety (see below).

6. REFERENCES (SCIENTIFIC AND / OR LEGAL)

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