UNIVERSITY OF NAPLES "FEDERICO II"
DEPARTMENT OF PHARMACY

PhD COURSE IN
"PHARMACEUTICAL SCIENCE"

XXX CYCLE

THE SAFETY ASSESSMENT OF HERBALS WITH A NEW AND ETHICAL APPROACH

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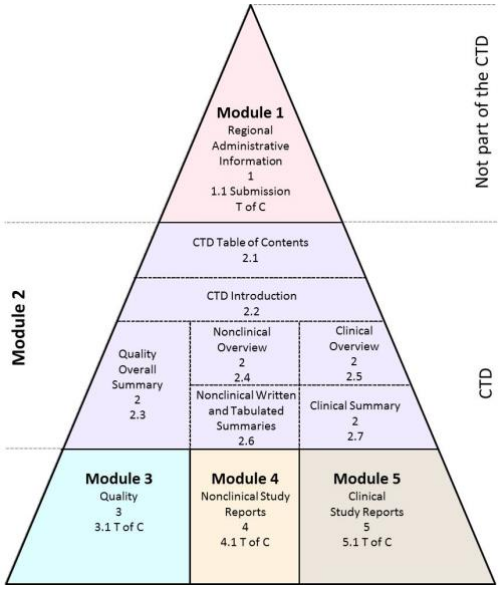
INTRODUCTION

The increasing use of plant medicines in Europe needs a shared methodology to determine the toxicity and daily exposure level to these drugs. For this reason, the European regulatory Agencies have undertaken a study that could meet popular uses and toxicological research in different countries of the Union. Here we list some examples of the most used herbal drug classes where we propose a decision-making process based on the plant's characteristics, their content in active principles and on the basis of the present scientific pharmacological and toxicological literature. The proposed decision tree actually makes easier for the assessor to quickly and accurately evaluate the accredited indexes for risk and toxicity assessment based on the preclinical literature data and using the correct classification that some of them may have because they are already present in medicinal products or used as food. Here is reported a systematic approach to assessing the toxicity of plant drugs based on their phytochemical composition and the conscious use of the parameters (e.g. NOEL, NOAEL, TTC etc.), which must be applied appropriately to the protection of both the manufacturer and the final consumer.

Before discussing the systematic approach, it is critical to have e view of the regulatory terms and guidelines which must be respected in order to obtain a marketing authorization and the different legal basis that a product of botanical origin can make use of the "full dossier" application request to the applicant to provide a multitude of documents. To make this application easier all those documents follow the Common Technical document grid (CTD) which were agreed in November 2000 within the International Conference on Harmonization (ICH) and is summarized in the following image.



1. *Regulatory*

The Common Technical Document is organized into five modules. The content of Module 1 is defined by the European Commission in consultation with the competent authorities of the Member States, the European Agency for the Evaluation of Medicinal Products and interested parties. Concerning the structure of Modules 2, 3, 4, and 5 they are common for all ICH regions. Administrative, regional or national information is provided in Module 1. This module contains the specific EU-requirements for the administrative data (e.g. the application form, the proposed summary of product characteristics, labelling and package leaflet, etc.).

Module 2 contains high level summaries (the Quality Overall Summary, the Non-Clinical Overview /Summaries, and the Clinical Overview/Summaries), which must be prepared by suitably qualified and experienced persons (experts). Although the term "Expert Report" must be maintained for legal reasons, the content is expected to be given in the Quality Overall Summary, the Non-Clinical Overview/Summaries, and the Clinical Overview/Summaries documents. Old Expert Reports are now replaced by Module 2. The experts have to sign and add brief information on their educational background and specific expertise in a special section in Module 1.4.

Chemical, Pharmaceutical and Biological documentation is provided by Module 3. This information should be structured as described in Guideline M4Q (M4Q (R1): QUALITY Module 2: Quality Overall Summary (QOS) Module 3: Quality The section of the application covering chemical and pharmaceutical data including data for biological/ biotechnological products).

The documentation on the Toxicological and Pharmacological Tests performed on drug/active substance and a drug/medicinal product is provided in the Non-Clinical Written Summaries (from Module 2) and by the Non-Clinical Study Reports (Module 4). These reports should be presented in the order described in Guideline M4S (M4S (R2): SAFETY Nonclinical Summaries and Organization of Module 4 The non-clinical section of the application).

The documentation on the Clinical Trials performed on the drug/medicinal product is provided in the Clinical Written Summaries (from Module 2) and in the Clinical Study Reports (Module 5). These reports should be presented in the order described in Guideline M4E (M4E (R1): EFFICACY Module 2: Clinical Overview and Clinical Summary Module 5 Clinical Study Reports The clinical section of the Application).

Shifting the attention on the part concerning the safety, here we report how to analyze the appropriate section of the module 2 and 4 underlining the information that should be provided by the applicant.

Module 2.4 Nonclinical Overview

Nonclinical Overview

The Nonclinical Overview should provide an integrated overall analysis of the information in the Common Technical Document. In general, the Nonclinical Overview should not exceed about 30 pages.

General Aspects

The Nonclinical Overview should present an integrated and critical assessment of the pharmacologic, pharmacokinetic, and toxicologic evaluation of the pharmaceutical. Where relevant guidelines on the conduct of studies exist, these should be taken into consideration, and any deviation from these guidelines should be discussed and justified. The nonclinical testing strategy should be discussed and justified. There should be comment on the GLP status of the studies submitted. Any association between nonclinical findings and the quality characteristics of the human pharmaceutical, the results of clinical trials, or effects seen with related products should be indicated, as appropriate.

Except for biotechnology-derived products, an assessment of the impurities and degradants present in the drug substance and product should be included along with what is known of their potential pharmacologic and toxicologic effects. This assessment should form part of the justification for proposed impurity limits in the drug substance and product, and be appropriately cross-referenced to the quality documentation. The implications of any differences in the chirality, chemical form, and impurity profile between the compound used in the nonclinical studies and the product to be marketed should be discussed. For biotechnology-derived products, comparability of material used in nonclinical studies, clinical studies, and proposed for marketing should be assessed. If a drug product includes a novel excipient, an assessment of the information regarding its safety should be provided.

Relevant scientific literature and the properties of related products should be taken into account. If detailed references to published scientific literature are to be used in place of studies conducted by the applicant, this should be supported by an appropriate justification that reviews the design of the studies and any deviations from available guidelines. In addition, the availability of information on the quality of batches of drug substance used in these referenced studies should be discussed.

The Nonclinical Overview should contain appropriate reference citations to the Tabulated Summaries, in the following format: (Table X.X, Study/Report Number).

Content and Structural Format

The Nonclinical Overview should be presented in the following sequence:

Overview of the nonclinical testing strategy Pharmacology
Pharmacokinetics
Toxicology

Integrated overview and conclusions

List of literature references

Studies conducted to establish the pharmacodynamic effects, the mode of action, and potential side effects should be evaluated and consideration should be given to the significance of any issues that arise.

The assessment of the pharmacokinetic, toxicokinetic, and metabolism data should address the relevance of the analytical methods used, the pharmacokinetic models, and the derived parameters. It might be appropriate to cross-refer to more detailed consideration of certain issues within the pharmacology or toxicology studies (e.g. impact of the disease states, changes in physiology, anti-product antibodies, cross-species consideration of toxicokinetic data). Inconsistencies in the data should be discussed. Inter-species comparisons of metabolism and systemic exposure comparisons in animals and humans (AUC, C_{max}, and other appropriate parameters) should be discussed and the limitations and utility of the nonclinical studies for prediction of potential adverse effects in humans highlighted.

The onset, severity, and duration of the toxic effects, their dose-dependency and degree of reversibility (or irreversibility), and species- or gender-related differences should be evaluated and important features discussed, particularly with regard to:

- pharmacodynamics
- toxic signs
- causes of death
- pathologic findings
- genotoxic activity - the chemical structure of the compound, its mode of action, and its relationship to known genotoxic compounds
- carcinogenic potential in the context of the chemical structure of the compound, its relationship to known carcinogens, its genotoxic potential, and the exposure data
- the carcinogenic risk to humans - if epidemiologic data are available, they should be taken into account
- fertility, embryofetal development, pre-and post-natal toxicity
- studies in juvenile animals
- the consequences of use before and during pregnancy, during lactation, and during pediatric development
- local tolerance
- other toxicity studies/ studies to clarify special problems

The evaluation of toxicology studies should be arranged in a logical order so that all relevant data elucidating a certain effect / phenomenon are brought together. Extrapolation of the data from animals to humans should be considered in relation to:

- animal species used
- numbers of animals used
- routes of administration employed
- dosages used
- duration of treatment or of the study
- systemic exposures in the toxicology species at no observed adverse effect levels and at toxic doses, in relation to the exposures in humans at the maximum recommended human dose. Tables or figures summarising this information are recommended.

- the effect of the drug substance observed in nonclinical studies in relation to that expected or observed in humans

If alternatives to whole-animal experiments are employed, their scientific validity should be discussed.

The Integrated Overview and Conclusions should clearly define the characteristics of the human pharmaceutical as demonstrated by the nonclinical studies and arrive at logical, well-argued conclusions supporting the safety of the product for the intended clinical use. Taking the pharmacology, pharmacokinetics, and toxicology results into account, the implications of the nonclinical findings for the safe human use of the pharmaceutical should be discussed (i.e., as applicable to labeling).

2.5.5 Overview of Safety

The purpose of this section is to provide a concise critical analysis of the safety data, noting how results support and justify proposed prescribing information. A critical analysis of safety should consider:

- adverse effects characteristic of the pharmacological class. Approaches taken to monitor for similar effects should be described.
- special approaches to monitoring for particular adverse events (e.g., ophthalmic, QT interval prolongation).
- relevant animal toxicology and product quality information. Findings that affect or could affect the evaluation of safety in clinical use should be considered.
- the nature of the patient population and the extent of exposure, both for test drug and control treatments. Limitations of the safety database, e.g., related to inclusion/exclusion criteria and study subject demographics, should be considered, and the implications of such limitations with respect to predicting the safety of the product in the marketplace should be explicitly discussed.
- common and non-serious adverse events, with reference to the tabular presentations of events with the test drug and with control agents in the Clinical Summary. The discussion should be brief, focusing on events of relatively high frequency, those with an incidence higher than placebo, and those that are known to occur in active controls or other

members of the therapeutic class. Events that are substantially more or less common or problematic (considering the duration and degree of the observed events) with the test drug than with active controls are of particular interest.

- serious adverse events (relevant tabulations should be cross-referenced from the Clinical Summary). This section should discuss the absolute number and frequency of serious adverse events, including deaths, and other significant adverse events (e.g., events leading to discontinuation or dose modification), and should discuss the results obtained for test drug versus control treatments. Any conclusions regarding causal relationship (or lack of this) to the product should be provided. Laboratory findings reflecting actual or possible serious medical effects should be considered.
- similarities and differences in results among studies, and their effect upon the interpretation of the safety data.
- any differences in rates of adverse events in population subgroups, such as those defined by demographic factors, weight, concomitant illness, concomitant therapy, or polymorphic metabolism.
- relation of adverse events to dose, dose regimen, and treatment duration.
- long-term safety.
- methods to prevent, mitigate, or manage adverse events.
- reactions due to overdose; the potential for dependence, rebound phenomena and abuse, or lack of data on these issues.
- world-wide marketing experience. The following should be briefly discussed:

  - the extent of the world-wide experience,

  - any new or different safety issues identified,

  - any regulatory actions related to safety.

- support for the applicability to the new region of data generated in another region, where appropriate (guideline ICH E5).

2.6.6 Toxicology written summary

The sequence of the Toxicology Written Summary should be as follows:

- Brief Summary
- Single-Dose Toxicity
- Repeat-Dose Toxicity
- Genotoxicity
- Carcinogenicity
- Reproductive and Developmental Toxicity
- Studies in Juvenile Animals
- Local Tolerance
- Other Toxicity Studies
- Discussion and Conclusions
- Tables and Figures (either here or included in text)

2.6.6.1 Brief Summary

The principal findings from the toxicology studies should be briefly summarized in a few pages (generally not more than 6). In this section, the extent of the toxicologic evaluation can be indicated by the use of a table listing the principal toxicologic studies (results should not be presented in this table), for example:

Toxicology programme

The scope of the toxicologic evaluation should be described in relation to the proposed clinical use. A comment on the GLP status of the studies should be included.

2.6.6.2 Single-Dose Toxicity

The single-dose data should be very briefly summarised, in order by species, by route. In some instances, it may be helpful to provide the data in the form of a table.

Study type and duration	**Route of administration**	**Species**	**Compound administered***
Single-dose toxicity	po and iv	Rat and mouse	Parent drug
Single-dose toxicity	po and iv	Rat and mouse	Metabolite X
Repeat-dose toxicity			

1 month	po	Rat and dog	Parent drug
6 months	po	Rat	" "
9 months	po	dog	" "
etc.			

***** This column required only if metabolite(s) are investigated.

2.6.6.3 Repeat-Dose Toxicity (including supportive toxicokinetics evaluation)

Studies should be summarised in order by species, by route, and by duration, giving brief details of the methodology and highlighting important findings (e.g., nature and severity of target organ toxicity, dose (exposure)/response relationships, no observed adverse effect levels, etc.). Non-pivotal studies can be summarized in less detail (pivotal studies are the definitive GLP studies specified by ICH Guideline M3).

2.6.6.4 Genotoxicity

Studies should be briefly summarised in the following order:

- *in vitro* non-mammalian cell system
- *in vitro* mammalian cell system
- *in vivo* mammalian system (including supportive toxicokinetics evaluation)
- other systems

2.6.6.5 Carcinogenicity (including supportive toxicokinetics evaluations)

A brief rationale should explain why the studies were chosen and the basis for high-dose selection. Individual studies should be summarised in the following order:

- Long-term studies (in order by species; including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics)
- Short- or medium-term studies (including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics)
- Other studies

2.6.6.6 Reproductive and Developmental Toxicity (including range-finding studies and supportive toxicokinetics evaluations)

Studies should be summarised in the following order, giving brief details of the methodology and highlighting important findings:

- Fertility and early embryonic development
- Embryo-fetal development
- Prenatal and postnatal development, including maternal function
- Studies in which the offspring (juvenile animals) are dosed and/or further evaluated, if

such studies have been conducted.
If modified study designs are used, the sub-headings should be modified accordingly.

2.6.6.7 Local Tolerance

If local tolerance studies have been performed, they should be summarised in order by species, by route, and by duration, giving brief details of the methodology and highlighting important findings.

2.6.6.8 Other Toxicity Studies (if available)

If other studies have been performed, they should be summarised. When appropriate, the rationale for conducting the studies should be provided.

- Antigenicity
- Immunotoxicity
- Mechanistic studies (if not reported elsewhere)
- Dependence
- Studies on metabolites
- Studies on impurities
- Other studies

2.6.6.9 Discussion and Conclusions

This section should provide an opportunity to discuss the toxicologic evaluation and the significance of any issues that arise. Tables or figures summarizing this information are recommended.

Module 4 – Nonclinical study report

The appropriate location for individual-animal data is in the study report or as an appendix to the study report.

4.1 Table of contents of module 4

A Table of Contents should be provided that lists all of the nonclinical study reports and gives the location of each study report in the Common Technical Document.

4.2 Study reports

The study reports should be presented in the following order:

4.2.1 Pharmacology

1. 4.2.1.1 Primary Pharmacodynamics
2. 4.2.1.2 Secondary Pharmacodynamics
3. 4.2.1.3 Safety Pharmacology
4. 4.2.1.4 Pharmacodynamic Drug Interactions

4.2.2 Pharmacokinetics

5. 4.2.2.1 Analytical Methods and Validation Reports (if separate reports are available)
6. 4.2.2.2 Absorption
7. 4.2.2.3 Distribution
8. 4.2.2.4 Metabolism
9. 4.2.2.5 Excretion
10. 4.2.2.6 Pharmacokinetic Drug Interactions (nonclinical)
11. 4.2.2.7 Other Pharmacokinetic Studies

4.2.3 Toxicology

4.2.3.1 Single-Dose Toxicity (in order by species, by route)

4.2.3.2 Repeat-Dose Toxicity (in order by species, by route, by duration; including supportive toxicokinetics evaluations)

4.2.3.3 Genotoxicity

4.2.3.3.1 In vitro

4.2.3.3.2 In vivo (including supportive toxicokinetics evaluations)

4.2.3.4.1 Long-term studies (in order by species; including range- finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics)

4.2.3.4.2 Short- or medium-term studies (including range-finding studies that cannot appropriately be included under repeat- dose toxicity or pharmacokinetics)

4.2.3.4.3 Other studies

4.2.3.5 Reproductive and Developmental Toxicity (including range-finding studies and supportive toxicokinetics evaluations) (If modified study designs are used, the following sub-headings should be modified accordingly.)

4.2.3.5.1 Fertility and early embryonic development

4.2.3.5.2 Embryo-fetal development

4.2.3.5.3 Prenatal and postnatal development, including maternal function

4.2.3.5.4 Studies in which the offspring (juvenile animals) are dosed and/or further evaluated.

4.2.3.6 Local Tolerance

4.2.3.7 Other Toxicity Studies (if available)

4.2.3.7.1 Antigenicity

4.2.3.7.2 Immunotoxicity

4.2.3.7.3 Mechanistic studies (if not included elsewhere)

4.2.3.7.4 Dependence

4.2.3.7.5 Metabolites

4.2.3.7.6 Impurities

4.2.3.7.7 Other

4.3 Literature references

That safety information should be provided by the applicant for a “full dossier” application, however some product, (especially those made by plant) can take advantage of other kind of application provided by the European Directive 2001/83/EC of 6 November 2001. Particularly the art. 16a and the art. of the above-mentioned Directive ratify the possibility to submit a dossier under the “traditional use” or “well-established use”. Due the choice of the applicant concerning the legal basis chosen for the product formulation also the information required should be modified.

Article 16a

1. A “simplified registration” procedure (hereinafter ‘traditional-use registration’) is hereby established for herbal medicinal products which fulfil all of the following criteria:

(a) they have indications exclusively appropriate to traditional herbal medicinal products which, by virtue of their composition and purpose, are intended and designed for use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment;

(b) they are exclusively for administration in accordance with a specified strength and posology;

(c) they are an oral, external and/or inhalation preparation;

(d) the period of traditional use as laid down in Article 16c(1)(c) has elapsed;

(e) the data on the traditional use of the medicinal product are sufficient; in particular the product proves not to be harmful in the specified conditions of use and the pharmacological effects or efficacy of the medicinal product are plausible on the basis of long-standing use and experience.

Concerning the safety information the article 16c 1. (b), (c), (d) and 2. must be considered:

> (b) any authorization or registration obtained by the applicant in another Member State, or in a third country, to place the medicinal product on the market, and details of any decision to refuse to grant an authorization or registration, whether in the Community or a third country, and the reasons for any such decision;
>
> (c) bibliographical or expert evidence to the effect that the medicinal product in question, or a corresponding product has been in medicinal use throughout a period of at least 30 years preceding the date of the application, including at least 15 years within the Community. At the request of the Member State where the application for traditional-use registration has been submitted, the Committee for Herbal Medicinal Products shall draw up an opinion on the adequacy of the evidence of the long-standing use of the product, or of the corresponding product. The Member State shall submit relevant documentation supporting the referral;
>
> (d) **a bibliographic review of safety data together with an expert report, and where required by the competent authority, upon additional request, data necessary for assessing the safety of the medicinal product.**

2. A corresponding product, as referred to in paragraph 1(c), is characterized by having the same active ingredients, irrespective of the excipients used, the same or similar intended purpose, equivalent strength and posology and the same or similar route of administration as the medicinal product applied for.

Referring to the “well-established use” submission the Part II of the Directive 2001/83/EC declaim:

Some medicinal products present specific features which are such that all the requirements of the marketing authorization application dossier as laid down in Part I of this Annex need to be adapted. To take account of these particular situations, an appropriate and adapted presentation of the dossier shall be followed by applicants.

1. Well-established medicinal use

For medicinal products, the active substance(s) of which has/have a 'well-established medicinal use' as referred to Article 10(1)(a)(ii), with recognized efficacy and an acceptable level of safety, the following specific rules shall apply.

The applicant shall submit Modules 1, 2 and 3 as described in part I of this Annex.

For Modules 4 and 5, a detailed scientific **bibliography** shall address non-clinical and clinical characteristics.

The following specific rules shall apply in order to demonstrate the well-established medicinal use:

a) Factors which have to be taken into account in order to verify a well-established medicinal use of constituents of medicinal products are:

— the time over which a substance has been used,

— quantitative aspects of the use of the substance,

—the degree of scientific interest in the use of the substance (reflected in the published scientific literature) and

— the coherence of scientific assessments.

Therefore, different periods of time may be necessary for verify well-established use of different substances. In any case, however, the period of time required for establishing a well-established medicinal use of a constituent of a medicinal product must not be less than one decade from the first systematic and documented use of that substance as a medicinal product in the Community.

b) The documentation submitted by the applicant should cover **all aspects of the safety and/or efficacy assessment and must include or refer to a review of the relevant literature**, **taking into account pre- and post-marketing studies and published scientific literature concerning experience in the form of epidemiological studies and in particular of comparative epidemiological studies**. All documentation, both favorable and

unfavorable, must be communicated. With respect to the provisions on 'well-established medicinal use' it is in particular necessary to clarify that 'bibliographic reference' to other sources of evidence (post marketing studies, epidemiological studies, etc.) and not just data related to tests and trials may serve as a valid **proof of safety** and efficacy of a product if an application explains and justifies the use of these sources of information satisfactorily.

2. *Toxicology*

The traditional definition of toxicology is "*the science of poisons*". As our understanding of how various agents can cause harm to humans and other organisms, a more descriptive definition of toxicology is "*the study of the adverse effects of chemicals or physical agents on living organisms*". The historical development of toxicology began with early cave dwellers who recognized poisonous plants and animals and used their extracts for hunting or in warfare. By 1500 *B.C.*, written recordings indicated that hemlock, opium, arrow poisons, and certain metals were used to poison enemies or for state executions. With time, poisons became widely used and with great sophistication. Notable poisoning victims include Socrates, Cleopatra, and Claudius. By the time of the Renaissance and Age of Enlightenment, certain concepts fundamental to toxicology began to take shape. The studies of Paracelsus (~1500 *A.D.*) and Orfila (~1800 *A.D.*) are well known. Paracelsus determined that specific chemicals were actually responsible for the toxicity of a plant or animal poison. He also documented that the body's response to those chemicals depended on the dose received. His studies revealed that small doses of a substance might be harmless or beneficial whereas larger doses could be toxic. This is now known as the dose-response relationship, a major concept of toxicology. Paracelsus was one of the founders of modern toxicology. His best-known quote: "*All substances are poisons; it is the dose that makes the poison*." Orfila, a Spanish physician, is often referred to as the founder of toxicology. It was Orfila who first prepared a systematic correlation between the chemical and biological properties of poisons of the time. He demonstrated effects of poisons on specific organs by analyzing autopsy materials for poisons and their associated tissue damage. The 20th century is marked by an advanced level of understanding of toxicology. DNA and various biochemicals that maintain body functions were discovered. Our level of knowledge of toxic effects on organs and cells is now being revealed at the molecular level. It is recognized that virtually all toxic effects are caused by

changes in specific cellular molecules and biochemical. Now toxicology can be divided in tree macro areas:

Molecular Toxicology concerning the Identification of biochemical and cellular molecular mechanisms by which substances exert their toxic effect.
Descriptive Toxicology targeted to produce the information necessary for the safety assessment (e.g. Pre-clinical studies, assessment of the LD_{50})
Regulatory Toxicology concerning the guidelines and other documents issued by regulatory Agency in FDA, EMA to assess drugs and food about safety.

Before discussing and analyze how to use a toxicological data here we report some basic information concerning definitions:

Xenobiotic is the general term that is used for a foreign substance taken into the body. It is derived from the Greek term *xeno* which means *"foreigner."* Xenobiotics may produce beneficial effects *(such as a pharmaceuticals)* or they may be toxic *(such as lead)*. As Paracelsus proposed centuries ago, dose differentiates whether a substance will be a remedy or a poison. A xenobiotic in small amounts may be non-toxic and even beneficial but when the dose is increased, toxic and lethal effects may result.

Toxicants	Substances that produce adverse biological effects of any nature. May be chemical or physical in nature. Effects may be of various nature (acute, chronic, etc.)
Toxins	Specific proteins produced by living organisms (mushrooms, bacteria, etc.)
Poisons	Toxicants that cause immediate death or illness when experienced in very small amounts

Toxicant, toxin, and poison are often used interchangeably in the literature; however, there are subtle differences as indicated in the table. Toxic substances may be systemic toxins or organ toxins. A systemic toxin is one that affects the entire body or many organs rather than a specific site. For example, potassium cyanide is a systemic toxicant in that it affects virtually every cell and organ in the body by interfering with the cell's ability to utilize oxygen. Toxicants may also affect only specific tissues or organs while not producing damage to the body as a whole. These specific sites are known as the target organs or target tissues.

Some examples: Benzene is a specific organ toxin in that it is primarily toxic to the blood-forming tissues. Lead is also a specific organ toxin; however, it has three target organs *(central nervous system, kidney, and hematopoietic system)*.

A toxic agent is anything that can produce an adverse biological effect. It may be chemical, physical, or biological in form. For example, toxic agents may be chemical *(such as cyanide)*, physical *(such as radiation)* and biological *(such as snake venom)*.

A distinction is made for diseases due to biological organisms. Those organisms that invade and multiply within the organism and produce their effects by biological activity are not classified as toxic agents. An example of this is a virus that damages cell membranes resulting in cell death.

If the invading organisms excrete chemicals which is the basis for toxicity, the excreted substances are known as biological toxins. The organisms in this case are referred to as toxic organisms. An example is tetanus. Tetanus is caused by a bacterium, *Clostridium tetani*. The bacteria *C. tetani* itself does not cause disease by invading and destroying cells. Rather, it is a toxin that is excreted by the bacteria that travels to the nervous system that produces the disease.

A toxic substance is simply a material which has toxic properties. It may be a discrete toxic chemical or a mixture of toxic chemicals. For example, lead chromate, asbestos, and gasoline are all toxic substances. Lead chromate is a discrete toxic chemical**.** Asbestos is a toxic material that does not consist of an exact chemical composition but a variety of fibers and minerals. Gasoline is also a toxic substance rather than a toxic chemical in that it contains a mixture of many chemicals. Toxic substances may not always have a constant composition. For example, the composition of gasoline varies with octane level, manufacturer, time of season, etc. Toxic substances may be systemic toxins or organ toxins. A systemic toxin is one that affects the entire body or many organs rather than a specific site. For example, potassium cyanide is a systemic toxicant in that it affects virtually every cell and organ in the body by interfering with the cell's ability to utilize oxygen. Toxicants may also affect only specific tissues or organs while not producing damage to the body as a whole. These specific sites are known as the target organs or target tissues.

A toxicant may affect a specific type of tissue *(such as connective tissue)* that is present in several organs. The toxic site is then referred to as the target tissue.

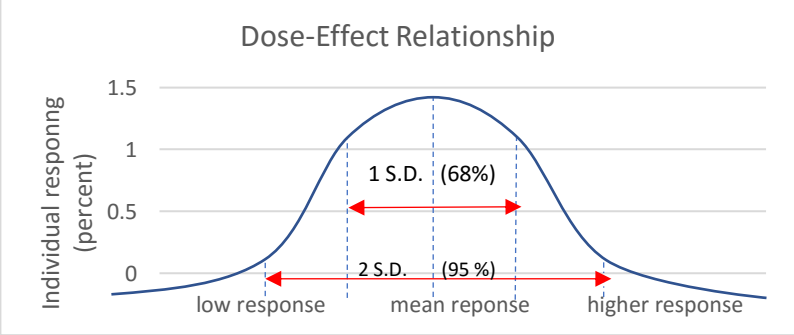
There are many types of cells in the body and they can be classified in several ways.

- basic structure (e.g., cuboidal cells)
- tissue type (e.g., hepatocytes of the liver)
- germinal cells (e.g., ova and sperm)
- somatic cells (e.g., non-reproductive cells of the body)

The main factors determining adverse effects are: **intrinsic toxicity** of the substance, **dose**, **exposure conditions** and **response of host** (such as detoxification abilities of the organism or the bioactivation). Dose by definition is the amount of a substance administered at one time. However, other parameters are needed to characterize the exposure to xenobiotics. The most important are the number of doses, frequency, and total time period of the treatment.

Exposure dose	The amount of a xenobiotic encountered in the enviroment
Absorbed dose	The actual amount of the exposed dose that enters the body
Administered dose	The quantity administered usually orally or by injection
Total dose	The sum of all individual doses

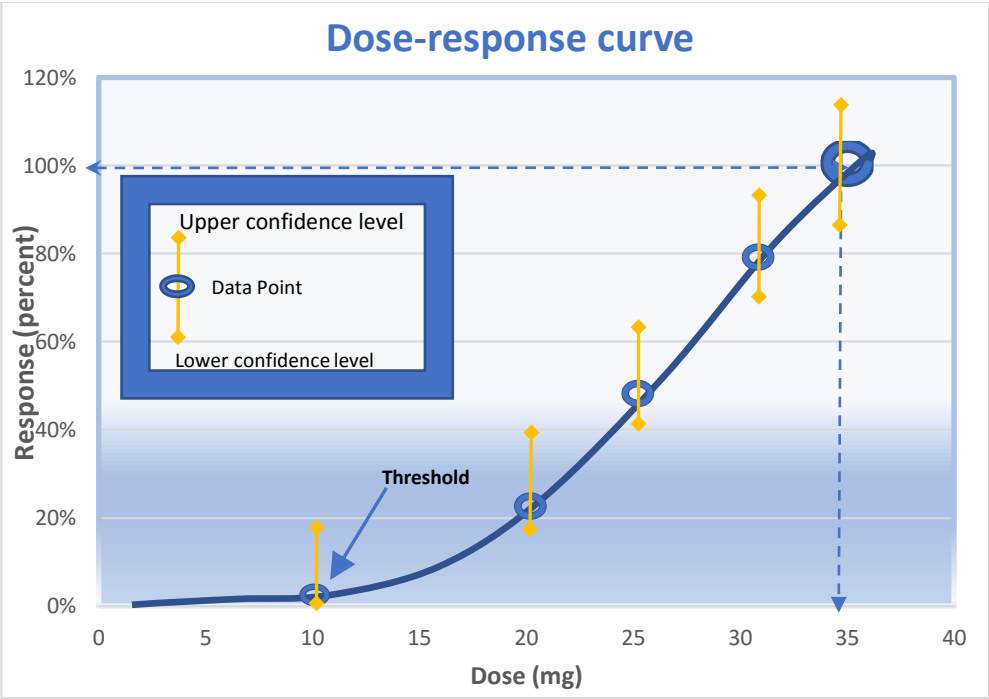
Fractionating a total dose usually decreases the probability that the total dose will cause toxicity. The reason for this is that the body often can repair the effect of each subtoxic dose if sufficient time passes before receiving the next dose. In such a case, the total dose, harmful if received all at once, is non-toxic when administered over a period of time. For example, 30 mg of strychnine swallowed at one time could be fatal to an adult whereas 3 mg of strychnine swallowed each day for ten days would not be fatal. The dose-response relationship is a fundamental and essential concept in toxicology. It correlates exposures and the spectrum of induced effects. Generally, the higher the dose, the more severe the response. The dose-response relationship is based on observed data from experimental animal, human clinical, or cell studies.



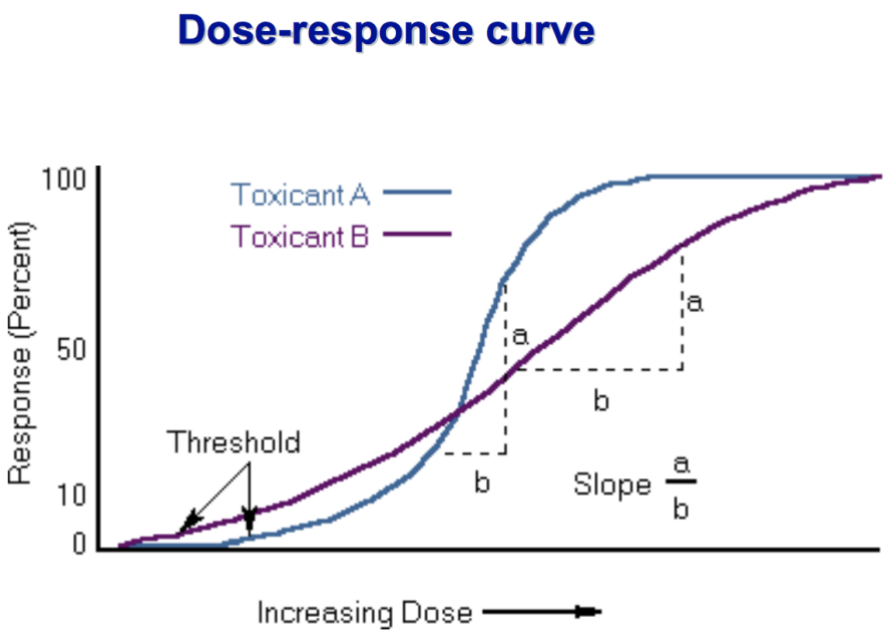
Knowledge of the dose-response relationship:

- - establishes causality that the chemical has in fact induced the observed effects
- - establishes the lowest dose where an induced effect occurs (the threshold effect)
- - determines the rate at which injury builds up (the slope for the dose response).

Within a population, the majority of responses to a toxicant are similar; however, a wide variance of responses may be encountered, some individuals are susceptible and others resistant. As demonstrated above, a graph of the individual responses can be depicted as a bell- shaped standard distribution curve. Dose responses are commonly presented as mean + 1 S.D. (*standard deviation*), which incorporates 68% of the individuals. The variance may also be presented as two standard deviations, which incorporates 95% of the responses. A large standard deviation indicates great variability of response. For example, a response of 12+5 mg indicates considerably more variability than 12+1 mg.



The dose-response curve normally takes the form of a sigmoid curve. It conforms to a smooth curve as close as possible to the individual data points. For most effects, small doses are not toxic. The point at which toxicity first appears is known as the threshold dose level. From that point, the curve increases with higher dose levels. In the hypothetical curve above, no toxicity occurs at 10 mg whereas at 35 mg 100% of the individuals experience toxic effects. There is always a relation between dose and effect/response, but for some agents there is a threshold below which no effect occurs. A threshold for toxic effects occurs at the point where the body's ability to detoxify a xenobiotic or repair toxic injury has been exceeded. For most organs, there is a reserve capacity so that loss of some organ function does not cause decreased performance. For example, the development of cirrhosis in the liver may not result in a clinical effect until over 50% of the liver has been replaced by fibrous tissue.



Knowledge of the shape and slope of the dose-response curve is extremely important in predicting the toxicity of a substance at specific dose levels. Major differences among toxicants may exist not only in the point at which the threshold is reached but also in the percent of population responding per unit change in dose (*i.e., the slope*). As illustrated above, Toxicant A has a higher threshold but a steeper slope than Toxicant B. Knowledge of the dose-response relationship permits one to determine whether exposure has caused an effect, threshold for the effect, and the rate of buildup of the effect with increasing dose levels. Rate of buildup of toxic effects is known as the "slope" of the dose-response curve.

MATHERIAL AND METHODS

In the light of what has just been said the selection of the toxicological data should be as representative as possible of the final use of the product under assessment e.g. same route of administration, pharmaceutical form etc. In any case correction factors or, in the end the TTC (Threshold of Toxicological Concern) approach, could always be applied if no representative data have been founded. The complex composition of herbal preparations presents an additional challenge because it is not always possible to make or find an *ad hoc* study and that is why it is possible to avail of numerous toxicological parameters that allow to assess the reliability of the security data. The well-known toxicological parameters usually considered for the safety assessment requirements are NOEL (Non-Observed Effect Level), LHRD (Lowest Human Recommended Dose), DL_{50} (lethal dose 50), TTC e PDE (Permitted Daily Exposure). These parameters can be easily calculated or derivate from literature data. For plant used both in medicinal products and in food, the ADI (admitted daily intake) should be considered as parameter also because is used by WHO and other competent Authorities in safety food assessments (e.g. EFSA).

In this complex evaluation process, the assessors can find a help in guidelines and the most used ones are published in the EMA website such as *Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities; guideline for residual solvents; Guideline on the limits of genotoxic on the impurities; updated on revision chapters 3 and 5 of the good manufacturing practices (GMP) guide: "dedicated facilities"; Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products, Guidance for Industry estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers; Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet.*

Here we suggest an overview of the safety requirements, raised by the enormous interest and growing market for medicinal products of botanical origin, that are contemplated in European pharmaceutical legislation, in order to apply a scheme that provides a practical method for assessing herbal substances and herbal preparations for which an adequate body of knowledge exists in the scientific literature and therefore without the must of needing an *ad hoc* study. This scheme uses the well-known toxicological parameters and the consistency of the obtained parameter is strongly influenced from the chosen datum. Usually to assess the risks related to

toxic substances were used experimental toxicology studies that are divided in: Acute toxicity studies, toxicity studies in the medium term (also called sub-acute or sub chronic) and long-term toxicity studies (also known as chronic toxicity studies) (C. Lu, Kacew 2002). **Acute toxicity** occurs almost immediately *(hours/days)* after an exposure. An acute exposure is usually a single dose or a series of doses received within a 24-hour period. Death is a major concern in cases of acute exposures. One example is that in 1989, 5,000 people died and 30,000 were permanently disabled due to exposure to methyl isocyanate from an industrial accident in Bhopal, India. **Subchronic toxicity** results from repeated exposure for several weeks or months. This is a common human exposure pattern for some pharmaceuticals and environmental agents. An example is that the ingestion of coumarines *(blood thinners)* for several weeks as a treatment for venous thrombosis can cause internal bleeding. **Chronic toxicity** represents cumulative damage to specific organ systems and takes many months or years to become a recognizable clinical disease. Damage due to subclinical individual exposures may go unnoticed. With repeated exposures or long-term continual exposure, the damage from these subclinical exposures slowly builds-up *(cumulative damage)* until the damage exceeds the threshold for chronic toxicity. Ultimately, the damage becomes so severe that the organ can no longer function normally and a variety of chronic toxic effects may result. Examples of chronic toxic effects are: cirrhosis in alcoholics who have ingested ethanol for several years; chronic kidney disease in workmen with several years exposure to lead; chronic bronchitis in long-term cigarette smokers. For a complete point of view, if that information has been investigated, also **carcinogenicity**, **development toxicity** and **genetic toxicity** should be analyzed.

Carcinogenicity is a complex multistage process of abnormal cell growth and differentiation which can lead to cancer. At least two stages are recognized. They are initiation in which a normal cell undergoes irreversible changes and promotion in which initiated cells are stimulated to progress to cancer. Chemicals can act as initiators or promoters.

Developmental toxicity pertains to adverse toxic effects to the developing embryo or fetus. This can result from toxicant exposure to either parent before conception or to the mother and her developing embryo-fetus. The three basic types of developmental toxicity are: Embryiolethality (failure to conceive, spontaneous abortion or stillbirth), embriotoxicity (growth retardation or delayed growth of specific organ system), teratogenicity (irreversible conditions that leave permanent birth defects in live offspring). Developmental toxicity should not be confused with the **reproductive toxicity** that involves toxicant damage to either the male or female reproductive

system and produce toxic effect such as decreased libido and impotence, infertility, chromosome abnormalities and births defect, etc.

Genetic toxicity results from damage to DNA and altered genetic expression. This process is known as mutagenesis. The genetic change is referred to as a mutation and the agent causing the change as a mutagen. There are three types of genetic change: multistage process, initiation, promotion.

The complete discussion and the analysis about the parameters that should be considered during the assessment of the safety aspects of a medicinal product of botanical origin with some practical examples of the application of the formulas to calculate parameters such as PDE are provided in "*2.PDE*" section.

1. NOEL and NOAEL

Here below the formulas and their respective correction factors are analyzed:

The **NOEL** is the maximum dose that did not cause any observable effect, the **NOAEL** (No Observable Adverse Effect Level) is the dose at which no observed adverse effects are evaluable, the **LOEL** (Lowest Observed Effect Level) is the lowest dose level (exposure) in which Observed Effect, the **LOAEL** (Lowest Observed Adverse Effect Level) is the lowest dose that show a detectable adverse effect and **FEL** (Frank Effect Level) level of exposure that produces unmistakable and irreversible effects (such as mortality or impairment) All these parameters are generally expressed in mg/kg and/or mg/kg body weight/day (in general are also known the corresponding exposure values in terms of C_{max} and AUC). It's really important to underline the difference from NOEL to NOAEL: the NOAEL is not the same as the no observed effect level (NOEL), which refers to any effect, not just an adverse one, although in some cases the two might be identical. The definition of the NOAEL, in contrast to that of NOEL, reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic environment and may not raise a safety concern. The differences between NOEL and NOAEL are shown also in the formulas for the

calculation that are the following:

NOAEL = RfD (Acute Reference Dose) x UF (Uncertain Factor) or ADI x UF[1]

NOEL = DL_{50} x Animal Weight / (UF) 200 - 3000

[1] Usually significant NOAELs and LOAELs are obtained directly by the toxicological study rather than calculated

UF is an empirical uncertainty factor, to obtain the toxicologically acceptable dose (Belitz, Grosch et. al. 2009). This is calculated and assigned by an expert judgment in force of the data and the existence in literature of an extensive knowledge of the substance in analysis. The minimum value of UF is 200 because 10 is the inter-species variability and another UF of 10 for intra-species variability. Now, considering the quality of the data, the absence of data about the chronic toxicity and the severity of the effect it is possible to have an additional factor of between 2-30 by multiplying with the previously obtained UF. The choice of these factors depends on the overall assessment of the available data and is based on an "expert judgment". Every assessor could decide to apply different UFs, this depend on the finished product's characteristics such as the route of administration, the issue to treat, the intrinsic toxicity of the substances under assessment; anyway, it is important to note that each different approach for the same product could be correct under an adequate scientific rational. The UF (as many default values) are under statistic rules such as inferential statistic and then anytime an experimental data is available in literature it must be considered.

2. *PDE*

Regarding the PDE (Permitted Daily Exposure) approach it could be calculated by the following formula:

$$\text{PDE} = \frac{\text{NOEL x Weight Adjustment}}{\text{F1 x F2 x F3 x F4 x F5}}$$

F1: a factor (between 2 and 12) which takes account of the extrapolation between species; F2: a factor of 10, which takes into account the variability between individuals; F3: a factor of 10, which takes account of the short-term toxicity studies with repeated doses e.g., Less than 4 weeks; F4: a factor (1-10) that can be applied in case of severe toxicity, e.g. non-genotoxic carcinogenicity, neurotoxicity or teratogenicity; F5: a factor that no effect can be applied if it has not been established.[2]

For the same substance, there could be available different values of ADIs and PDEs the first parameter is related to an intake the other is related to an exposure, indeed the PDE is defined as a pharmaceutically acceptable intake of residual solvents and then is more restrictive. For the purposes of calculation, when is available only the LOEL, a factor of up to 10 may be used depending on the severity of the toxicity.

[2] For the exact factors, please see the document: Guideline on the Limits of Genotoxic on the Impurities, EMEA/CHMP/QWP/251344/2006

NOEL/F1 is equivalent to another toxicological parameter called **HED** (Human Equivalent Dose), that just represent the extrapolation.

It should be noted that the PDE is more restrictive than the NOEL approach indeed one of its main application is mentioned in the guideline of “cleaning validation” to evaluate residual impurities, so is referred to substances that should not be in the finished product or something that should be avoided. About the PDE calculation, if different critical side effects were identified for the substance under assessment, the calculation of multiple values of PDEs should be considered and a decision as regards the most appropriate PDE must be made with an appropriate justification. Usually, it will use the lowest value of PDE. Different administration routes, except in exceptional cases, change the bioavailability. If there are obvious differences (>40%), it is necessary, therefore, to apply correction factors. The correction factors for route-to-route extrapolation should preferably be based on human data. If these data are unavailable, it is possible to proceed, by example extrapolating from the route of oral administration to the inhalation one, assuming 100% of the bioavailability of the substance as worst case. Consequently, the derived PDE based on oral data can be corrected by multiplying the oral absorption percentage for 100% inhalation. Unfortunately, it is not always possible to find a toxicological data in literature that is representative of the substance for which safety is to be assessed, especially when we are talking about of a pool of substances like a botanical fluid extract. In this case, the phytocomplex should be considered as representative for the toxicity unless the toxicity of the other components, different by the most potentially toxic one, is well known and negligible. In the worst case, not even the phytochemistry composition of the product under assessment is available and then the most restrictive approach (TTC value of 0,15 μg/person) provided by Kroes et al. 2004 and Cramer et. Al 1978 should be applied. About those issues can be very useful the “Class structure” published by Cramer et al. 1978 which help to understand the most appropriate value of TTC to apply also in absence of toxicological data.

3. *TTC*

The threshold of toxicological concern (TTC) is defined as a level of human intake or exposure considered to be of negligible risk, despite the absence of chemical-specific toxicity data.

The TTC is a pragmatic risk assessment tool that is based on the principle of establishing a human exposure threshold value for all chemicals, below which there is a very low probability of an appreciable risk to human health. In 1978 Cramer proposed (Cramer et al, 1978) a decision tree

for the application of the TTC concept to non-cancer endpoints. This is probably the most commonly used approach for classifying and ranking chemicals on the basis of their expected level of oral toxicity and this approach could be considered as a priority setting tool in the safety assessment of food additives which would make expert judgments more transparent, explicit and rational, and thus more reproducible and trustworthy. The scheme was derived from the authors' earlier experience in classifying food flavors (Oser and Hall, 1977) and their subsequent work in evaluating a range of carcinogens, pesticides and industrial chemicals. The original Cramer classification consists in three different classes (I, II and III):

Class I. Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.

Class II. Substances that possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.

Class III. Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

Recently, March 2016, the WHO and EFSA published a document concerning the application of the TTC approach and the Cramer's scheme where is possible to read "*The expert group concluded that the currently available information do not warrant major revisions to the Cramer scheme. The scheme is well suited for its intended purpose and when used in conjunction with the associated TTC values is protective. The group acknowledged that the sorting process of the Cramer decision scheme does work, is reproducible and the TTC values have been substantiated by post hoc comparison with numerous newer databases. In consequence, the expert group concluded that there is no scientifically-based justification for major restructuring of the decision scheme. The Expert group recommended minor suggestions to modify the Cramer decision scheme to remove ambiguity, improve its clarity and to harmonize with the electronic tool Toxtree. The expert group recognized that there are a number of efforts underway, including those of the US Food and Drug Administration (FDA) and the International Organization of the Flavour Industry (IOFI), that propose significant modifications to the Cramer decision scheme, indicating that the developers interpret a need for revision. However, major modification to and restructuring of the Cramer decision scheme could result in a situation in which the original TTC values derived by Munro et al. (1996) and subsequently substantiated using different databases may be altered, and the implications for existing safety assessments need to be evaluated. Because the Cramer decision*

scheme has been applied for the evaluation of flavouring agents for over 15 years, there is a need for broad acceptance of any future changes".[3]

The Threshold of Toxicological Concern, as said before, is an approach that can be used in the absence of chemical-specific toxicity data. It is based on the establishment of levels of human exposure (TTCs) that would not represent a safety concern using toxicological data for other chemicals sharing some structural similarities. The approach does not involve quantitative structure–activity relationships (QSAR) for specific endpoints but rather is based on the distribution of potencies for chemicals that share similar broad structural characteristics with the chemical under evaluation. In principle, the TTC approach can be considered for the safety evaluation of any chemical, which is not covered by the exclusion criteria in the current TTC approach, for which information on the chemical structure is available and for which an estimate of the extent of human exposure can be made. It allows an assessment of whether human exposure is so low that a more in-depth evaluation is not needed, or if chemical-specific toxicity data and/or control of human exposure are required. The TTC values can also be used to set an upper limit for human exposure for a chemical in the absence of intake data. The history of the TTC, the supporting databases and the extension of the original concept have been considered in numerous papers (Barlow et al., 2001; Brown et al., 2009; Cheeseman et al., 1999; Gold et al., 2005; Kroes et al., 2004, 2007; Munro et al., 1996, 2008; SCCP, 2008). The approach has been controversial because it seeks to provide risk characterization advice in the absence of the usual toxicity database; the validity of the approach is critically dependent on the validity of the databases used to derive the TTC values.

The TTC evolved following a review by Munro (1990) of the Threshold of Regulation (TOR), which is being used by the US Food and Drug Administration in the context of regulating food contact materials with low exposures and relates to a dietary concentration giving an intake of 1.5 μg per person per day, equivalent to 0.025 μg/kg body weight (bw) per day (US FDA, 1993). The TOR was derived by low-dose extrapolation of carcinogenicity data from animal studies to define human exposures associated with an upper-bound estimate of one in a million-cancer risk (US FDA, 1993; Munro, 1990). The TOR of 1.5 μg per person per day was considered to provide an adequate margin of safety for other forms of toxicity for chemicals that do not have a structural alert for genotoxicity/DNA reactivity. This approach led to the development of TTC values for non-cancer

[3] Review of the Threshold of Toxicological Concern (TTC) approach and development of new TTC decision tree – 10 March 2016

effects by Munro et al. (1996), which were based on analyses of the no-observed (adverse) effect levels (NOAELs) from repeated dose toxicity data for chemicals separated into three structural classes using the Cramer et al. (1978) decision scheme.

The intent of Munro et al. (1996) was to develop a database consisting mainly of NOAELs from chronic toxicity studies in animals. However, in many cases, the lowest and thus most conservative NOAEL for a chemical came from a subchronic study. In order to group NOAELs for chemicals with only subchronic studies with those with chronic studies to derive the cumulative distribution of NOAELs, subchronic NOAELs were divided by a factor of three to approximate the most likely NOAEL that would be derived from a chronic study. This conversion factor was based on research defining the relationship between subchronic and chronic NOAELs available at the time. Based on an analysis of 222 NOAEL ratios of subchronic/chronic rat studies and 99 NOAEL ratios of subchronic/chronic mouse studies (Zarn et al. 2011) taken from publicly available mouse and rat feeding toxicity studies, and the EFSA (2012b) recommended a factor of 2 for extrapolating from subchronic to chronic study duration in rodents, which means that the factor of three used by Munro et al. (1996) can be considered to be conservative. A TTC value was calculated by Munro et al. (1996) from the respective distribution of NOAELs for each of the 3 Cramer structural classes, using a database of 613 chemicals with 2941 NOAELs, representing a range of industrial chemicals, pharmaceuticals, food chemicals and environmental, agricultural and consumer chemicals likely to be encountered in commerce with good supporting toxicological data, yielding 137, 28 and 448 chemicals in Cramer class I, II and III, respectively. For each of the 613 chemicals, the most conservative NOAEL was selected, based on the most sensitive species, sex and endpoint. The fifth percentile NOAEL (in mg/kg bw/day) was calculated for each structural class and this was converted to the intake for a 60kg person following the application of a safety factor to calculate the TTC value. In converting the fifth percentile NOAELs to a TTC value for the three structural classes, a 100-fold safety factor was used, the default safety factor used for establishing health-based guidance values for chemicals using toxicity data from animal studies. This procedure resulted in TTC values of **1800, 540, 90 µg/person/day** for Cramer classes I, II and III, respectively.

A number of criticisms of the TTC approach were raised at a workshop organised by the ILSI Europe TTC Task Force in 1999 (Barlow et al., 2001), principally in relation to some potentially sensitive endpoints: immunotoxicity, developmental toxicity, neurotoxicity and developmental neurotoxicity, endocrine active chemicals and allergenicity. These issues were considered, where possible, by analyses of databases selected for these endpoints (Kroes et al., 2000). Such selective

databases are considered conservative since chemicals are likely to have been selected for special endpoint study for some a priori reason. For developmental toxicity, the distribution of NOAELs divided by 100 was about 3 orders of magnitude higher than the distribution of 1 in a million upper-bound lifetime risk estimates derived from carcinogenicity data. Importantly, the cumulative distribution of NOAELs was similar to that of Class III chemicals in the Munro et al. (1996) database, i.e. developmental toxicity was not more sensitive than other non-cancer endpoints, indicating that a specific TTC was not necessary. For adult neurotoxicity, the distribution of NOAELs was lower by about one order of magnitude than those for other non-cancer endpoints, including developmental neurotoxicity (Kroes et al., 2000).

For allergies, hypersensitivity reactions and intolerances, none of the current testing strategies were considered adequate for such effects and, therefore, there is no database to develop TTC values and these endpoints are not included in the TTC approach. However, in the absence of suitable animal models, the TTC is not different to any of the other approaches to risk assessment.

These analyses led to further refinements of the TTC approach: Kroes et al. (2004) developed a TTC value for chemicals with certain structural alerts for genotoxic carcinogenicity (0.15 μg/day, calculated by linear extrapolation to a theoretical upper-bound risk of one in a million from the TD_{50}[4]) and a separate TTC value for organophosphate chemicals (18 μg/day). It was acknowledged that organophosphate chemicals are usually regulated products and it was clearly stated that the TTC approach should not be considered an alternative to testing procedures required for regulatory approval.

Removing organophosphate and carbamate chemicals from Cramer Class III, being the most potent chemicals in that class, would have an impact on the existing TTC value for Cramer Class III. Kroes et al. (2004) did not propose to revise the TTC value for Cramer class III and the EFSA (2012a) stated that, pending any future revision of the TTC approach, it would be prudent to maintain the value for Cramer Class III at 90 μg/person per day. On the other hand, Felter et al. (2009) proposed to revise the existing TTC value for Cramer Class III to 180 μg/person per day after removing organophosphate and carbamate chemicals from Cramer Class III. Munro et al. (2008) noted that exclusion of organophosphate and carbamate chemicals would give a corrected Class III TTC value of 180 μg/person/day instead of 90 μg/person/day and, in addition, if

[4] The TD_{50} is defined as the daily dose-rate in mg/kg body weight per day for life to induce tumours in half of the test animals that would have remained tumours-free at zero dose.

organohalogen chemicals are also excluded from Cramer class III, the resulting corrected Class III TTC value would be about 600 μg/person/day. Kroes et al. (2004) also developed a step-wise decision tree which incorporates the various TTC values in decreasing order of concern and increasing numerical values. At the beginning, they included an exclusion category for certain types of chemicals that should not be assessed by a TTC approach. The reason for this was either that similar structures were not represented in the database and/or that established risk assessment approaches already exist (heavy metal and TCDD-like chemicals), or that they represent high potency genotoxic carcinogens (aflatoxin-like chemicals, N-nitroso-chemicals and azoxy-chemicals – the so-called cohort of concern (CoC)). Separate TTC values could be developed for the CoC, but it was considered that the resulting TTC values would to be too low to be of practicable use.

The use of the TTC approach as a pragmatic risk assessment or prioritization tool has become established in several areas of chemical risk assessment in the regulatory context, including food contact materials (US FDA, 1995), food flavouring agents evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) (WHO, 1997; Munro et al., 1999; Renwick, 2004) and impurities in pharmaceutical products (EMEA, 2006, 2007; US FDA, 2008). Areas currently under consideration for the future uses of TTC include metabolites of plant protection products (pesticides) (Brown et al., 2009), cosmetics (Kroes et al., 2007; SCCP, 2008) and consumer products (Blackburn et al., 2005).

The application of the TTC approach would also permit prioritization of risk assessment resources (fiscal, time and human expertise) to chemicals posing potentially greater risks. Although the TTC approach has traditionally been used in human health assessments for oral exposures there is no reason why it could not be adapted for other routes of exposure including dermal (Kroes et al., 2007; Safford, 2008) and inhalation (Carthew et al., 2009; Escher et al., 2010).

RESULTS

Aesculus hippocastanum

cortex

Main components

The most characteristic compounds are coumarin derivatives (up to 7%):
- **Glucosides**: Esculin (6-(*β*-D-glucopyranosyloxy)-7-hydroxy-2*H*-1-benzopyran-2-one, or 6,7- dihydroxycoumarin 6-glucoside), a glucoside of esculetin (6,7-dihydroxy-2*H*-1-benzopyran-2- one, or 6,7-dihydroxycoumarin), Fraxin (8-(*β*-D-glucopyranosyloxy)-7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one, or 7,8- dihydroxy-6-methoxycoumarin-8-*β*-D-glucoside), a glucoside of fraxetin (7,8-dihydroxy-6- methoxy-2*H*-1-benzopyran-2-one, or 7,8-dihydroxy-6-methoxycoumarin); Scopolin (7-(*β*-D-glucopyranosyloxy)-6-methoxy-2*H*-1-benzopyran-2-one), a glucoside of scopoletin (7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one, or 7-hydroxy-6-methoxy- coumarin); **Aglycones**: esculetin, fraxetin and scopoletin.
Other constituents are: **tannins** (up to 2 %), **flavonoids**, **anthocyanins**, **catechins** derivatives, traces of aescin.

Toxic component	Approach	Remarks	Acceptable amount	UF
esculin	$LHRD_{aescin\ oral}$ $LHRD_{water\ extract\ oral}$	France "traditional use": Traditionally used in the symptomatic treatment of functional disorders of cutaneous capillary fragility, such as ecchymosis, petechias, etc. Traditionally used in subjective signs of venous insufficiency, such as heavy legs. Traditionally used in haemorrhoidal symptoms. As there are no clinical studies conducted with horse chestnut bark in children and adolescents under the age of 18 years, horse chestnut bark should not be used in this target population and should be limited to adults and elderly. Kidney failure has been documented in children and adults after receiving injections of aescin, and in adults after taking high doses of aescin (Chandler 1993)	40 mg/die 400 mg/die	/

References:

- Bézanger-Beauquesne L, Pinkas M, Torck M and Trotin F. Aesculus hippocastanum L. Plantes médicinales des régions tempérées. Paris: Maloine, 1980, 2è ed., 164-165
- Bombardelli E, Morazzoni P and Griffini A. Aesculus hippocastanum L. Fitoterapia 1996, 67:483–511
- Chandler RF. Herbal medicine: horse chestnut. Can. Pharm. J., 1993, 126, 297, 300-306
- Fournier P. Marronnier d'Inde – Aesculus hippocastanum L. Le livre des plantes médicinales et vénéneuses de France. Paris: P. Lechevalier Éditeur, 1948, T.2, 475-479
- Paris M, Moyse H. Abrégé de matière médicale, Tome 1. Paris: Masson, 1981, 147-150
- Wichtl M et al. Plantes thérapeutiques, 2è ed. Paris: Editions Tec et Doc/EM Inter, 2003, 294-296

Althaeae officnalis

radix

Main components

Mucilage: polysaccharides 5-11.6
Pectins: 11 %
Starch: 25-35 %
Mono-, Di-saccharides saccharose: 10 %; **crude mucilages** contained 5 % glucose in spring and 20 % glucose in winter
Flavonoids 0,14 – 0.28 %
Phenolic acids
Coumarins
Other compounds: phytosterols, calcium oxalate, fat, tannins acids, 2 % asparagine, glycine betain

Toxic component	Approach	Remarks	Acceptable amount	UF
/	NOEL $_{\text{human oral}}$		125 mg/Kg b.w./day	2000

References:

- Benbassat N, Kostova B, Nikolova I, Rachev D. Development and evaluation of novel lozenges containing marshmallow root extract. Pak J Pharm Sci 2013, 26(6):1103-1107
- Deters A, Ziffel J, Hellenbrand N, Pappai D, Possemeyer C, Hensel A. Aqueous extract and polysaccharides from Marshmallow roots (Althaea officinalis L.): Cellular internalisation and stimulation of cell physiology of human epithelial cells in vitro. J Ethnopharmacology 2010, 127:62-69
- EMA/HMPC/436680/2015
- ESCOP Monographs 2nd ed. Althaeae radix–Marshmallow root. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 32-35
- Mascolo N, Autore MG, Capasso F, Menghini A, Fasulo MP. Biological screening of Italian medical plants for anti-inflammatory activity. Phytother Res 1987, 1:28-31
- Rouhi H, Ganji F. Effect of Althaea officinalis on cough associated with ACE inhibitors. Pakistan J Nutr 2007, 6(3):256-258
- Schmidgall J, Schnetz E, Hensel A. Evidence for bio adhesive effects of polysaccharides and polysaccharide-containing herbs in an ex vivo bioadhesion assay on buccal membranes. Planta Med 2000, 66:48-53

Angelicae sinensis

radix

Main components

Alkyl phthalides (ligustilide, (Z)-ligustilide, (Z)-6,7-epoxyligustilide, angelicide, (Z)-butylidenephthalide, butylphthalide, 2,4-dihydrophthalic anhydride), which are the major components of the essential oil of the roots.
Oil fraction: terpenes (β-cadinene and cis-β-ocimene).
Aromatic compounds: phenol, o-cresol, p-cresol, guaiacol, 2,3-dimethylphenol, pethylphenol, m-ethylphenol, 4-ethylresorcinol, isoeugenol, carvacrol, 2,4-dihydroxyacetophenonecadinene, safrole, isosafrole and vanillin.
Among characteristic non-volatile constituents are phenylpropanoids ((E)-ferulic acid, coniferyl ferulate); benzenoids (valerophenone-o-carboxylic acid and vanillic acid) and **coumarins** (angelol G, angelicone and umbelliferone), and also 6-methoxy-7-hydroxycoumarin (scopoletin), 6-ethoxycoumarin.

Furthermore, have been found osthole and furocoumarins: bergapten (5-methoxypsoralen, 5-MOP), imperatorin, psoralen, oxypeucedanin.

Toxic component	Approach	Remarks	Acceptable amount	UF
Safrole Isosafrole Umbrelliferone	TTC	There are no quantitative data on the content of safrole and isosafrol. The essential oil contains also safrole, described in the EFSA compendium on botanicals containing toxic substances of concern (2009) as a weak carcinogen; as well as in rats and mice and a known genotoxic carcinogen, and isosafrole, a weak hepatocarcinogen in rats and mice.	0,15 µg/day	/

References:

- Bradley RR, Cunniff PJ, Pereira BJG, Jaber BL. Hematopoietic effect of Radix angelicae sinensis in a hemodialysis patient. *American Journal of Kidney Diseases* 1999, 34:349-354
- Chen SW, Min L, Li WJ, Kong WX, Li JF, Zhang Yj. The effects of angelica essential oil in three murine tests of anxiety. *Pharmacology Biochemistry and Behavior* 2004, 79:377-382
- Deng S, Chen S-N, Yao P Et al. Serotonergic activity-guided phytochemical investigation of the roots of Angelica sinensis. *J Nat Prod* 2006, 69:536-541
- EFSA. Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern. European Food Safety Authority. *EFSA Journal* 2009, 7:281
- EMA/HMPC/614586/2012
- Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet (Kroes et. al, 2003)

Arnica montana

flos

Main components

Sesquiterpenes: The most relevant constituents so far are helenalin and 11,13-dihydrohelenanin and their derivatives (0,3%-1%).

More recent investigations led to the detection of **methylated flavonoids** (0.4 to 0.6%) and further **sesquiterpene lactones**

essential oil (0.2 to 0.35%)

Toxic component	Approach	Remarks	Acceptable amount	UF
helenalin flavonoil	PDE_{oral}	Dermal use represents 99% of all preparations Genotoxicity: The mutagenic potential of an extract of Arnica (100 µl of extracts contain 100 mg dried Arnica, extract not further specified) was determined in the AMES-test using S. typhimurium TA98 and TA100 (with and without metabolic activation). The Arnica extract (10-400 ml) produced a 2-4fold increase in the number of revertants (except TA100 without metabolic activation). The authors ascertained that the mutagenic effects could be ascribed to flavonols present in Arnica montana.	0,000717 mg/kg/day	200 (NOEL) 1200 (PDE)

References:

- CIR Expert Panel (2001) Final report on the safety assessment of Arnica montana extract and Arnica montana. Int J Toxicol 20 (Suppl 2):1-11
- Göggelmann W, Schimmer O, Mutagenic activity of phytotherapeutic drugs. Prog Clin Biol Res, 1986, 206, 63-72
- EMA/HMPC/198794/2012
- Alonso D et al., Effects of topical Arnica gel on post-laser treatment bruises. Dermatol Surg, 2002, 28, 686-688
- Bergonzi MC et al., Evaluation of skin permeability of sesquiterpenees of an innovative supercritical carbon dioxide arnica extract by HPLC/DAD/MS. Pharmazie, 2005, 60, 36-38
- Corazza M et al., Use of topical herbal remedies and cosmetics: a questionnaire based investigation in dermatology out patients. Journal compilation European Academy of Dermatology and Venerology, 2009, 10.1111, 1468-3083
- Spettoli E et al., Contact dermatitis caused by sesquiterpene lactones. Contact Dermatitis, 1998, 9 (1), 49-50

Avena sativa

Herba cum fructus

Main components

Sugar fraction: mucilage (beta-glucan); 3 to 4% sugar (fructose, glucose),
Protein fraction: contains glutelin (> 50%) and avenin. The globulin of oats fruits could be separated into an acidic (32,500 - 37,500 Dalton) and a basic part (22,000 - 24,000 Dalton). The unreduced protein exists as disulfide-linked alpha/beta species of molecular weight 53,000 to 58,000. There is a considerable heterogeneity within both groups of polypeptides. The protein fraction of oats contains more lysine as compared to other cereals. Endosperm, hulls (outer shells), embryonic axis and scutellum are rich in glutamic acid. Various enzymes were identified, including alpha-amylase, phosphatase, tyrosinase, maltase and lipase. From a practical point of view the lipases are the most important. Hydrolysis of the triglycerides is undesirable, due to the soapy and bitter flavours which can result.
Lipid fraction: the grains of oats contain the highest lipid fraction among all feeding crops belonging to the family of the Poaceae. Unsaturated hydroxy fatty acids are formed by lipid peroxidase activity. Avenothionin was identified as a viscotoxin-like purothionin low molecular weight lipoprotein. It could be separated into alpha and beta avenothionin, of which the former had 47 amino acids.
Alkaloids: The indole alkaloid gramine is thought to be responsible for a weak sedative effect similar to *Passiflora incarnata.*
Organic acids: Diverse organic acids: malic, citric, malonic, aconitic, oxalic acid: (the latter up to 0.04%). Caffeic and ferulic acid have antioxidant properties. Avenanthramides are described as polyphenols in oats seeds. The latter represents a group of phenolic compounds which are not present in other cereal grains. Steaming and flaking of dehulled oat groats (inner kernel) resulted in moderate losses of avenanthramide Bp, while ferulic acid and vanillin increased. Avenanthramides Bc and Bf were not affected by steaming.
Flavonoids: To date, 28 flavonoids have been identified in the seeds and the green parts of the plant. Rhamnosylisoswertisin may have phytoalexin properties, protecting the plant against mycoses. Also 3 flavonolignanes derived from the flavone, triticin, were isolated from *Avena sativa* herb. In the known compounds a coniferyl alcohol moiety is linked to the flavone by an ether bond. In a new natural product, it is linked by C-C bonds.
Saponins may also protect oats against fungal infections. They are of the triterpene saponin type.
Steroids in the seeds like avenasterin and stigmasterin.
Vitamins: A, B1, B6, E

Toxic component	Approach	Remarks	Acceptable amount	UF
avenanthramide avenacoside A avenacoside B	Food Approach	Due the presence of saponins, of course, the intravenous route of administration must be excluded *Avena sativa* L. has been known for more than 4000 years as a food and the traditional medicinal usage of *Avena sativa* has been documented since the 12th century	3700 mg/die 1100 mg/die	/

References:

- EMEA/HMPC/202967/2007
- Hänsel R., Keller K., Rimpler H., Schneider G. Hager's Handbuch der Pharmazeutischen Praxis. Drogen A-D. Springer-Verlag, Berlin 1992: 437-46.
- Heinoven M., Ollilainen V., Linkola E., Varo P., Koivistoinen P. Carotenoids and retinoids in Finnish foods: cereal and bakery products. Cereal Chem. 1989; 66 (4): 270-3.
- Karmally W., Montez MG., Palmas W. Et al. Cholesterol-lowering benefits of oat-containing cereal in Hispanic Americans. J. Am. Diet. Assoc. 2005; 105: 967-70.
- Scientific Opinion on the substantiation of a health claim related to oat beta-glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006 - EFSA Journal 2010;8(12):1885

Camelia sinensis
folium non fermentatum

Main components
Methylxanthines: caffeine (2.5 to 4.2%), theophylline (0.02-0.04%), theobromine (0.15-0.2%)
Flavonoids: Flavonols: quercetin, kaempferol, myricetin mainly as 3-O-glycosides Flavones: apigenin, luteolin as C-glucuronides Flavanols: (flavan-3-ols 10-25%): (-)-epicatechin (EC), (-)-epicatechin-3-O-gallate (ECG), (-)- epigallocatechin (EGC) and (-)-epigallocatechin-3-O-gallate (EGCG)
Phenolic acids: including among others, chlorogenic acid, gallic acid, theogallin
Amino acids: 19 amino acids, amongst which theanine [5-N-ethyl glutamine (3% w/w)]
Terpene saponins (theafolia saponins): aglycones including among others, barringtogenol C, R1-barringenol
Polysaccharides (13 %) Proanthocyanidins (tannins**)**

Toxic component	Approach	Remarks	Acceptable amount	UF
EGCG Saponins	LHRD $NOEL_{EGCG}$ human oral $NOEL_{EGCG}$ human parenteral	In vitro studies by Schmidt et al., 2005 showed that high concentrations (100-500 µg/ml) of green tea extracts (containing 47.5-52.5% polyphenols) can damage rat hepatocytes. In a separate series of experiments EGCG (at concentration of 200 µM) was identified as the cytotoxic compound, in contrast with EC, caffeine and theanine. The authors concluded that extremely high concentrations were required in vivo, taking into account the low oral bioavailability of catechins. Polyphenons (extracts with a mixture of catechins) have even higher LD_{50}, suggesting extremely low toxicity of these (Hara, 2001)	250 mg (Spain TU)[5] 34,75 mg/Kg b.w./day 4,875 mg/Kg b.w./day	2000 2000

References:

- Gruenwald J., Brendler T., Jaenike C. PDR for Herbal Medicines. 2nd ed. Montvale, NJ: Medical Economics Company, Inc, 2004, 408-414
- Hara Y. Green tea- Health benefits and application, Marcel Dekker Inc, 2001, 18:178
- Schmidt M., Schmitz H.-J., Baumgart A., Guedon D., Netsch M.I., Kreuter M.-H., Schmidlin C.B., Schrenk D. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. Food Chem Toxicol 2005, 43:307–314

[5] On the Spanish market, as "traditional use"

Capsella bursa-pastoris

herba

Main components

Flavonoids (a.o. flavonglycosides): quercetin, tricin, diosmetin, kaempferol, luteolin, hesperitin and derivated glycosides (e.g. rutin, diosmin, hesperidin, luteolin-7-rutinoside, luteolin-7-galactoside, quercetin-3-rutinoside).
Amines: choline (1%), acetylcholine, histamine
Aminoacids: (22, a.o. proline, tyramine, and ornithine), (poly)peptides (a.o. α- and γ-aminobutyric acid, α-aminoadipic acid) and proteins
Aliphatic and phenolic acids: chlorogenic, vanilic, syringic, fumaric acid
Volatile oil, with at least 74 components identified, with camphor as major constituent (0.02%).
Resin
Saponins
Other constituents: 9-methylsulfinylnoyl and 10-methylsulfinyldecyl glucosinulates (in seeds), carotenoids, ascorbic acid, vitamin K, cardenolide, calcium and potassium salts, unidentified alkaloids, mustardoil glucoside (e.g. sinigrin, possibly responsible for the off-flavours in dairy products,).

Young leaves contain **Vitamin A** (5,000 IU/100 g) and **ascorbic acid** (91 mg/100 g)

Toxic component	Approach	Remarks	Acceptable amount	UF
sinigrin saponins	$NOEL_{human}$ $NOEL_{human\ parenteral}$	Signs of toxicity were described as sedation, enlargement of pupils, paralysis of hind limbs, difficulty in respiration, and death by respiratory paralysis A quaternary ammonium salt has been isolated from the herb Analysis of herbs and their decoctums and tinctures by ICP-OES revealed that Capsella contains essential elements as Ca, Cu, Cr, Fe, K, Mg, Mn, Na and Zn, of which some in relatively high amounts (Ca, Cr, K and Na) compared to the other 9 analysed plant species NOEL parenteral is calculated just as example. given the presence of saponins, the parenteral route of administration must be avoided	787,5 mg/Kg b.w./day 37,5 mg/Kg b.w./day	2000 2000

References:

- Barnes J, Anderson L, Philipson JD. Shepherd's Purse. Herbal Medicines, 3rd edition, Pharmaceutical Press, London 2007, 541-542.
- Martindale: The Complete Drug Reference. Shepherd's Purse.S. C. Sweetman. The Pharmaceutical
- Press 36th edition. London 2009
- Kuroda K, Kaku T. Pharmacological and chemical studies on the alcohol extract of Capsella bursapastoris. Life Sciences, 1969, 8(I):151-155.
- Szentmihalyi K, Taba G, Lado C, Fodor J, Then M, Szoke E. Medicinal plant teas recommended as natural source for element supplementation. Acta Alimentaria, 2005, 34(2):161-167

Carum carvi

Herba cum fructus

Main components

Caraway fruit

Caraway fruit contains 3-7 % v/m of essential oil, consisting largely of d-carvone (50-65 %), and (+)-limonene (up to 45 %), with less than 1.5 % of carveol and dihydrocarveol. It also contains 10-18 % of fixed oil, of which the main components are petroselinic (30-43 %), linoleic (34-37 %), oleic (15-25 %) and palmitic (4-5 %) acids. Other constituents include about 20 % of protein, about 15 % carbohydrates, phenolic acids, mainly caffeic acid, and traces of flavonoids such as quercetin, kaempferol and their glycosides. Carvenone, carvacrol and peril alcohol are found as distillation and storage artefacts.

Caraway oil

According to the European Pharmacopeia, caraway oil should contain 0.1-1 % β-myrcene, 30-45 % limonene, 50-65 % carvone and a maximum of 2.5 % of trans-dihydrocarvone and trans-carveol, respectively (Ph. Eur. 01/2008:1817).

d-Carvone: Carvone (p-mentha-6,8-dien-2-one) is a monoterpene ketone representative of the terpenes

Toxic component	Approach	Remarks	Acceptable amount	UF
Carvone	$NOEL_{oil\ human\ oral}$ $NOEL_{carvone\ human\ oral}$	The European Food Safety Authority (EFSA) Scientific Committee has published a safety assessment of d-carvone. In the assessment report, unpublished data from a 90-day NTP study in rats from the year 1982 is presented. The EFSA Scientific Committee concluded that relative liver weight of the surviving dose groups (93, 187 and 375 mg/kg) was statistically significantly increased compared with controls. For what above mentioned was not chosen the PDE approach for carvone but an UF 3000 for a restrictive approach	167,5 mg/Kg b.w./day 27,3 mg/Kg b.w./day	2000 3000

References:

- ESCOP Monographs 2nd ed. Carvi fructus – caraway fruit. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 64-69
- NTP Toxicology and Carcinogenesis Studies of d-Carvone (CAS No. 2244-16-8) in B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser 1990, 381:1-113
- European Food Safety Authority (EFSA). Scientific opinion on the safety assessment of carvone, considering all sourse of exposure. EFSA Journal 2014, 12 (7): 3806 74pp
- European Pharmacopoeia Online 8.2. Caraway fruit – Carvi fructus. Council of Europe. 01/2008:1080
- European Pharmacopoeia Online 8.2. Caraway oil – Carvi aetheroleum. Council of Europe. 01/2008:1817

Centaurium erythraea

herba

Main components

Secoiridoid glucosides are the characteristic bitter-tasting constituents, principally (75%) swertiamarin and smaller amounts of gentiopicroside (gentiopicrin) and sweroside (bitterness value ca. 12,000) and centapricin (bitterness value ca. 4,000.000). Other iridoids include bitter m-hydroxybenzoyl esters of sweroside, and de-acetylcentapicrin, centauroside (a dimeric secoiridoid), secologanin, 6'-m-hydroxy-benzoyl-loganin, dihydrocornin (a cyclopentane iridoid), gentioflavoside.
Secoiridoid alkaloids: gentianine and gentianidin**;**
Xanthones: 6 methoxylated xanthones, including eustomin (1-hydroxy-3,5,6,7,8-penta-methoxyxanthone) and 8-demethyl-eustomin and others;
Organic/Phenolic acids such as p-coumaric, O-hydroxyphenylacetic, ferulic, protocatechuic, sinapic, vanillic, syringic, hydroxyterephthalic and 2,5-dihydroxy-terephthalic acids and oleanolic acid (0.1%);
Phytosterols: β-sitosterol, stigmasterol, campesterol and others;
Coumarins: 5-formyl-2,3-dihydroisocoumarin**;**
Miscellaneous: flavone components and anthocyanes**.**

Toxic component	Approach	Remarks	Acceptable amount	UF
/	$PDE_{human\ oral}$	The authors stated that because of the lack of toxicity of the CE-extract given by the oral route, and relatively high NOAEL values for the i.p. dose in the acute study in mice, as well as lack of mortality or clinically significant adverse changes in the biological and haematological parameters, and the morphology of liver and kidneys in rats after 90 days of daily dosing, it may be concluded that the CE- extract is relatively non-toxic. Also, in view of the doses consumed empirically in traditional medicine in Morocco, there is a wide margin of safety for the therapeutic use of Centaurium erythraea (Tahraoui et al., 2010).	62,5 mg/Kg/day[6]	12000

References:

- Capasso F, Mascolo N, Morrica P, Ramundo E Phytotherapeutic profile of some plants used in folk medicine. Boll Soc Ital Biol Sper 1983, 59:1398-1404 (source: ESCOP Monograph)
- ESCOP monographs 2nd ed. Centaurii herba - Centaury. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 70
- European Pharmacopoeia 6th ed. Centaury – Centaurii herba. Council of Europe. 01/2008:
- Kumarasamy Y, Nahar L, Sarker SD. Bioactivity of gentiopicroside from the aerial parts of Centaurium erythraea. Fitoterapia 2003a, 74(1-2):151-154
- Tahraoui A, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of a lyophilized aqueous extract of Centaurium erythraea in rodents. J Ethnopharmacol 2010, 132(1):48-55

[6] ethanolic extract

Centella asiatica

herba

Main components

According to the European Pharmacopoeia the herbal substance consists of the dried, fragmented aerial parts, containing minimum 6% of total triterpenoid derivatives, expressed as asiaticoside ($C_{48}H_{78}O_{19}$; Mr 959.15)

Essential oil (0.1% of the plant): Terpene acetate, Germacrene, Caryophyllene, p-Cymol, Pinene;
Flavone derivatives: Quercetin glycoside, Kaempferol, glycoside and in free form Astragalin;
Sesquiterpenes: Caryophyllene, Elemene and bicycloelemene, Trans-farnesene, Ermacrene D;
Triterpenic steroids: Stigmasterol, Sitosterol;
Triterpenic acids: Asiatic acid, 6-hydroxy asiatic acid, Madecassic acid, Madasiatic acid, Betulinic acid, Thankunic acid, Isothankunic acid;
Triterpenic acid sugar esters (= saponins or pseudosaponins) (1-8% depending on country or origin): Asiaticoside (major component), Asiaticoside A, Asiaticoside B, Asiaticoside A (Madecassoside) and B, Braminoside, Brahmoside, Brahminoside, Thankuniside, Isothankuniside;

Toxic component	Approach	Remarks	Acceptable amount	UF
asiaticoside	$NEOL_{human\ oral}$ $LHRD_{asiaticoside\ topical}$	Chronic oral administration of the extract to rats at 150 mg/kg for 30 days led to no significant differences in body weight or consumption of food and water, nor to changes in plasma glucose, proteins, cholesterol or triglycerid levels, compared to controls. No macroscopic alteration in internal organs was evident (ESCOP 2009)	16,875 mg/Kg b.w./day (70% ethanolic dry extract from Centella 6:1) 12 mg[7]	2000

References:

- ESCOP Monographs 2nd ed. Supplement 2009. Centellae asiaticae herba. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2009
- Laerum OD, Iversen OH. Reticuloses and epidermal tumors in hairless mice after topical skin applications of cantharidin and asiaticoside. Cancer Res 1972, 32:1463-69
- Siddique YH, Ara G, Beg T, Faisal M, Ahmad M, Afzal M. Antigenotoxic role of Centella asiatica L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. Toxicol In Vitro 2008, 22:10-17
- Yen GC, Chen HY, Peng HH. Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food Chem Toxicol 2001, 39:1045-53

[7] Marketing authorization in Italy; AIC: 016222073 - https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_000143_016222_RCP.pdf&retry=0&sys=m0b1l3

Chelidonium majus

herba

Main components

Benzylisoquinoline type (0.01-1%): with at least three subgroups**:**
Benzophenanthridines: chelerythrine, chelidonine, sanguinarine, isochelidonine
Protoberberines: berberine, coptisine, dihydrocoptisine, stylopine
Protopine;
Acids: chelidonic, malic, citric, caffeic (0.4%) ferulic (0.02%), p-coumaric (0.06%), gentisic and phydroxybenzoic acids
Hydroxycinnamic acid derivates: (-)-2-(E)-caffeoyl-D-glyceric acid, (-)-4-(E)-caffeoyl-L-threonic acid, (-)-(2)-(E)-coffeoyl threonic acid lactone, (+)-(E)-caffeoyl-L-malic acid
Others: a saponine, carotenoids, a phytocytostatin (chelidocystatin), sparteine and flavonoids.

Toxic component	Approach	Remarks	Acceptable amount	UF
chelidonine and other alkaloids Berberine	PDE_{oral}	It was found that the fresh plant can cause acute toxicity due to the latex. Drying of the plant considerably reduces the toxicity. The use of therapeutic doses is safe due to the low quantity of alkaloids in the plant preparations. Large doses can irritate the gastro-intestinal tract. An excessive use for long periods should be avoided because of the risk of hepatotoxic effects, including severe hepatitis, severe cholestasis and fibrosis. A mechanism for C. majus induced hepatotoxicity has not been established. In the literature, deadly poisonings have been described with children after eating the plant (Hänsel et al. 1992). The NOEL of Chelidonium fluid extract is the highest administered dose, 1820 mg/kg body weight/day, corresponding to 2.68 mg/kg body weight total alkaloids. (Mheddhbi 2002)	182 mg/kg/day	500

References:

- ESCOP Monographs 2nd ed. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 74-78
- Hänsel R, Keller K, Rimpler H, Schneider G, editors. Hagers Handbuch der Pharmazeutischen Praxis. 5th ed. Springer Verlag, Berlin 1992
- Mazzanti G, Di Sotto A, Franchitto A et al. Chelidonium majus is not hepatotoxic in Wistar rats, in a 4-week feeding experiment. Ethnopharmacol 2009, 126: 518-24
- Mheddhbi, S. Four-week toxicity study by oral route in rats. Weleda 2002
- Stiborova M, Vostalova J, Zdarilova A, Ulrichova J, Hudecek J, Tschirner K, Simanel V. Macleaya cordata extract and Sangrovit genotoxicity assessment in vivo. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2008, 152 (1): 35-39

Cola nitida

semen

Main components

Purine alkaloids: Cola seeds contain purine bases chiefly represented by caffeine, ranging from 1.5 to 3.2% (2.5% on average in the dried drug)

Phenolic compounds: The tannin content of Cola seeds is 5-10%. Other notable constituents are the flavan-3-ol type

polyphenols: (+)-catechin, (-)-epicatechin, and proanthocyanidin dimers of group B, Tannins also include colatin, colatein, colanin .

Mineral contents: Ca (0.07-0.09%), Na, K (1.01-1.47%), Mg (0.2-0.27%) and Fe. Other trace elements determined were Zn, Co, Mn, Cu and Cr.

Others: dimethylamine, pyrrolidine and piperidine methylamine, ethylamine, isobutylamine and isopentylamine, aminocids.

Toxic component	Approach	Remarks	Acceptable amount	UF
Cr dimethylamine	food $NOEL_{caffeine\ human\ oral}$	The Cola nitida semen herbal substances/preparations are in the list of ingredients that may be used in food supplements in Italy updated on 1 April 2009 with the following indications: Tonic; physical and mental tiredness. Stimulation of metabolism. Because caffeine and methylxanthines are principal components of Cola seeds, at least some of the potential toxicities could be due to them. Methylxanthine toxicity has been assessed extensively elsewhere.	3,75 mg/Kg b.w./day	2000

References:

- Burdock GA, Carabin IG, Crincoli CM. Safety assessment of kola nut extract as a food ingredient. Food Chem Toxicol 2009; 47: 1725-32
- EMA/HMPC/722365/2010
- Eteng MU, Eyong EU, Akpanyung EO, Agiang MA, Aremu CY. Recent advances in caffeine and theobromine toxicities: a review. Plant Foods Hum Nutr 1997; 51: 231-43
- European Pharmacopoeia 7th ed. Colae semen. Council of Europe. 01/2008:1504 corrected 6.0
- FDA. Listing of Food Additive. http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm [accessed Nov. 2011]
- The Merck Index, 11th edition

Curcuma xanthorrhiza

rhizoma

Main components

Carbohydrates: 69.4% of total mass.
Curcuminoids: this is a mixture of curcumin (diferuloylmethane), monodexmethoxycurcumin and bisdesmethoxycurcumin. Curcumin makes up approximately 90% of the curcuminoid content in turmeric. The phenolic groups in the structure of curcumin explain the ability of curcumin to eliminate oxygen-derived free radicals. The free radicals which can be eliminated by curcumin are hydroxyl radical, singlet oxygen, superoxide radical, nitrogen dioxide and NO.
Curcumin content of the *Curcuma longa* rhizome varies from 0.6 to 5% of the dry mass. The dry turmeric rhizomes contain 3-5% curcumin, the curcumin content of turmeric oleoresin is 40%.
Essential oil: 5.8% of total mass, constituents are: a-phellandrene 1%, sabinene 0.6%, cineol 1%, borneol 0.5%, zingiberene 25%, and sesquiterpenes 53%. The mono- and sesquiterpenes include zingiberene, curcumene, α- and β-turmerone.
Mineral matter: 3.5% of total mass.
Moisture: 13.1% of total mass.
Polypeptides.
Protein: 6.3% of total mass.
Fatty oil.

Toxic component	Approach	Remarks	Acceptable amount	UF
Essential oil	$food_{ADI\ curcumin}$	Donatus et al. (1990) observed curcumin to be moderately cytotoxic in vitro, inducing slightly increased LDH-leakage from rat hepatocytes, accompanied by an increase in GSH-depletion. For the national toxicity programme (NTP) longterm (103-weeks) dietary exposure studies were performed in rats and mice. Based on the findings in rats the NOEL for gastrointestinal irritation (ulcers, hyperplasia and inflammation) was established at 440 mg curcumin/kg/day. In mice, there were absolute and relative increases in liver weights after 15 months of treatment, with a NOEL of 220 mg/kg/day (NTP, 1993). Based on these results and reckoned with a **safety factor of 200**, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established at its 44th meeting the temporary ADI to 0–1 mg/kg for human, pending submission of the results of a study on reproductive toxicity	0-1 mg/Kg	/

References:

- Donatus AA, Sardjoko, Vermeulen NPE. Cytotoxic and cytoprotective activities of curcumin. Biochem Pharmacol 1990, 39(12):1869-1875
- EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific Opinion on the reevaluation of curcumin (E 100) as a food additive. EFSA Journal 2010, 8(9):1679 doi: 10.2903/j.efsa.2010.1679
- JECFA: Joint FAO/WHO Expert Committee on Food Additives, 44th meeting: WHO Food Additives Series 35: Curcumin.
- Lin SC, Lin CC, Lin YH, Supriyatna S, Teng CW. Protective and therapeutic effects of Curcuma xanthorrhiza on hepathotoxin-induced liver damage. Am J Chin Med 1995, 23:243-254
- National Toxicology Program (NTP). Toxicology and Carcinogenesis Studies of Turmeric Oleoresin (Cas No. 8024-37-1) in F344/N Rats and B6C3F1 Mice (Feed Studies). Technical Report Series No. 427, NIH Publication No. 93-3158. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC 1993

Eleutherococcus senticosus maxi

radix

Main components

phenyl propane compounds: eleutheroside B – 0.5%, chlorogenic acid – up to 0.3% , coniferyl aldehyde and its glucoside, caffeic acid derivates;
lignanes: eleutheroside E 0.1%, Eleutheroside E2, eleutheroside B4 0.023%, Eleutherosid D 0.10%, Eleutherosid E1, Syringaresinol, (+)-pinoresinol di-O- β-Dglucoside;
coumarins: isofraxidin and its O-glucoside eleutherosid B1, 7-ethylumbelliferone;
triterpensaponines: daucosterol (eleutheroside A), β -hederin (Eleutheroside K), 2-protoprimulagenin A-glycoside – 0.125%.
polysaccharides: heteroglycans, eleutherans
Other constituents: steroids, carbohydrates and essential oil 0.8%

Toxic component	Approach	Remarks	Acceptable amount	UF
isofraxidine	$NOAEL_{human}$ from an in human study (6 month)	The toxicity of Eleutherococcus extracts is reported to be extremely low. The oral acute LD_{50} of powdered Eleutherococcus in mice is reported to be in the range of about 30 g/kg	500 mg	/

References:

- EMA/HMPC/680615/2013
- Gardner Z. and McGuffin M., 2013. Eleutherococcus senticosus (Rupr. & Maxim.) Maxim., Araliaceae. American Herbal Association's Botanical Safety Handbook. CRC Press. Boca Raton (Florida, US), 329-331.
- Lee YJ, Chung HY, Kwak HK, Yoon S, 2008. The effects of A. senticosus supplementation on serum lipid profiles, biomarkers of oxidative stress, and lymphocyte DNA damage in postmenopausal women. Biochem Biophys Res Commun, 375(1):44-48.
- Lee D, Park J, Yoon J, Kim MY, Choi HY, Kim H, 2012. Neuroprotective effects of Eleutherococcus senticosus bark on transient global cerebral ischemia in rats. J Ethnopharmacol, 139 (1):6-11.
- Kuo J, Chen KW, Cheng IS, Tsai PH, Lu YJ and Lee NY, 2010. The effect of eightweeks of supplementation with Eleutherococcus senticosus on endurance capacity andmetabolism in human. Chin J Physiol, 53(2):105-111.

Epilobium angustifolium

herba

Main components

Tannins and related compounds (4-14%): e.g. oenothein B, oenothein A, tri-, tetra-, and penta-Ogalloylglucose

Flavonoids (1-2%): e.g. kaempferol, quercetin, myricetin.

Phenolic acids and their derivatives: e.g. ellagic acid, valoneic acid dilactone, chlorogenic acid, neochlorogenic acid, coumaroylyquinic acids, feruloylquinic acids, gallic acid, cinnamic acid, protocatechuic acid, caffeic acid, ferulic acid.

Steroids (ca.0.4%) and **triterpenes** (ca. 1.5%): e.g. cholesterol, campesterol, stigmasterol, β-sitosterol, ursolic acid, corosolic acid, oleanolic acid

Other constituents: e.g. linoleic acid, palmitic acid, stearic acid, eicosenoic acid, behenic acid, arachidic acid

Toxic component	Approach	Remarks	Acceptable amount	UF
/	$LHRD_{decotion\ oral}$ $LHRD_{ethanolic\ extract}$ $NOEL_{human}$	On the market as herbal tea since 11/04/2005 in Hungary, and as oral drops since 25/07/2002 (only as combination products therefore this information cannot sustain the safety and thus is reported as additional information.) herbal tea is widely distributed in the food sector in Poland, also in combination products. However, no further product-specific details are given	350 mg/die 100 mg/die 35 mg/Kg b.w./day	2000

References:

- EMA/HMPC/712510/2014
- Kujawski R, Ozarowski M, Derebecka-Holysz N, Bartkowiak-Wieczorek J, Bogacz A, Karasiewicz M, et al. Effect of Willow herb (Epilobium angustifolium L.) extract on gene expression of selected P450 cytochromes in rat liver – preliminary study. Herba Pol 2009, 55(4):52-64
- Roman A, Rusu MA, Puica C, Borsa M. Citotoxic effects of three speies of Epilobium (Onagraceae) herbal extracts in rats. Studia Universitatis "Vasile Goldis", Seria Stiintele Vietii 2010, 20(1):19-23
- Vitalone A, Bordi F, Baldazzi C, Mazzanti G, Saso L, Tita B. Anti-proliferative effect on a prostatic epitheliala cell line (PZ-HPV-7) by Epilobium angustifolium L. Il Farmaco 2001, 56:483-489

Equisetum arvense

herba

Main components

Inorganic constituents: with 5-7.7% silicic acid (or silicates respectively) of witch 10% are water- soluble, 1.5% aluminium chloride, potassium chloride and manganese

Flavonoids: mostly kaempferol- and quercetin glycosides and their malonyl esters, luteolin-5-O-β-D-glucoside, apigenin-5-O-β-D-glucoside und 6-chloroapigenin; the pharmacopoeial standard is minimum 0.3% of total flavonoids**.**

Alkaloids: small amounts of nicotine, 3-methoxypyridine, traces of palustrin are possible; equisetonin, a saponin-complex, i a mixture of sugars and flavonoids; Schneider & Kubelka (1989) suggested, equisetonin should be cancelled from the list of compounds of horsetail, as it does not exist

Toxic component	Approach	Remarks	Acceptable amount	UF
apigenin	$PDE_{human\ oral}$	Tago et al. (2010) evaluated the influence of administration of an aqueous extract of Equisetum arvense (no further information) in diet at doses of 0, 0.3, 1 and 3% for 13 weeks in male and female F344 rats (1% was thought to mirrow a proximate dosage level of 500 mg/kg). No death or obvious clinical signs were noted in any of the animals. The NOAEL was determined to be >1.79 g/kg/day (males) and >1.85 g/kg/day (females) under the condition of the study.	179 mg/Kg/die	500 (PDE on NOAEL of male rats)

References:

- Dos Santos JG Jr, Do Monte FH, Blanco MM, Lanziotti VM, Maia FD, Leal LK. Cognitive enhancement in aged rats after chronic administration of Equisetum arvense L. with demonstrated antioxidant properties in vitro. Pharmacology Biochemistry and Behaviour 2005b, 81(3):593-600
- European Pharmacopoeia 8th ed. Equisetum Stem - Equiseti herba.
- Joksić G, Stanković M, Novak A. Antibacterial medicinal plants Equiseti herba and Ononidis radix modulate micronucleus formation in human lymphocytes in vitro. Journal of Environmental Pathology, Toxicology and Oncology 2003, 22(1):41-48
- Schneider K, Kubelka W. Zur Hämolytischen Wirkung von Schachtelhalmkraut (Herba Equiseti). *Scientia Pharmaceutica* 1989, 57(3):214-215
- Tago Y, Wei M, Ishii N, Kakehashi A, Wanibuchi H. Evaluation of the subchronic toxicity of dietary administered Equisetum arvense in F344 rats. Journal of Toxicologic Pathology 2010, 23(4):245-251

Euphrasia officinalis

herba

Main components

Flavonoids: 0.38% apigenin, luteolin, kaempferol, rhamnetin, quercetin
Polyphenols: 1.47%
Phenolic acids: caffeic acid and its ester derivatives, chlorogenic acids and coumaric acids
Hydroxycinnamic derivatives: 1.97%
Tannins: 0.56%
Iridoids: aucubin 0.05%

Toxic component	Approach	Remarks	Acceptable amount	UF
Apigenin	TTC[8]	From a phytopharmacological point of view Euphrasia may be associated with different effects: adstringent (due to the tannins) and anti-inflammatory (due to the iridoids). However, no studies have been performed. Tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed. Only one study concerning the acute toxicity of aqueous eyebright extract on six mice is in litterature.	1800 µg/day	/

References:

- British Herbal Pharmacopoeia. Scientific Committee, British Herbal Medicine Association, Bournemouth 1983, 89–90
- Chudnicka A, Matysik G. Research of enzymatic activities of fresh juice and water infusions from dry herbs. J Ethnopharmacol 2005; 99(2):281-286
- EMA/HMPC/246799/2009
- Porchezhian E, Ansari SH, Shreedharan NK. Antihyperglycemic activity of Euphrasia officinale leaves. Fitoterapia 2000; 71(5):522-526
- Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet (Kroes et. al, 2003)

[8] TTCs values are set under the munro's scheme because are not intended for a chronic use.

Filipendula ulmaria

herba

Main components

Salicylates are the main components of the **volatile oil,** mainly salicylaldehyde (up to 70%). The amount of salicylates, mostly present in the form of glycosides, is assumed to be less than 0.5%

Flavonoids: from 3-4% in the flowering herb up to 6% in the fresh flowers, in particular spiraeoside (quercetin-4'-glucoside), also hyperoside, other quercetin and kaempferol derivatives, as kaempferol- 4'-glucoside.

Tannins: hydrolysable type, ranging from 1% in ethanolic extracts to 12% in aqueous extracts, predominantly the dimeric compound rugosin D.

Miscellaneous: coumarin (trace), mucilage, carbohydrates, ascorbic acid.

Toxic component	Approach	Remarks	Acceptable amount	UF
/	$LHRD_{oral}$ $NOEL_{human\ parenteral}$	The Council of Europe categorises Filipendulae ulmariae herba as a natural source of food flavouring that can be added to foodstuffs in small quantities, with a possible limitation of an active principle (as yet unspecified) in the final product (Barnes et al., 2007) Due to the presence of salicylates, Filipendula ulmaria should not be used in cases of hypersensitivity to salicylates (Wichtl, 1994). In the ESCOP Monographs (2003), it is stated: "Herb is used as supportive therapy for the common cold and to enhance the renal elimination of water."	200 mg[9] 0,1363 mg/Kg b.w./day	2000 (NOEL Rabbit) 2500 (PDE)

References:

- Barnaulov OD, Kumkov AV, Khalikova NA, Kozhina IS, Shukhobodskij BA. [Chemical composition and primary evaluation of the properties of preparations from Filipendula ulmaria (L.) Maxim flowers.] Rastit Resur 1977, 13:661-669
- ESCOP Monographs 2nd ed. Filipendulae ulmariae herba – Meadowsweet. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 157-161
- European Pharmacopoeia 6th ed. Meadowsweet – Filipendulae ulmariae herba. Council of Europe. 01/2008:1868, 2344-2345
- Wichtl M. Herbal drugs and Phytopharmaceuticals. 1st ed. Medpharm Scientific Publishers GmbH, Stuttgart 1994, 480-482

Fraxinus excelsior

[9] On the French market, as "traditional use"- powdered herbal substance

folium

Main components

Simple coumarins: only in traces (0.01-0.05%). These compounds are represented in the herbal substance by: esculin, fraxin; esculetin, fraxetin; cichoriin; scopoletin, isoscopoletin.
Iridoids: Very limited data are available on the occurrence of these compounds in the drug: deoxysyringoxide, hydroxynuezhenide, syringoxide; deoxysyringoxidine.
Secoiridoids: A larger variety of these compounds were isolated: 10-hydroxyligstroside, 7-β-D-glucopyranosyl-11- methyl-oleoside, oleoside-11-methyl-ester; oleoside-7,11-dimethyl-ester; excelsioside; oleuropein, ligstroside.
Flavonoids: 1.4% rutoside (= rutin) 0.5%, the amount of these compounds can vary between 0.6-2.2%, from which 0.1-0.9% can be rutoside, but kaempferol-3-O-glucoside, quercetrin-3-O-glucoside, and their respective 3-O- rhamnoglucosides can also be found.
Triterpenes: Data on the occurrence of these compounds are very limited: β-sitosterol, betuline, betulinic and ursolic acid (the latter in 0.7-2.5%).
Simple phenolic acids 3.2%: ferulic, caffeic, p-coumaric, p-hydroxybenzoic, protocatehuic, sinapic, syringic and vanillic acids can be found in the drug
Alkanes: hentriacontane, nonacosane, tetratriacontane.
Other components*:* Mucilaginous 15.3% (broader interval from 9.5% to 22.2%); 2.5% of tannins (the range of 0.6-4%).
Minerals: potassium 1.7%.

The aqueous dry extract contains approximately 5.7% phenolic components, while the aqueous-ethanolic (70% EtOH V/V) contains 8.8% (Gaedcke, 1993).

Toxic component	Approach	Remarks	Acceptable amount	UF
	$LHRD_{human\ oral}$ TTC	Esculetin was proven to be of sensitising potency within the frame of studies performed on coumarins used in perfumery, cosmetics and therapeutic ointments. Esculetin, esculin and isoscoloplamine showed a local photosensitising effect in an animal study. Coumarin compounds detected so far in ash leaf do not possess the minimum structural requirements (a C-4 hydroxyl substituent and a C-3 non-polar carbon substituent) for anticoagulant activity. Toxicological data is very limited. Due to the lack of data on acute and chronic toxicity, repeated dose toxicity, genotoxicity, mutagenicity, carcinogenicity, reproductive and developmental toxicity, the safety of the therapeutic application of ash leaf cannot be substantiated. Nonetheless, neither the chemical composition nor the long-term widespread use in the European Union suggests that there is any risk associated with the use of ash leaf products, thus the use can be suggested to be deepened.	750 mg/die[10] 540 µg/day	/

References:

- Barnes J, Anderson LA, Phillipson JD. Chamomile, Roman. In: Barnes J, Anderson LA, Phillipson JD. Herbal Medicines. 3rd Edition. Pharmaceutical Press, London, Chicago 2007, 157
- Gaedcke F. Fraxinus. In: Hänsel R, Keller K, Rimpler H, Schneider G, editors. Hagers Handbuch der Pharmazeutischen Praxis. Drogen E-O, Vol 5. 5th ed. Springer-Verlag, Berlin, 1993, 188-200
- Kostova I, Iossifova T. Chemical components of Fraxinus species. Fitoterapia 2007, 78:85-106
- Masamoto Y. [Sensitization and crossreaction of simple coumarins.] Yakugaku Zasshi. 2001, 121:97- 103 [Japanese]
- Ph. Eur. 6th ed. Ash leaf - Fraxini folium. Council of Europe. 01/2008:1600; 1222-1223

Fucus vescicolosus

[10] On the Spanish market, as “traditional use” - powdered leaves in capsules - since 1987 - indication: arthritis and diuretic

thallus

Main components

Minerals: iodine (mostly bound in organic substances), with a minimum of 0.03 and a maximum 0.2 per cent of total iodine determined on the dried drug. sodium, potassium, calcium, magnesium, iron, phosphor, sulphates, copper, chrome, chloride, zinc, manganese, silicon and selenium.
Polysaccharides: laminarin. The content of alginic acid is estimated at 12%. Alginic acid is a linear polymer with various sequences of beta-(1-4)-Dmannuronic acid and alpha-(1-4)-L-guluronic acid residues; fucans of varying structure such as fucoidancomposed mainly of alpha-(1-2)-L-fructose-4-sulphate residues.
Polyphenols: ca. 15%, composed of phloroglucinol units. Most are high in molecular weight (25% greater than 10,000), phlorotannins consisting of carbon-carbon or ether linked phloroglucinol units in linear chains with numerous side branches.
Lipids: glycosyldiacylglycerids, phosphatidylethalolamin, phosphatidylcholin, eicosapentaeenacid (EPA), arachidonic acid (AA).
Sterols: fucosterol, β-sitosterol
Polyphenols: phlorotanin
Pigments: fucoxanthin, zeaxanthinlutein, violaxanthin, neoxanthin, fucoxanthinol, β-carotene, squalene.
vitamins: C, B1, B2, B3, B6, folic acid, choline, vitamin K
Other constituents: pectin-like membrane slime, ethereal oil, phloroglucinol, mannitol, sorbitol, aminoacids, proteins.

Toxic component	Approach	Remarks	Acceptable amount	UF
Phlorotannins Fucophlorethols	$LHRD_{human\ oral}$	Possible contamination with heavy metals Phlorotannins from Fucus vesiculosus inhibited α-amylase and α-glucosidase and inhibited the accumulation of advanced glycation products by scavenging reactive carbonyls. Fucophlorethols from Fucus vesiculosus inhibited CYP1A and aromatase.	120 mg/die[11] 130 mg/die[12]	/

References:

- Barnes J, Anderson AA, Phillipson JD. Herbal medicines. 3rd ed. Pharmaceutical Press London, Chicago 2007, 273-275
- British Herbal Compendium. Vol. 1. British Herbal Medicine Association, Dorset 1992, 37-39
- EFSA. Tolerable upper intake levels of vitamins and minerals. European Food Safety Authority, 2006 135-150
- Zaragoza MC, Lopez D, Saiz MP, Poquet M, Pérez J, Puig-Parellada P et al. Toxicity and antioxidant activity in vitro and in vivo of two Fucus vesiculosus extracts. J Agr Food Chem 2008, 56:7773-7780
- Williamson E, Driver S, Baxter K. Stockley's Herbal Medicines Interactions. 1st ed. Pharmaceutical
- Press, 2009, 265-266

Fumaria officinalis

[11] On the United Kingdom market, as "traditional use" - Fucus Aqueous Powdered Extract 5:1

[12] On the French market, as "traditional use"- powder of Fucus vesiculosus

herba

Main components

Alkaloids: (0.3-1% calculated as protopine (0.13%)
protopines, the quantitatively predominant type, as protopine (fumarine) and cryptopine, Protoberberines: aurotensine, stylopine, N-methylsinactine and others, Spirobenzylisoquinolines: fumaritine, fumaricine anf fumariline and others.
Benzophenanthridines such as sanguinarine and corydamine (traces),
Indenobenzazepines: fumaritrididine and fumaritrine
Flavonoids: principally glucosides of quercetin such as isoquercitrin, rutin, quercetin-3,7-diglucoside- 3-arabinoglucoside
Acids: chlorogenic and caffeic acids, also fumaric acid, caffeoylmalic acid and other aliphatic acids

Toxic component	Approach	Remarks	Acceptable amount	UF
sanguinarine corydamine	$LHRD_{human\ oral}$ $TTC_{ethanolic}$	The clinical experience with a Fumaria officinalis nebulisate (water extract of 4-6:1) as an amphocholinergic agent used in 64 patients suffering from biliary disorders (dyskinesia, hepatopathy etc.) has been published by Zawodsky (1974). All patients were treated with 3 tablets containing 250 mg of the extract daily, for 21 days. No adverse effects were noticed and an excellent tolerability was reported. (also if some human data are available the literature is poor, no representative data of LD_{50} is found so for an ethanolic extrac is suggested to apply this value of TTC to observe a restrictive approach)	200 mg/day[13] 540 µg/day	/

References:

- Bradley PR editor. British Herbal Compendium. Vol 1. Fumitory - Fumariae herba. British Herbal Medicine Association, Bournemouth 1992, 102-104.
- European Pharmacopoeia 6th ed. Fumariae herba. Council of Europe. 1/2008: 1952.
- Forgacs P, et al. Alcaloides des Papavéracées II: Composition chimique de dix-sept espèces de Fumaria. Plantes Med Phytother 1986, 20: 64-81.
- Wynne PM, Vine JH, Amiet RG. Protopine alkaloids in horse urine. J Chromatogr B Analyt Technol Biomed Life Sci 2004, 811: 85-91.
- Zawodsky, L. Klinische Erfahrungen mit dem Amphocholeretikum Oddibil. [Clinical experience with Fumaria officinalis nebulisate, an amphocholinergic agent.] Wiener Medizinische Wochenschrift 1974, 124(46): 677-680.

[13] On the Austrian market, as "well-established use" - Dry extract, extraction solvent water, DER 5:1, not less than 2.5 mg alkaloids calc. as protopine per film coated tablet. 1 film-coated tablet contains 250 mg extract

Gentiana lutea

radix

Main components

Bitter constituents (2-8%) are located mostly in the cortex of the root. The most bitter constituents belong to the class of secoiridoid glycosides, with gentiopicroside (also known as gentiamarine and gentiopicrine) as main component and a lower amount of amarogentine (0.025 – 0.4%)
Up to 1% **xanthones**: gentisine, isogentisine, methylgentisine, gentiseine xanthones are also responsible for the yellow colour of the root.
Carbohydrates: 30-55% carbohydrates in the dried root including monosaccharides (glucose and fructose), disaccharides (saccharose and gentiobiose), trisaccharide (gentianose) and polysaccharides (e.g. pectins).
volatile oil 0.1 – 0.2%; used mainly in the liqueur-production for giving its characteristic flavour.
Other constituents: phytosterols, triterpenes

Toxic component	Approach	Remarks	Acceptable amount	UF
/	LHRD	Different cases of poisoning in humans are described. The most cases were due to an adulteration or mistaken use of Veratrum album.	1200 mg/die[14] 1000 mg/die[15] Proved to be on the European market for a period of 30 years	/

References:

- Commission E Monographs. Enzianwurzel - Gentianae radix. Bundesanzeiger 223, 1985. Translated in Blumenthal M., Busse W.R., Goldberg A., Gruenwald J., Hall T., Riggins W., Rister R., Klein S. The Complete German Commission E Monographs.
- EMA/HMPC/578322/2008
- ESCOP Monographs 2nd ed. Gentianae Radix – Gentian Root. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart-New York 2003, 174-177.
- European Pharmacopoeia. Ph. Eur. 6.0, 1380 (01/2008)
- Leslie G.B., Salmon G. Repeated dose toxicity studies and reproductive studies on nine Bio-Strath herbal remedies. Swiss Med 1979, 1: 43-45.
- Matsushima T., Araki A., Yagame O., Muramatsu M., Koyama K., Ohsawa K. et al. Mutagenicities of xanthone derivatives in Salmonella typhimurium TA100, TA98, TA97 and TA2637. Mutation Res 1985, 150: 141-146.
- Wegner T. Anwendung eines Trockenextraktes aus Gentianae luteae radix bei dyspeptischem Symptomkomplex. Z Phytotherapie 1997, 19: 163-164.

[14] Marketing authorization in Germany - (commonly as dry extract from Gentianae radix (4.5-5.5:1) ethanol 53% v/v)

[15] As fluid extract (1:1); ethanol 45% v/v

Ginkgo biloba
folium

Main components
Terpenes: Triterpenelactones (diterpenes: ginkgolides A, B, C, J (0.06-0.23%) sesquiterpene: bilobalide (up to 0.26%)) Triterpenes (steroids, phytosterols) Carotenoids, Polyprenols (di-trans-poly-cis-octadecaprenol) concentration ranges from 0.04% to 2.0%, Volatile terpenes
Flavonoids not less than 0.5%, Flavanols (catechins), Flavones (aglycones, monoglycosides and biflavones with a concentration of 0.4% to 1.9%)
Flavonols (the aglycones isorhamnetin, kaempferol, quercetin and myricetin have a concentration of 0.2% to 1.4% w/w)
Organic acids Polyacetate derived compounds, Alkyl phenolic acids and alkyl phenols (ginkgolic acid, cardanols (approx. 0.1%)), Long chain hydrocarbons (waxes)
Lipids
Others: carbohydrates, miscellaneous organic compounds, inorganic compounds

Toxic component	Approach	Remarks	Acceptable amount	UF
alkylphenols	$LHRD_{human\ oral}$ $NOEL_{human\ oral}$	In crude ginkgo extracts a group of alkylphenols (e.g. ginkgolic acids, ginkgol, bilobol) has been described to exhibit potential contact allergenic and toxic properties. A maximum concentration of 5 ppm has to be maintained to comply with the Ph. Eur. and to ensure safety of use for Ginkgo biloba leaf extracts. no chronic toxicity reported. There was no evidence of organ damage or impairment of hepatic and renal functions when EGb 761 was administered orally to rats and mice over a period of 27 weeks in doses ranging from 100 to 1,600 mg/kg.	120 mg/die [16] 47,5 mg/Kg b.w./day	2000

References:

- Bebbington A, Kulkarni R, Roberts P. Persistent bleeding after total hip arthroplasty caused by herbal self-medication. The Journal of Arthroplasty 2005, 20(1):125-126
- DeFeudis FV. Ginkgo biloba extract EGb 761: from chemistry to the clinic. Ullstein Medical, Wiesbaden 1998
- ESCOP Monographs 2nd ed. Ginkgo folium – Ginkgo leaf. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 178-210
- Esposito M, Carotenuto M. Ginkgolide B complex efficacy for brief prophylaxis of migraine in school- aged children: an open-label study. Neurological Sciences 2011, 32(1):79-81
- Guideline on medicinal products for the treatment of Alzheimer's disease and other dementias from Committee for medicinal products for human use (CHMP). Doc. Ref. CPMP/EWP/553/95 Rev. 1. European Medicines Agency, London 2009
- Leistner E, Drewke C. Ginkgo biloba and ginkgotoxin. Journal of Natural Products 2010, 73(1):86-92
- Salvador RL. Herbal medicine - Ginkgo. Canadian Pharmaceutical Journal 1995, 39-41, 52
- NTP technical report on the toxicology and carcinogenesis studies of Ginkgo biloba extract (CAS No. 90045-36-6) in F344/N rats and B6C3F1/N mice (gavage studies). National toxicology program, Research triangle park, North Carolina 2013

[16] "well-estabilished use" on the German, Belgian and Denmark market.

Grindelia robusta

herba

Main components

resin (5-20% depending on the species) consisting mainly of diterpenic acids such as grindelic acid, 7-8-epoxygrindelic acid and 17-acetoxygrindelic acid; acetylenic compounds such as matricarianol and marticarianol acetate

flavonoids such as kaempferol-3-methylether and kaempferol-3,7-dimethylether and various quercetin-methylethers and main compounds quercetin-3-methyl-ether and 6-OH-kaempferol-3,6- dimethylether

triterpenoid saponins with grindelia sapogenin D, bayogenin and oleanolic acid as the sapogenins

phenolic acids such as chlorogenic, p-hydrobenzoic and p-coumaric acids

approximately 5% of **tannins**

approximately 0.2% of **essential oil** consisting mainly of mono- and sesquiterpenes, and especially for G. robusta - borneol (15.2%), alpha-pinene (10.3%), trans-pinocarveol (7%), bornyl acetate (4.5%), limonene (4.3%)

Toxic component	Approach	Remarks	Acceptable amount	UF
Essential oil	TTC	The lack of subrchronic, genotoxicity, carcinogenicity as well as reproductive and developmental toxicity studies do not allow the establishment of rappresentative NOEL also if some acute toxicity studies are available. No mortality occurred and no toxic effects were apparent in rats after a single oral dose of a Grindelia robusta dry extract (80% ethanol) at 2.5 g/kg body weight In sensitive persons, irritation of the gastric mucosa might occur (ESCOP 2009) while side effects listed in older scientific literature include gastric irritation and diarrhoea (Gruenwald *et al.* 2007) as well as irritation of kidney and/or stomach at high doses	540 µg/day	/

References:

- EMA/HMPC/748221/2011
- ESCOP Monographs. 2nd ed. Grindeliae Herba monograph. European Scientific Cooperative on Phytotherapy, editor. Georg Thieme Verlag, Stuttgart, Supplement 2009, 131-134
- Goetz P. Plantes médicinales d'Amérique du Nord: apport à la phytothérapie française du traitement des affections des voies respiratoires. Phytothérapie 2005, 1 :19-27
- Gruenwald J, Brendler T, Jaenicke C, LaGow B, editors. PDR for Herbal Medicines. 4th ed. Thomson PDR, Montvale 2007, 429-430
- Pharmacopée Française - French Pharmacopoeia 10 th ed. Grindélia - Grindelia sp. 1998

Hamamelis Virginiana

cortex, folium et ramunculus

Main components

Leaf

3-10% **tannins:** a mixture of catechins, gallotannins, plus cyanidin and delphinidin type proanthocyanidins; catechins: mainly (+)-catechin, (+)-gallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate; phenolic acids: caffeic and gallic acids
flavonoids such as kaempherol, quercetin, quercitrin, and isoquercitrin**;**
0.01-0.5% **volatile oil** among which 40% are aliphatic alcohols, 25% carbonyl compounds, 15% aliphatic esters, and a maximum of 0.2% safrol; a small amount of hamamelitannin.

Bark

8-12% **tannins,** Cortex tannins are qualitatively similar to folium tannins, but have a higher content of hamamelitannin (1-7%) monogalloylhamamelose, free gallic acid, condensed catechin tannins, and small amount of **flavonols;** approximately 0.1% **volatile oil** with a very complex composition. The bark contains significantly higher levels of phenylpropanoids and sesquiterpenoids in the volatile fractions compared to the leaves, which contain higher amounts of monoterpenoids. The bark is richer in hydrolysable tannins and the leaves mainly contain condensed tannins.

Toxic component	Approach	Remarks	Acceptable amount	UF
safrole	$LHRD_{dermal\ human}$ $PDE_{human\ oral}$	Internal use: Haemorrhoids, varicose veins, heavy legs External use: skin & mucous inflammation, minor skin injuries. Neurodermitis atopica, Haemorrhoids, Heavy legs, Bruises. The safe use of hamamelis distillates has been accepted by the MLWP and HMPC, taking into account the data in the AR toxicology section (II.2.3) of the document EMA/HMPC/114585/2008	5350 mg[17] 10 mg/day	500

References:

- Bernard P, Balansard P, Balansard G, Bovis A. Valeur pharmacodynamique toniveineuse des préparations galéniques à base de feuilles d'hamamélis. [Venitonic pharmacodynamic value of galenic preparations with a base of Hamamelis leaves]. J Pharm Belg 1972, 27(4): 505-512.
- Hamamelidis aqua (Hamamelis water). European Scientific Cooperative on Phytotherapy, Exeter 2003. In: ESCOP Monographs, 2nd edition, 2003.
- Hamamelidis folium (Hamamelis leaf). European Scientific Cooperative on Phytotherapy, Exeter 2003. In: ESCOP Monographs, 2nd edition, 2003.
- Hamamelis. In: WHO monographs on selected medicinal plants. WHO, Geneva 2002, 124-136.
- Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. Salmonella Mutagenicity tests: II. Results from the testing of 270 Chemicals. Envir Mutagen 1986, 8(7): 1-27.

[17] Cream, 100 g containing 5.35 g distillate (1:1,6); Distillate of fresh *Hamamelis virginiana* L. leaves and branches (1:1.12-2.08), distillation agent ethanol 6% m/m. – Marketing authorization for the Italian market

Harpagophytum procumbens

radix

Main components

iridoid glucosides (0.5-3%), principally harpagoside, extremely bitter (0.8-3.0% in H. procumbens, 0.7-1.7% in H. zeyheri), together with 8-(p-coumaroyl)-harpagide (0.03-0.17% in H. procumbens, 0.61-1.84% in H. zeyheri) and small amount of harpagide, procumbide and their 6'-p-coumaroyl esters. The secondary tubers contain approximately twice as much harpagoside as the primary tubers.
phenolic glycosides acteoside (verbascoside) and isoacteoside, and sugars (about 51%), mainly the tetrasaccharide stachyose (up to 46%) with smaller amount of raffinose (a trisaccharide), sucrose and monosaccharides are also present.
acylated phenolic glycoside 6-acetylacteoside has been found in H. procumbens but not in H. zeyheri, so can be used to distinguish between the two Harpagophytum species the ratio of 8-O-p-coumaroylharpagide to the sum of harpagoside and 8-O-p-coumaroylharpagide is a distinguishing feature between H. procumbens and H. zeyheri. In H. procumbens it is below 10% while it is above 31% in H. zeyheri
Other compounds in small amounts: triterpenes, mainly oleanolic acid, 3β-acetyloleanolic acid and ursolic acid
Phytosterols: mainly β-sitosterol, stigmasterol and their glucosides
aromatic acids: caffeic, cinnamic and chlorogenic acids
flavonoids including kaempferol and luteolin**,** harpagoquinone

Toxic component	Approach	Remarks	Acceptable amount	UF
phenolic glycosides harpagoside	$LHRD_{human\ oral}$	Adulteration: Devil's claw (Harpagophytum procumbens**)** is occasionally adulterated with harpagoside-poor primary roots or with other bitter plants such as Elephantorrhiza and Acanthosicyos. The medicinal use has been documented in well-known handbooks dating from 1976. Dosage: (3 times daily) dried tuber 0.10-0.25 g; liquid extract (1:1, 25% ethanol), 0.1-0.25 ml; tincture 1:5 in 25% alcohol, 0.5-1 ml In a randomised, double-blind, placebo-controlled study, 63 patients (31 verum group and 32 placebo), between 18-62 years old, with slight to moderate muscular tension or slight muscular pain of the back, shoulder and neck received 2x480 mg Harpagophytum dry extract (DER 4.4-5.0:1, extraction solvent ethanol 60% V/V) or placebo daily for 4 weeks (Göbel et al., 2001). The efficacy of verum treatment was clear from the clinical global score and patient and physician ratings.	300 mg/day[18]	

References:

- Brendler T, Gruenwald J, Ulbricht C, Basch E. Devil's Claw (Harpagophytum procumbens DC): an evidence-based systematic review by the Natural Standard Research Collaboration. J Herb Pharmacother 2006, 6:89–126
- Bruneton J. Phytothérapie. Les données de l'évaluation Ed. TEC & DOC- EM Inter, 2002, 101-107
- ESCOP. Harpagophyti radix (Devil's Claw root). In ESCOP Monographs. 2nd ed. Supplement 2009, 135- 147
- Göbel H, Heize A, Ingewersen M, Niederberger U, Gerber D. Harpagophytum-Extrakt LI 174 (Teufelskralle) bei der behandlung unspezifischer rûckenschmerzen. Schmerz 2001, 15:10-18
- Grigorescu EM, Ciulei I, Stanescu U.- Index Fitoterapeutic, Edit. Medicală, Bucureşti 1986, 199-200
- WHO monographs on selected medicinal plants. Vol. 3. Radix Harpagophyti. World Health Organisation. Geneva 2007, 182-194
- Wichtl M. Herbal drug and Phytopharmaceuticals 3rd ed. MedPharm GmbH Scientific Publishers 2004, 271-273
- Zimmermann W. Planzliche Bitterstoffe in der Gastroenterologie. Z Allgemeinmed 1976, 54:1178-1184

[18] On the German market, as "well-established use" since 1976 - Dry extract of Harpagophyti radix (1.5- 2.0:1), extraction solvent ethanol 40% (V/V)

Hieracium pilosella

Herba cum radice

Main components

Hydroxycoumarins: umbelliferone (mainly as 7-glucoside; about 0.60% of the dry plant material), skimmine
Flavonoids: luteolin, luteolin-7-O-glucoside, apigenin-7-O-glucoside (about 0.25% of the dry plant material), isoetin 4'-O-β-D-glucopyranoside
Tannins
Triterpenoids: alpha- and beta-amyrin, taraxerol, taraxasterol and fern-7-en-3-beta-ol
Organic acids: caffeic acid, chlorogenic acid (about 20% of the dry plant material)
Ascorbic acid

Toxic component	Approach	Remarks	Acceptable amount	UF
umbrelliferone	$LHRD_{human\ oral}$	The traditional use of the following Hieracium pilosella preparations is well documented, on the basis of the information on the availability of products in the market since 1986, together with the information on the use of such preparations, throughout a period of at least 30 years According to Stanojević et al. (2009) the content of total phenolic compounds is about 240 mg gallic acid equivalents/g of dry extract	140 mg/day[19]	/

References:

- Beaux D, Fleurentin J, Mortier F. Effect of extracts of Orthosiphon stamineus Benth, Hieracium pilosella L., Sambucus nigra L. and Arctostaphylos uva-ursi (L.) Spreng. in rats. Phytother Res 1999, 13(3):222-225
- British Herbal Pharmacopoeia. Part 2. 1st ed. British Herbal Medicine Association, Exeter 1979, 141-143
- Bruneton J. Farmacognosia: fitoquimica, plantas medicinales. 2nd ed. Acribia, Zaragoza 1998
- Gawrońska-Grzywacz M, Krzaczek T. Identification and determination of triterpenoids in Hieracium pilosella L. J Sep Sci 2007, 30(5):746-750
- Stanojević L, Stanković M, Nikolić V, Nikoli L, Ristić D, Canadanovic-Brunet J, et al. Antioxidant Activity and total Phenolic and Flavonoid Contents of Hieracium pilosella L. extracts. Sensors (Basel) 2009, 9(7):5702-5714
- Van Hellemont J. Compendium de Phytothérapie. 1st ed. Association Pharmaceutique Belge, Brussels 1986
- Wren RC. Potter's New Cyclopedia of Botanical Drugs and Preparations. The C.W. Daniel Company Limited, Safron Walden, Essex 1998

[19] On the spanish market as "traditionl use" - powdered dried herbal substance

Hypericum perforatum

herba

Main components

Phloroglucinol derivates: 0.2-4%, depending on the age of the herbal drug, mainly hyperforin and its homologue adhyperforin, furanohyperforin

Naphtodianthrones: 0.06-0.4%, mainly pseudohypericin and hypericin, protohypericin, protopseudohypericin, cyclopseudohypericin, skyrinderivatives. The amount of pseudohypericin is about 2-4 times higher than that of hypericin**.**

Flavonoids: 2-4%, mainly glycosides of the flavonol quercetin: hyperoside, rutin, isoquercitrin, quercitrin; also biflavones (I3, II8-Biapigenin, Amentoflavone)

Procyanidines: e.g. procyanidine B2, tannins with catechin skeletal (6-15%)

Xanthones: in trace amounts

Essential oil: 0.1-0.25%; the essential oil of dried flowering tops contains as main compounds 2-methyloctane (16%) and α-pinene (10.6%). In the essential oil of leaves of Indian origin 58 components were identified, α-pinene (67%) being dominant; the other components included caryophyllene, geranyl acetate and nonane (each about 5%)

Other constituents: include small amounts of chloregenic acid and other caffeoylquinic and p-coumaroylquinic acids, and also free amino acids

Toxic component	Approach	Remarks	Acceptable amount	UF
α-pinene	LHRD	Although there are no controlled studies with children and adolescents published it can be concluded that there is a widespread documented use of *Hypericum* extracts among adolescents. However, there are no data available on the efficacy and safety in this population. Therefore, the use in children and adolescents below 18 years of age is not recommended. The absorbance spectrum of the Hypericum extract revealed maxima in the whole UV	600 mg/day[20]	/

References:

- Bernd, A., Simon, S., Ramirez Bosca, A., Kippenberger, S., Diaz Alperi, J., Miquel, J., Villalba Garcia, J.F., Pamies Mira, D., Kaufmann R., Phototoxic effects of Hypericum extract in cultures of human keratinocytes compared with those of psoralen. Photochem Photobiol. 69, 218-221. 1999
- ESCOP Monograph. HYPERICI HERBA (St. John's Wort). ESCOP Monographs Second edition. ESCOP 2003
- Greeson, J.M., Sanford, B., Monti, D.A. St. John's wort (Hypericum perforatum): a review of the current pharmacological, toxicological, and clinical literature. Psychopharmacology. 153, 402-414. 2001
- Leuschner, J. Preclinical toxicological profile of Hypericum extract LI 160. Abstracts of the 2nd International Conference on Phytomedicine, Munich. SL 80, 1996
- Okpanyi, S.N., Lizba, H., Scholl, B.C., Miltenburger, H.G. Genotoxicity of a standardized Hypericum extract. Arzneimittelforschung. 40, 851-855. 1990

[20] Marketing authorization in Italy - NERVAXON AIC: 033894078

Juglans regia
folium

Main components

10% tannins of the ellagitannins type. not less than 2%, calculated as pyrogallol.

Naphtalene derivatives: The most known naphthoquinone constituent is juglone (5-hydroxy-1,4-naphtoquinone) which occurs in fresh plant (leaf, stain) as glycoside of reduced form: 4β-D-glucoside of α-hydrojuglone=4β-D-glucoside of 1,4,5-trihydroxynaphthalene (2% in the stain, 0.6% in the leaves), but also in free state, particularly in the epicuticular leaf wax (to about 30%)

Phenolic acids/phenolic composition: p-hydroxybenzoic, vanillic, genistic, protocatechuic, p-coumaric, caffeic, ferulic, gallic, chlorogenic (3-caffeoylquinic) acids (Łuczak et al. 1989) and neochlorogenic (5-caffeoylquinic acid). Additionally, the presence of neochlorogenic and p-coumaric acids in the analysed cultivars were detected; all samples exhibited the same phenolics profile, whereas 3-caffeoylquinic acid was the major constituent (about 19.7%) and p-coumaric acid was the minor compound, representing ca. 1.4% of total phenolics.

Volatile oil: about 4 ppm, with monoterpene and sesquiterpene hydrocarbons. Monoterpenes are represented by: (E)-β- ocimene (12%), β-pinene (11%), limonene (10%), with traces of sabinene, α-pinene, myrcene, and linalool, whereas sesquiterpenes by caryophyllene (15%), germacrene D (13%), with minor amounts of (E)-β-farnesene and α-farnesene

Other compounds: Ascorbic acid (0.85-1%), cyclitols (11.2%), mucilage (7.6%), calcium (1.9%), potassium (1.4%)

Toxic component	Approach	Remarks	Acceptable amount	UF
Juglone α-pinene	LHRD $PDE_{juglone}$	The pharmacokinetics of 3H-juglone (0.02 mg/kg, i.v.) were studied by Aithal et al. (2011) in C57/BL mice. After the bolus dose about 35% of juglone accumulated within 15 min in the kidneys, with a half-life of about 2 h. Juglone induced cell death by apoptosis and necrosis through diverse mechanisms such as induction of oxidative stress, cell membrane damage and clastogenic effects. Cytotoxic activity of juglone was demonstrated after 72 h of incubation against leukemia (HL-60), melanoma (MDA-MB435), brain (SF-295) and colon (HCT-8) human cancer cell lines and against peripheral blood mononuclear cells (PMBC) as the control normal cell lines. The cytotoxicity of all compounds was tested using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). Juglone was dissolved in DMSO 1% and was added to each well and incubated for 72 h.	680 mg[21] 0,014 mg/day	200 (NOEL) 5000 (PDE)

References:

- Aithal BK, Sunil Kumar MR, Rao BN, Upadhya R, Prabhu V, Shavi G, Arumugam K, Sajankila SP, Udupa N, Satyamoorthy K, Satish Rao BS. Evaluation of pharmacokinetic, biodistribution, pharmacodynamic, and toxicity profile of free juglone and its sterically stabilized liposomes. J Pharm Sci 2011, 100:3517- 3528.
- Edenharder R and Tang X. Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1- nitropyrene by flavonoids, coumarins, quinones and other phenolic compounds. Food Chem
- Babula P, Adam V, Havel L, Kizek R. Noteworthy Secondary Metabolites Naphthoquinones – their Occurrence, Pharmacological Properties and Analysis. Curr Pharm Anal 2009a, 5:47-68.
- Li ZB, Wang JY, Jiang B, Zhang XL, An LJ, Bao YM. Benzobijuglone, a novel cytotoxic compound from Juglans mandshurica, induced apoptosis in HeLa cervical cancer cells. Phytomedicine 2007, 14:846- 852.

[21] On the Hungarian market, as "traditional use" only available in combination products therefore this information cannot sustain the safety and thus is reported as additional information.

Juniperus communis,
pseudo-fructus

Main components
Monoterpenes (about 58% of the essential oil); the essential oil contains mainly α-pinene (20%), limonene (8.7%), myrcene (8.5%) and β-pinene, myrcene, sabinene, 1,4-cineol, camphene, Δ3-carene, terpinen-4-ol, terpinolene, 4-terpineol, β-elemene-7-ol
Sesquiterpenes: cadinene, α-cadinene, β-cadinene
Diterpenic acids: isocommunic acid; labdane diterpenes
C12 terpenoid: geijerone
Tannins: proanthocyanidines (condensed), gallocatechin and epigallocatechin
Flavonoids: amentoflavone, quercitin, isoquercitrin, apigenin and various glucosides
Invert sugar (30%); glucose + fructose (about 30%) and pectin
Organic acids: malic acid, ascorbic acid, glucuronic acid
Lignan: desoxypodophyllotoxin
Cerin
Resins
The cone berries may not contain less than 10 ml/kg of essential oil. The amount of essential oil can be up to 3%. The essential oil of Juniper cone berries contains about 105 constituents

Toxic component	Approach	Remarks	Acceptable amount	UF
essential oil (unidentified compounds) α-pinene	PDEoral PDEoral	The dried herbal substance is used in a dose of 2 g with a maximum dose equivalent to 10 g per day. According to some authors, this posology corresponds with respectively 20 and 100 mg essential oil (Hänsel et al. 1993; Barnes et al. 2007; Ph. Eur. 2008) The therapeutic indications do not relate to life threatening conditions and they are supported by traditional use evidence. There are more potent conventional medicines with known benefits based on well-established use. Groups at risk can be defined as constitutional: **elderly, pregnant and breastfeeding mothers and children**	250 mg/day (standardised 80% ethanolic extract of Juniper pseudo-fructus) 300 mg/day (lyophilized water extract of Juniper pseudo-fructus)	500 500

References:

- Barnes J, Anderson LA, Phillipson JD. Herbal Medicines, 3rd ed. Pharmaceutical Press, London 2007; 386-388.
- British Herbal Pharmacopoeia. 4th ed. British Herbal Medicine Association, Exeter 1996; 117-118
- De Smet PAGM, Keller K, Hänsel R, Chandler RF. Adverse Effects of Herbal Drugs Vol. 2. Springer-Verlag, Berlin / Heidelberg 1993; 217-228.
- ESCOP Monographs 2nd ed. Juniperi pseudo-fructus – Juniper. European Scientific Cooperation on Phytotherapy, editor. Thieme Verlag, Stuttgart 2003; 282-285.
- Hänsel R, Keller K, Rimpler H, G. Schneider. Hagers Handbuch der Pharmazeutischen Praxis. Drogen E- O, Springer Verlag, Berlin 1993; 561-579.
- Weiss RF, Fintelmann V. Lehrbuch der Phytotherapie. 9th ed. Hippokrates Verlag, Stuttgart 1999; 253- 255.

Juniperus communis,

oil

Main components

α-pinene (24.1-55.4%) (range Ph. Eur. 2008 = 20-50%), β-pinene (2.1-6.0%) (range Ph. Eur. 2008 = 1.0-12%), β-myrcene (7.3-22.0%) (range Ph. Eur.2008 = 1.0-35%), sabinene (1.4-28.8%) (range Ph. Eur. 2008 = less than 20%), terpinene-4-ol (0.7-17.0%) (range Ph. Eur. 2008 = 0.5-10%), α-terpineol (up to 1.7%), α-thujene (0.6-1.9%), caryophyllene (1.3-2.3%) (Ph.Eur. 2008 for β–caryophyllene = less than 7.0%), γ-muurolen (7.6%), humulen (2.1%), α-muurolen (1.1%), β-elemen (1%), β-farnesen (0.9%), α-cubeben (0.9%), 4-thujanol (0.8%), α-cadinol (0.8%), γ-cadinen (0.7%), aromadendren (0.6%), α-copaen (0.4%), bornylacetate (0.4 %) (range Ph.Eur. less than 2.0%), camphen (0.3%), campholenaldehyd (0.2%), p-cymene (0.2%), verbenon (0.2%). Additionally, the Ph.Eur. limits the concentration of α–phellandrene to less than 1.0%

Toxic component	Approach	Remarks	Acceptable amount	UF
essential oil (unidentified compounds) α-pinene	TTC	There is a lot of discussion about the safe use of the oil of Juniperus. Some sources refer to the hyperemic effect of terpenes in the essential oil fraction to explain the diuretic action. Their action should be based on hyperemia of the glomeruli which is considered as an irritative action. Experimental pharmacological and toxicological data will be important in a constructive therapeutic approach. The indication for external use (promoting blood circulation in the skin) can be questioned as being too close to a health claim for cosmetics but no medicinal indication. The effect of Juniper oil (Juniper species not stated) on the cardiovascular and respiratory system was evaluated using 20 rabbits, anesthetized with urethane. When Juniper oil was administered intramuscularly as well as orally (concentrations 0.5%, 2.5% and 5% in corn oil: dose 1 mg/kg) a prolonged and slowly developing hypotonia occurred.	540 µg/day	/

References:

- Barnes J, Anderson LA, Phillipson JD. Herbal Medicines, 3rd ed. Pharmaceutical Press, London 2007; 386-388.
- British Herbal Pharmacopoeia. 4th ed. British Herbal Medicine Association, Exeter 1996; 117-118
- De Smet PAGM, Keller K, Hänsel R, Chandler RF. Adverse Effects of Herbal Drugs Vol. 2. Springer-Verlag, Berlin / Heidelberg 1993; 217-228.
- ESCOP Monographs 2nd ed. Juniperi pseudo-fructus – Juniper. European Scientific Cooperation on Phytotherapy, editor. Thieme Verlag, Stuttgart 2003; 282-285.
- Glisic SB, Milojevic SZ, Dimitrijevic SI, Orlovic AM, Skala DU. Antimicrobial activity of the essential oil and different fractions of Juniperus communis L. and comparison with some commercial antibiotics. *J Serb Chem Soc* 2007, 72:311-320.
- Hänsel R, Keller K, Rimpler H, G. Schneider. Hagers Handbuch der Pharmazeutischen Praxis. Drogen E- O, Springer Verlag, Berlin 1993; 561-579.
- Rossi PG, Berti L, Panighi J, Luciani A, Maury J, Muselli A. Antibacterial Action of essential oils from Corsica. J Essent Oil Res 2000, 19:176-182.
- Weiss RF, Fintelmann V. Lehrbuch der Phytotherapie. 9th ed. Hippokrates Verlag, Stuttgart 1999; 253- 255

Lavandula angustifolia,
flos

Main components

Essential oil (1-3%)
Coumarin derivatives: umbelliferon, herniarine
Flavonoids
Sterols (traces): cholesterol, campesterol, stigamsterol, β-sitosterol
Triterpenes (traces): mictomeric acid, ursolic acid
Tannins: up to 13% in the herbal substance
Phenylcarboxylic acids such as rosmarinic acid, ferulic acid, isoferulic acid, α-cumaric acid, p-cumaric acid, gentisinic acid, p-OH-benzoic acid, caffeic acid, melilotic acid, sinapinic acid, sytingic acid, vanillinic acid.

Lavender oil
The main components of the essential oil are **monoterpene alcohols** (60-65%) such as linalool (20-50% of the fraction), linalyl acetate (25-46% of the fraction). Others include cis-ocimen (3-7%), terpinene-4-ol (3-5%), limonene, cineole, camphor, lavandulyl acetate, lavandulol and α-terpineol, β- caryophyllene, geraniol, α-pinen. Non-terpenoid aliphatic components: 3-octanon, 1-octen-3-ol, 1- octen-3-ylacetate, 3-octanol

Toxic component	Approach	Remarks	Acceptable amount	UF
umbrelliferone	$NOEL_{human\ oral}$	Toxicity of lavender oil is not a major concern. Some components like linalool and linalyl acetate are not mutagenic. The essential oil did not demonstrate mutagenic activity towards two strains of Salmonella typhimurium and one of Escherichia coli with and without metabolic activation. The number of strains used for testing and the procedure used are not according to the recent regulatory guidelines. A Community list entry cannot be established for Lavandula. Lavender flowers were used for investigating a diuretic action. Experimental pharmacological data point to an activity in the central nervous system: anticonvulsive effects, sleep prolongation, locomotor activity, explorative or anticonflict behaviour and anxiety. Well known inflammatory and nociceptive experimental models were used	55 mg/Kg b.w./day	2000

References:

- Bickers D, Calow P, Greim h, Hanifin JM, Rogers AE, Saurat JH, Sipes IG, Smith RL, Tagami H. A toxicological and dermatological assessment of linalool and related esters when used as fragrance ingredients. Food and Chemical Toxicology 2003; 41: 919-942
- Bruneton J. Pharmacognosie: Phytochimie Plantes Médicinales. Ed. Tec & Doc, Paris 1999; 529-530
- Buchbauer G, Jäger W, Jirovetz L, Limberger J, Dietrich H. Therapeutic properties of essential oils and fragrances. In: Teranishi R, Buttery RG, Sugisama H, editors. Bioactive Volatile Compounds from Plants. AC Symposium Series 525. Washington DC: American Chemical Society, 1993b
- ESCOP Monographs 2nd ed. Lavandulae Flos, Lavandulae Aetheroleum. European Scientific Cooperative on Phytotherapy, editor. Supplement 2009. Georg Thieme Verlag, Stuttgart 2009; 147-156
- European Pharmacopoeia 6.0 (2008a) Lavender flower oil. Council of Europe. 1/2008: 1534
- Hänsel R, Keller K, Rimpler H, Schneider G. Hager's Handbuch der Pharmazeutischen Praxis. Spinger Verlag, Berlin 1993; 630-644

Melaleuca alternifolia

Tea Tree Oil (TTO)

Main components

The **oil** contains 42.35% terpinen-4-ol, 20.65% γ-terpinene, 9.76% α-terpinene, 3.71% terpinolene, 3.57% 1,8-cineole, 3.09%, α-terpineol, 2.82% p-cimene, 2.42% α-pinene, 1.75% limonene, 1.05% δ-cadinene, 0.94% α-thujene, 0.94% aromadendrene, 0.87% myrcene, 0.73% β-pinene, 0.40% sabinene, and 0.34% α-phellandrene

Toxic component	Approach	Remarks	Acceptable amount	UF
α-terpinene	PDE_{oral} $NOAEL_{\alpha\text{-terpinene}}$	Several reports of oral toxicity can be found in the literature. Data indicate that due to its systemic toxicity, TTO should only be used as a topical agent. Based on the available information on repeated dose systemic toxicity of TTO constituents, the SCCP opinion estimated a derived NOAEL for TTO of 117 mg /kg/day for renal effects (SCCP 2008, Norwegian Food Safety Authority)	11,7 mg/Kg/day 60 mg/Kg/day	500

References:

- Carson CF, Riley TV and Cookson BD. Efficacy and safety of tea tree oil as a topical antimicrobial agent Journal of Hospital Infection 1998, 40: I 75-I 78
- Council of Europe Committee of Experts on Cosmetic Products. Melaleuca alternifolia. Plants in Cosmetics: Plants and plant preparations used as ingredients for cosmetic products Volume II Committee of experts on cosmetic products. Cedex, France: Council of Europe Publishing 2001
- EFSA Journal - Scientific Opinion on the safety and efficacy of aliphatic and alicyclic ethers (chemical group 16) when used as flavourings for all animal species 2012, 10(11):2967
- Hammer KA, Carson CF, Riley TV, Nielsen JB. A review of the toxicity of Melaleuca alternifolia (tea tree) oil. Food Chem Toxicol 2006, 44(5):616-625
- Martindale. The extra pharmacopeia. 30th ed. Melaleuca Oil. The Pharmaceutical Press, London 1993, 1385
- Norwegian Food Safety Authority (Matthylsynet). RISK PROFILE Tea tree oil – TTO CAS No. 85085-48- 9, 68647-73-4, and 022-72-8 Date of reporting 10.08.2012
- World Health Organization. Aetheroleum Melaleucae Alternifoliae. WHO Monographs on Selected Medicinal Plants, Volume 2, Geneva 2004

Mentha piperita
folium

Main components

The leaves contain 1.2 – 3.9% **Essential oil**:
α-Pinene (0.32%), Sabinene (0.26%), β-pinene (0.58%), 1,8 Cineole (6.69%), cis-Sabinene hydrate (0.50%), Menthone (2.45%), Menthofuran (11.18%), Neomenthol (2.79%), Menthol (53.28%), Neomenthyl acetate (0.65%), Menthyl acetate (15.10%), Isomenthyl acetate (0.61%), β-Bourbonene (0.37%), (z)-Caryophyllene (2.06%), E-β-farnesene (0.30%), Germacrene (2.01%), carvone (up to 1%)
Bicyclogermacrene (0.22%), pulegone (until 4%).

flavonoids including luteolin and its 7-glycoside, rutin, hesperidin, eriocitrin and highly oxygenated flavones. Other constituents include phenolic acids and small amounts of triterpenes. Eriocitrin, with a concentration range of 6.6-15.0%, is the dominant flavonoid glycoside, accompanied by luteolin 7-0-rutinoside, hesperidin and rosmarinic acid.

Toxic component	Approach	Remarks	Acceptable amount	UF
α-Pinene Menthone Carvone Pulegone Menthofuran	PDEdry $_{\text{leaves extract oral}}$ $NOEL_{\text{pulegone oral}}$ $NOEL_{\text{menthone oral}}$ $NOEL_{\text{carvone human oral}}$	About 75% of the polyphenolic compounds present in the leaves are extracted in an infusion (Mackay, Blumberg 2006). An infusion of dried leaves is reported to contain 21% of the original oil (25mg/L) (Duband et al, 1992). Maximum levels for pulegone in foodstuff and beverages to which flavourings or other food ingredients with flavouring properties have been added: 25 mg/kg in foodstuff, 100 mg/kg in beverages, with the exception of 250 mg/kg in peppermint or mint flavoured beverages and 350 mg/kg in mint confectionery (Annex II of Directive 88/388/EEC).	166,67 mg/day 20 mg/kg bw/day 200 mg/kg bw/day 27,3 20mg/kg bw/day	1200 (pde oral) 3000 (carvone)

References:

- Akdogan M, Ozguner M, Kocat A, Oncu M, Cicek E., Effects of peppermint teas on plasma testosterone, folliclestimulating hormone and luteinizing hormone levels and testicular tissues in rats. Urology, 2004c, 64:394-398.
- Akdogan M., Gultekin F., Yontem M. Effect of Mentha piperitae (Labiatae) and Mentha spicata (Labiatae) on iron absorption in rats. Toxicol. Health, 2004a, 20:119-122
- Akdogan M., Klinic I., Oncu M., Karaoz E, Delibas N. Investigation of biochemical and histopathological effects of Mentha piperitae L., and Mentha spicata L., on kidney tissue in rats. Hum Exp Toxicol 2003, 22: 213-219.
- Duband F, Carnat AP, Carnat A, PetitJean-Freytet C, Clair G, Lamaison JL. Composition aromatique et polyphénolique de l'infusé de Menthe, Mentha x piperita L. Ann. Pharm. Franç. 1992; 50(3):146-155.
- EMEA/HMPC/138386/2005
- Evaluation of certain food additives. Fifty-first report of the Joint FAO/WHO Expert Committee on the Food Additives; WHO Geneve 2000.
- Mohammad Jamal Saharkhiz, Marjan Motamedi, Kamiar Zomorodian, Keyvan Pakshir, Ramin Miri, and Kimia Hemyari Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of Mentha piperita L., doi: 10.5402/2012/718645

Myroxylon balsamum

Main components

esters of cinnamic and benzoic acids, especially benzyl cinnamate (cinnamein), cinnamyl cinnamate (styracine) and benzyl benzoate. Small amounts of vanillin and free cinnamic acid are also present (55-66 %).

high-boiling volatile oil called **cinnamein** (50-64%), along with 20-28 % of resin. The volatile oil consists mainly of benzoic and cinnamic acid esters such as benzyl benzoate,
benzyl cinnamate and cinnamyl cinnamate (styracine), with small amounts of nerolidol, free benzyl alcohol, and free benzoic and cinnamic acids also present. In addition, traces of styrene, vanillin, and
coumarin have been identified in the material

Toxic component	Approach	Remarks	Acceptable amount	UF
/	$NOEL_{human\ oral}$	In a report from Germany, 59334 patients were tested for contact allergy to Peru balsam (25 % in petrolatum) between 1996 and 2002. The result for positive reaction varied from 7.3 % to 11.5 % (Schnuch et al., 2004).	100 mg/Kg b.w./day	2000

References:

- EMA/HMPC/712650/2014
- Martindale. The Extra Pharmacopoeia. 30th ed. The Pharmaceutical Press, London 1993, 1401
- Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. Contact Dermatitis 2008, 58(3): 134-141
- Opdyke DLJ. Peru balsam. Food Cosmet Toxicol 1974, 12: 951-952
- Schnuch A, Lessmann H, Geier J, Frosch PJ, Uter W. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK. Contact Dermatitis 2004, 50(2): 65-76

Oenothera biennis

oleum

Main components

at least 65% (cis)linoleic acid, 7-14% (cis)gamma-linolenic acid (γ- linolenic acid) and a maximum of 0.5% is alpha-linolenic acid. Other substances are 5-12% oleic acid, 1-4% stearic acid, 4-10% palmitic acid and a maximum of 0.3% saturated fatty acids of chain length less than C_{16}.

Toxic component	Approach	Remarks	Acceptable amount	UF
/	PDE_{oral} $LHRD_{oral}$	Patients with atopic eczema and premenstrual syndrome have a deficit in D6D, the enzyme that converts linoleic acid in γ-linolenic acid. Oenothera oil contains γ-linolenic acid. Based on this biochemistry, its therapeutic use can be hypothesised in patients with atopic eczema and PMS with a D6D deficit. For the treatment and symptomatic relief of neurodermatitis, especially of the associated pruritus.	12 ml/day 500[22] mg	20 (PDE)

References:

- European Pharmacopoeia 7th ed. Council of Europe. 1/2010: 2104
- Everett DJ, Greenough RJ, Perry CJ, et al. Chronic toxicity studies of Efamol evening primrose oil in rats and dogs. Med Sci Res 1988a; 16: 863-864
- Everett DJ, Perry CJ, Bayliss P. Carcinogenicity studies of Efamol evening primrose oil in rats and mice. Med Sci Res 1988b; 16: 865-866
- Hänsel R, Keller K, Rimpler H, Scheidner G. Hagers Handbuch der pharmazeutischer Praxis, Springer- Verlag, Berlin 1993, 929-936
- Kerscher MJ, Korting HC. Treatment of atopic eczema with evening primrose oil: rationale and clinical results. Clin Investig 1992; 70: 167-171
- Riaz A, Khan RA, Ahmed SP. Assessment of anticoagulant effect of evening primrose oil. Pak J Pharm Sci 2009; 22(4): 355-9

[22] Marketing authorization in Germany - 500 mg oil per capsule - Soft capsules since 1990

Ononis spinosa

radix

Main components

Isoflavones: formononetin (aglycone), ononin (formononetin 7-O-glucoside), pseudobaptigenin glucoside, genistein (1.7 – 3.8 mg/100g herbal substance), biochanin A 7-O-glucoside, biochanin A 7- O-glucoside 6"-malonate (biochanin A 0.08 – 0.70 mg/100g), formononetin 7-O-glucoside 6"-malonate (3.2 – 5.9 mg/100g), 2.3-dihydro-ononin and also tectoridin, trifolirhizin, rothinidin.
Glucosides: Spinonin, a glucoside with unusual structure has been detected, as well as medicarpin, a pterocarpan derivative.
Triterpenes include particularly α-onocerin (4.1 mg/1g herbal substance), also known as onocol.
Sterols: mainly β-sitosterol, stigmasterol, campesterol, cholesterol, α-spinasterol.
Saponins: triterpenoid saponin (e.g. 3-O-[α-L-rhamnopyranosyl-(1→2)-β -D-xylopyranosyl-(1→2)- β - D-glucuronopyranosyl]-3β ,22α-dihydroxyolean-13-en-11).
Phenolic acids: p-hydroxybenzoic, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, cinnamic acid, sinapin acid, salicylic acid, gentisin acid etc. can be detected in the Ononis spinosa L., radix.
Lectins: Index Nomenclature LECp.Ono.Spi.ro.Hga1
Small amounts of **essential oil** (0.02- 0.2%) are found, containing trans-anethole as the major constituent, with carvone, menthol, menthone, isomenthone, linalool, estragole, borneol and cis- anethole.
Other constituents are: tannins, sucrose, lipids, citric acid.

Toxic component	Approach	Remarks	Acceptable amount	UF
Saponins	$LHRD_{tea\ infusion}$ $LHRD_{oromucosal}$ $PDE_{oral\ ethanolic\ extract}$	Extracts of Restharrow (Ononis spinosa) roots in combination products are present on the market more than 30 years (e.g. since 1951 in Italy) 100 g of oral solution contain: Ononis spinosa radix liquid extract (DER 1:1, extraction solvent ethanol about 23% - formomonethin content 0.02%) 0.0104 g Without any further specification it is mentioned that Restharrow root should not be used in the presence of accumulation of water (oedema) due to impaired cardiac or renal function and dehydration [Blumenthal et al. 1998]	2000 mg[23] 10,4 mg[24] 8,332 mg/day	12000 (PDE)

References:

- Blumenthal M, Busse WR, Goldberg A, Gruenwald J, et al., editors. The Complete German Commission E Monographs. American Botanical Council, Austin Texas 1998, 213
- Bolle P, Faccendini P, Bello U, Panzironi C, Tita B. Ononis spinosa L. Pharmacological effect of ethanol extract. Phamacol Res 1993, Vol. 27, (suppl 1):27-28
- ESCOP Monographs 2nd ed. Ononidis radix - Restharrow root. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 351-353
- European Pharmacopoeia 7.0. Restharrow root – Ononidis radix. Directorate for the Quality of Medicines & Healthcare of the Council of Europe 2010, 01/2008:1879
- Joksić G, Stanković M, Novak A. Antibacterial medicinal plants Equiseti herba and Ononidis radix modulate micronucleus formation in human lymphocytes in vitro. J Environ Pathol Toxicol Oncol 2003, 22:41-4
- Wichtl M. Herbal drugs and Phytopharmaceuticals. 3rd ed. Medpharm Scientific Publishers GmbH, Stuttgart 2004, 421-423Evaluation of certain food additives.

[23] (ESCOP 2003)

[24] On the italian market as "well- established use" Ononis spinosa radix liquid extracts (DER 1:1, extraction solvent ethanol about 23%: only as combination products therefore this information cannot sustain the safety and thus is reported as additional information.

Orthosiphon stamineus

folium

Main components

The most characteristic compounds are **minerals** (potassium 3%), **diterpenes** (orthosiphols A-E 0.2%), **triterpenes**, **essential oil**, (0.02 -0.06%) (sesquiterpenes), **lipophilic flavones** like sinensetin (0.1 – 0.19%), isosinensetin and eupatorin **flavonol glycosides**; rosmarinic acid (0.1 – 0.5%), and other caffeic acid depsides like mono and dicafeyl tartric acid as well as lithospermic acid, pytosterols as b-sitosterol and up to 0.7% of essential oil, isositol, pimarane, isopimarane and staminane diterepnes, triterpenes and chromenes.

Toxic component	Approach	Remarks	Acceptable amount	UF
/	PDE_{oral} $LHRD_{oral}$	Based on decreased ALT and/or AST levels, it is concluded that the extracts possess beneficial effects on the liver. This statement cannot be supported, because the biological significance (e.g. dose- relationship) and the cause of these effects were not investigated. For example, potential causes of decreased serum activities of ALT and AST are reported to include: decreased hepatocellular production or release of the enzymes, inhibition or reduction of the enzyme's activity, interference with the enzyme assay	50 mg/day 832,5 mg[25] 750 mg[26]	5000 (PDE)

References:

- Chin Jin Han1, Abas Hj Hussin, Sabariah Ismail - Toxicity study of Orthosiphon stamineus Benth (Misai Kucing) on Sprague Dawley rats, Tropical Biomedicine 25(1): 9–16 (2008)
- Chow SY, Liao JF, Yang HY, Chen CF. Pharmacological effects of Orthosiphonis herba. J Formosan Med Assoc, 1979. 78: 953-960.
- Commission E monographs. Orthosiphonis folium (Orthosiphonblätter). 1986, revised in 1990.
- Englert J and Harnischfeger G. Diuretic action of aqueous Orthosiphon extract in rats. Planta Med, 1992. 58 :237-8.
- ESCOP, ESCOP Monographs (2nd edition completely revised and expanded) European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003.
- EMEA/HMPC/107079/07
- Tezuka Y, et al. Constituents of the Vietnamese Medicinal Plant Orthosiphon stamineus. Chem Pharm Bull, 2000. 48 (11): 1711-1719.

[25] Dry extract (solvent: ethanol 70% V/V, DER 7-8:1)

[26] powder

[23, 24] On the German and Belgian market as "well-established use"

Peumus boldus

folium

Main components

Alkaloids: Isoquinoline-type 0.25-0.7%. Boldine, isoboldine, 6a, 7-dehydroboldine, isocorydine, isocorydine-N-oxide, norisocorydine, laurolitsine, laurotetanine, N-methyllaurotetanine, reticuline, (-)-pronuciferine, sinoacutine. Boldine is usually the major alkaloid (reported as 14-36% of total alkaloids).

Volatile oil: 2.0-2.6% (Vogel *et al.,* 1999). Major constituents reported as: ascaridole (16-38%), 1,8 cineole (11-39%) and *p*-cymene (9-29%) (Bradley, 2006).

principal components of the **oil** are determined genetically and have reported levels: **ascaridole** (34.6%), p-cymene (3.9%), 1,8-cineole (0.5%). Other constituents include: α-pinene, camphene, β-pinene, sabinene, Δ3-carene, terpinolene, limonene, γ-terpinene, 2-nonanone, fenchone, 1-methyl-4-isopropenylbenzene, α-fenchol, terpinen-4-ol, α-terpineol and methyl eugenol

Polyphenols & Flavonoids: proanthocyanidins, flavonol glycosides: quercetin glycosides, kaempferol derivatives, isorhamnetin glycosides, phenolic acids, caffeoylquinic acid glycoside and proanthocyanidins. Isorhamnetin glucosyl-di-rhamnoside was the most abundant flavonol glycoside in the male boldo sample, whereas isorhamnetin di-glucosyl-di-rhamnoside was the main phenolic compound in female boldo leaves infusion

Other constituents: coumarin, resin, tannin.

Toxic component	Approach	Remarks	Acceptable amount	UF
ascaridole	$NOEL_{human\ oral}$ $NOEL_{total\ alkaloids\ human\ oral}$ $NOEL_{boldine\ human\ parenteral}$ $NOEL_{boldine\ human\ oral}$ $PDE_{ascaridole}$	Doses of 50 mg/kg/day did not produce any significant changes over the 90-day period. Neither the boldo extract nor boldine caused any overt signs of toxicity in the heart or kidneys but steatosis was observed in two animals at doses of 800 mg/kg Ascaridole is highly toxic and this raises concerns about the suitability of boldo leaf in traditional herbal medicinal products. 0.1% of total **alkaloids**, expressed as boldine ($C_{19}H_{21}NO_4$) 2-4% of volatile oil. Major constituents reported as: **ascaridole** (16-38%), 1,8 cineole (11-39%) and p-cymene (9-29%)	75 mg/Kg b.w./day 10,5 mg/Kg b.w./day 6,25 mg/Kg b.w./day 12,5 mg/Kg b.w./day 0.004 mg/Kg/day	2000 (ethanolic extract oral) 2000 (total alkaloids oral) 2000 (boldine parenteral) 2000 (boldine oral) 5000 (ascaridole PDE)

References:

- Levy-Appert-Collin M-C, Levy J. Sur quelques preparations galeniques de feuilles de Boldo (Peumus boldus, Monimiacees J Pharm Belg 1977; 32: 13-22
- Magistretti MJ. Remarks on the pharmacological examination of plant extracts. Fitoterapia 1980; 51:67-79.
- Kreitmar H. Pharmakologische wirkung des alkaloids aus Peumus boldus Molina. Pharmazie 1952; 7: 07-511.
- Opdyke DL. Monographs on fragrance raw materials: Boldo oil. Food and Chemical Toxicology 1982; 20 (Suppl.6): 643-644.

Pimpinella anisum

seeds and oil

Main components

The essential oil, obtained by steam distillation of crushed fruits, varies between 1.5% and 6% and contains mainly trans-anethole (80-95%)

Trans-anethole 76.7- 93.0%
Estragole 0.5- 6.1%
Anisaldehyde 0.1- 3.5%
Linalol 0.1- 1.5%
Alpha-terpineol 0.1-1.5%
Cis-anethole <0.5%

Toxic component	Approach	Remarks	Acceptable amount	UF
Trans-anethole	NOEL $_{\text{human oral}}$	Trans-anethole exerted dose-dependent anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy In 90-day experiments in rats, 0.1% trans-anethole in the diet induced no toxic effects, whereas a dose-related oedema of the liver was reported at levels between 0.3 and 3.0%	1,25 mg/Kg b.w./day	2000

References:

- European Pharmacopoeia 7th ed., Aniseed - Anisi fructus. Council of Europe. 1/2012: 0262; 3858- 3859.
- Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM et al. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet Toxicol 1967, 5: 141- 157.
- JECFA: Joint FAO/WHO Expert Committee on Food Additives. Safety evaluation of certain food additives. Trans-anethole. WHO Geneva, 1999.
- Le Bourhis B. Propriétés biologiques du trans-anethole. Essai de détermination de la dose journalière acceptable. Parfums Cosmet Sav Fr 1973, 3: 450-456.
- Lin FSD. Trans-anethole. In: Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series 28. WHO Geneva, 1991: 135-152.
- Samojlik I, Mijatović V, Petković S, Skrbić B, Božin B. The influence of essential oil of aniseed (Pimpinella anisum, L.) on drug effects on the central nervous system. Fitoterapia 2012.

Plantago ovata

seminis tegumentum

Main components

Plantago ovata, seminis tegumentum consists of 85% **water-soluble fibre.** The active polysaccharidic fraction comprises 65% D-xylose, 20% L-arabinose, 6% rhamnose and 9% D-galacturonic acid. The polysaccharide is shown to be a highly branched acidic arabinoxylan, the xylan backbone having both 1→4 and 1→3 sugar linkages.

Toxic component	Approach	Remarks	Acceptable amount	UF
/	$LHRD_{oral}$	The data available support the use of Plantago ovata, seminis tegumentum as laxative and as an adjuvant to diet in hypercholesterolemia, even if the mechanism is not fully understood. The non-clinical data on toxicology of P. ovata preparations are incomplete, but available data indicate no signals of toxicological concern There are only unpublished data (e.g. LD_{50}=3360 mg/Kg in rats) available concerning Plantago ovata, (semini tegumentum) and psyllium without an exact definition of the test preparation. however, given the composition, no toxicity concern is expected.	3200 mg[27]	/

References:

- Attaluri A, Donahoe R, Valestin J, Brown K, Rao SSC. Randomised clinical trial: dried plums (prunes) vs. psyllium for constipation. Aliment Pharmacol Ther 2011, 33:822-828
- Chouinard LE. The role of psyllium fibre supplementation in treating irritable bowel syndrome. Can J Diet Pract Res 2011, 72(1): e107-114
- Federal Register. The Daily Journal of the United States Government. Rule. Laxative Drug Products for Over-the-Counter Human Use; Psyllium Ingredients in Granular Forms. A Rule by the Food and Drug Administration on 03/29/2007
- Marlett JA, Kajs TM, Fischer MH. An unfermented gel compound of psyllium husk promotes laxation as a lubricant in humans. Am J Clin Nutr 2000, 72(3):784-789
- Study 6114-184, 187, 188. Procter & Gamble. Subchronic toxicity in the rat. Pharmaco-toxicological expert report for Metamucil, January 2001
- Turley SD, Daggy BP, Dietschy JM. Psyllium augments the cholesterol-lowering action of cholestyramine in hamsters by enhancing sterol loss from the liver. Gastroenterology 1994, 107(2):444-452

[27] On the Belgian market, as “well-established use” - granules since 1963; powder since 1984

Polygonum aviculare

herba

Main components

Tannins (3.5-4%): rhatannin, gallo- and catechol tannins
Phenolic carboxylic acids: caffeic, chlorogenic, gallic and protocatechuic acids
Naphthoquinone: 6-methoxyplumbagin
Hydroxycoumarins: umbelliferone, scopoletin
Lignans: lignin glycoside, aviculin
Sterols: mainly β-sitosterol
Saponins: triterpenoid saponins, mostly oleanolic acid
Anthraquinones: emodin
Silicic, tartaric and formic acids (1%): present as water-soluble silicates
Carbohydrates: glucose, galactose, arabinose, sucrose, rhamnose, galacturonic acid
Other constituents: essential oils, carotene, vitamins C and K

Toxic component	Approach	Remarks	Acceptable amount	UF
Naphthoquinone Umbrelliferone	TTC	Germany Pharmaceutical form: herbal tea containing (in 1 sachet (1.6 g)): 240 mg Foeniculi amari fructus, 208 mg Thymi herba, 192 mg Tiliae flos, 192 mg Polygoni avicularis herba, 176 mg Lichen islandicus, 96 mg Primulae flos, 64 mg Lamium album flos, 64 mg Verbascum thapsus flos Indication: traditional herbal medicinal product to liquefy mucus. Posology: 4-6 times daily 1 cup of tea (1 sachet/cup) On the market since: at least 1978 [only as combination products therefore this information cannot sustain the safety and thus is reported as additional information.] Only few toxicological data are available, with an adequate risk assessment, for each compound, the TTC could be set on 1800 µg/day	540 µg/day	/

References:

- European Pharmacopoeia 8h ed. Knotgrass – Polygoni avicularis herba. Council of Europe. 07/2013: 1885
- Kazemivash N, Jamali M. Investigation of cytotoxic effects of methanolic total extract of the aerial part of polygonum aviculare L. in vegetative and generative phase on nalm-6 (pre-B-cell leukaemia) cell line. Journal of Biology and Today's World 2013, 11:506-508
- Roman I, Rusu MA, Puică C, Borşa M. [Emphasis of some preparate of Polygonum action on the vital organs in laboratory animals]. Annals of the Romanian Society for Cell Biology 2008, 13:183-198
- EMA/HMPC/143659/2015

Potentilla erecta

rhizoma

Main components

Tannins: 15-22% total tannins (15-20% condensed tannins, about 3.5% hydrolysable tannins)
Flavonoids: kaempferol, cyanidinglucoside and leucoanthocyanidin and the tannin monomers catechin, epicatechin, gallocatechin and epigallocatechin
Phenol carboxylic acids: p-coumaric acid, 3,4-dihydroxybenzoic acid, gallic acid, sinapic acid and caffeic acid
Triterpene saponins: quinovic acid, tormentillic acid and tormentosid
Fatty acids: in extracts prepared with supercritical CO_2 the following constituents are found: lauric acid, linoleic acid, linolenic acid, palmitic acid, palmitoleic acid, pentadecanoic acid, stearic acid and oleic acid.

Toxic component	Approach	Remarks	Acceptable amount	UF
/	PDE_{oral} PDE_{oral}	The medicinal use of Potentilla erecta, rhizoma can be traced in literature back to the 15th century. Potentilla erecta, rhizoma is **traditionally** used for acute, unspecified diarrhoea, externally for haemostasis, mild inflammation of the oral and pharyngeal mucosa, prosthetic pressure points, frostbite, burns, haemorrhoids and poorly healing wounds The Danish Food Agency has accepted 200 mg Potentilla erecta, radix in a food supplement. This is not an upper limit but a specific assessment in a specific case. Risk – benefit Since no specific risks are known regarding the oral and oromucosal use of herbal preparations of Potentilla erecta, there are no limitations from the herbal preparation when used in adults.	145 mg/day (A dry extract prepared by maceration with water) 12,5 mg/day (extraction solvent acetone/water 75:25)	5000 1200

References:

- European Pharmacopoeia 6.0. Tormentil tincture – Tormentillae tinctura. Council of Europe. 01/2008: 1895
- Lund K, Rimpler H. Tormentil rhizome: isolation of an ellagitannin and pharmacological screening. Dtsch Apoth Ztg 1985, 125:105-08
- Madaus G. Lehrbuch der biologischen Heilmittel. Georg Thieme Verlag, Leipzig 1938
- Shushunov S, Balashov L, Kravtsova A, Krasnogorsky I, Latte KP, Vasiliev A. Determination of acute toxicity of the aqueous extract of Potentilla erecta (tormentil) rhizomes in rats and mice. J Med Food 2009, 12:1173-76
- Huber R, von Ditfurth A, Amann F, Güthlin C, Rostock M, Tritler R, Kümmerer K, Merfort I. Tormentil for active ulcerative colitis. J Clin Gastroenterol 2007, 41:834-38

Prunus africana

cortex

Main components

phytosterols (approximately 0.05%), e.g. beta-sitosterol, beta-sitosterol 3-glucoside and beta- sitostenone, free C, -C 24 fatty acids, pentacyclic triterpenic acids are present (14%) (ursolic and oleanolic acid derivatives) and long chain aliphatic alcohols (n-docosanol, n-tetracosanol and their trans-ferulic acid esters)
The proposed active constituents of a lipophilic extract of Pruni africanae cortex include **docosanol** (0.6%) and **beta-sitosterol** (15.7%).
alkanols (tetracosanol [0.5%] and trans-ferulic acid esters of docosanol and tetracosanol), fatty acids (which are 12-24 carbons in length, 62.3%, comprising myristic, palmitic, linoleic, oleic, stearic, arachidic, behenic and lignoceric acids); sterols (sitosterone [2.0%] and daucosterol) and triterpenes (ursolic acid [2.9%], friedelin [1.4%], 2-a-hydroxyursolic acid [0.5%], epimaslinic acid [0.8%] and maslinic acid)
Tannins.

Toxic component	Approach	Remarks	Acceptable amount	UF
/	PDE_{oral} $LHRD_{oral}$	France “well-established use”: Soft extract; Solvent: stabilised chloroform; DER 114-222:1 (stabilised by 1.2% of ethanol >99.9%) since 1969 Indication: Treatment in miction moderate disorders connected with benign prostatic hyperplasia (BPH). Posology: A capsule twice daily. A capsule contains 50 mg of extract. Duration of use: 6 weeks (+ 2 weeks)	75 mg/day 100 mg	500 (PDE)

References:

- Andro MC, Riffaud JP. Pygeum africanum extract for the treatment of patients with benign prostatic hyperplasia: a review of 25 years of published experience. Curr Ther Res 1995, 56:796–817
- Bombardelli E, Morazzoni P. Prunus africana (Hook f.) Kalkm. Fitoterapia 1997, 68:205-218
- ESCOP Monographs 2nd ed. Pruni africanae cortex – Pygeum bark. European Scientific Cooperative on Phytotherapy, editor. Thieme Verlag, Stuttgart Supplement 2009, 206
- European Pharmacopoeia. 8th ed. Pygeum africanum bark - Pruni africanae cortex. Council of Europe. 1/2015:1886
- Schleich S, Papaioannou M, Baniahmad A, Matusch R. Activity-guided isolation of anantiandrogenic compound of Pygeum africanum. Planta Med 2006a, 72(6):547-551
- Schleich S, Papaioannou M, Baniahmad A, Matusch R. Extracts from Pygeum africanum and other ethnobotanical species with antiandrogenic activity. Planta Med 2006b, 72(9):807-813
- Verschaeve L, Kestens V, Taylor JLS, Elgorashi EE, Maes A, Van Puyvelde L, et al. Investigation of the antimutagenic effects of selected South African medicinal plant extracts. Toxicology in Vitro 2004, 18(1):29-35
- WHO Monographs on Selected Medicinal Plants. Vol 2. Pygeum africanum. World Health Organization, Geneva 2009, 83-91

Rhodiola rosea

rhizoma

Main components

Phenylalkanoids: Phenylethanoids (e.g salidroside [syn. rhodioloside]: p-hydroxyphenylethyl-O-ß- D-glucopyranoside), phenylpropenoids (e.g. rosin: cinnamyl-O-ß-D- glucopyranoside; rosarin: (cinnamyl-(6'-O-α-L-arabinofuranosyl)- O-ß-D- glucopyranoside; rosavin: (cinnamyl-(6'-O- α-L-arabinopyranosyl)- O-ß-D- glucopyranoside), phenylpropanes (e.g. tyrosol). Only limited data are available regarding the quantitative composition
Essential oil: The dried rhizome contains approximately 0.05% of essential oil. Main components are α-pinene, geraniol, limonene, ß-phellandrene, linalool, n-octanol, n-decanol, dodecanol, 1,4-p- menthadien-7-ol.
Monoterpene derivatives: rosiridol, rosiridin, rhodiolosides A-E.
Cyanogenic glycosides: rhodiocyanoside A, lotaustralin
Proanthocyanidines: prodelphinidine-gallate esters
Flavonolignans: rhodiolin
Flavonoids: *Rhodiola*-specific flavonoids like rhodionidin, rhodiolgin, rhodalidin, rhodionin, rhodiolgidin, rhodalin, rhodiosin; tricin and kaempferol derivatives.
Phenolic acids: chlorogenic acid, hydroxycinnamic acid.

Toxic component	Approach	Remarks	Acceptable amount	UF
α-pinene	$LHRD_{oral}$	Hellum *et al.* 2010 report a considerable variability between clones of *Rhodiola rosea* in Norway based on data obtained from ethanolic extracts (primary extraction solvent ethanol 96%). Irrespective of the plant origin (cultivated in Lithuania or naturally occurring in Altai mountains). Kucinskaite *et al.* (2007) found in aqueous- ethanolic extracts 1.35-1.62 mg/ml of salidroside, while the profile of rosavins differed considerably. Ethanol 70% v/v yields extracts with a low content of salidroside compared to ethanol 40% v/v; in contrast rosavins are more efficiently extracted by ethanol 70% (Kucinskaite *et al.* 2007). The clinical trials as well as the traditional use do not give reasons for special safety concerns. No serious adverse events are reported. The *in vitro* observed inhibition of CYP3A4 and P-glycoprotein was not confirmed *in vivo*. Additionally, no case reports on interactions are published. Because of the limited duration of use of 2 weeks, the *in vitro* data seem to be of minor clinical relevance.	400 mg/day[28]	/

References:

- Battistelli M, De Sanctis R, De Bellis R, Cucchiarini L, Dacha M, Gobbi P. Rhodiola rosea as antioxidant in red blood cells: ultrastructural and haemolytic behaviour. Eur J Histochem 2005, 49(3):243-54.
- Burgos R, Hancke J. Long-term toxicity test. 3 months subchronic toxicity test for Sedum rosea. Swedish Herbal Institute, Gothenburg, Sweden, 1991 unpublished results.
- EMA/HMPC/232100/2011
- Hellum BH, Tosse A, Hoybakk K, Thomsen M, Rohloff J, Georg Nilsen O. Potent in vitro inhibition of CYP3A4 and P-glycoprotein by Rhodiola rosea. Planta Med 2010, 76(4):331-8.
- Kucinskaite A, Poblocka-Olech L, Krauze-Baranowska M, Sznitowska M, Savickas A, Briedis V. Evaluation of biologically active compounds in roots and rhizomes of Rhodiola rosea L. cultivated in Lithuania. Medicina (Kaunas) 2007, 43(6):487-94.
- Zhu J, Wan X, Zhu Y, Ma X, Zheng Y, Zhang T. Evaluation of salidroside in vitro and in vivo genotoxicity. Drug Chem Toxicol 2010, 33(2):220-6.

[28] On the italian market as "taditional use" – 200 mg dry extract

Viscum album

herba

Main components

Viscum lectins (ML I, ML II,ML III; glycoproteins with the ability to bind specifically to galactose, N-acetylgalactosamine and cell surfaces), proteins and polypeptides (in particular the viscotoxins which are composed of 46 amino acids), phenylpropanes and lignans, caffeic acid derivatives, flavonoides (especially derivatives of quercetin), biogenic amines (tyramine etc.), polysaccharides (particularly galacturonans and arabinogalactans), membrane lipids (vesicles) and other substances in low concentrations

Toxic component	Approach	Remarks	Acceptable amount
ML I ML II ML III	$PDE_{Viscum\ extract\ i.v.}$ $PDE_{Viscum\ juice\ parenteral}$ $PDE_{Viscotoxins\ i.v.}$	Even if in Germany there are some authorized products made from Viscum album (various preparations) due the route of administration, the uncertain balance of risk-benefit, the lack of toxicity data and the high toxicity of some come compounds, the LHRD approach can't be used to sustain the safety of the product.	$5{,}83\ 10^{-4}$ mg/die $3{,}73\ 10^{-5}$ mg/die $1{,}16\ 10^{-9}$ mg/die

References:

- Barnes J, Anderson LA, Philipson JD. Herbal medicines. 3rd edition. Pharmaceutical press 2007, 436-46. Available at the EMA-library
- Doehmer J, Eisenbraun J. Assessment of Extracts from Mistletoe (Viscum album) for Herb–Drug Interaction by Inhibition and Induction of Cytochrome P450 Activities. Phytother Res 2012, 26(1):11- 17, doi 10.1002/ptr.3473
- EMA/HMPC/246778/2009
- Hänsel R, Keller K, Rimpler H, Schneider G. editors. Hagers Handbuch der Pharmazeutischen Praxis. Springer-Verlag, Berlin 1994, 1160-1179
- Kienle GS, Kiene H. Die Mistel in der Onkologie - Fakten und konzeptionelle Grundlagen. Schattauer Verlag, Stuttgart, New York, 2003
- Stein GM. Toxicology of mistletoe and their components. In: Büssing A, editor. Mistletoe. The Genus Viscum. Hardwood Academic Publishers, Amsterdam 2000, 183-194

DISCUSSION

One of the first but still most used toxicological data, applied in calculation of others toxicological parameters (as PDE, NOEL, etc), is the LD_{50}. The considerable differences among species are one of disadvantage to using animal LD_{50} data, as well as differences among strains within a species. A similar disadvantage exists in the context of risk assessment for chronic exposures. The general approach to address this disadvantage for chronic exposure risk assessment, in part, is to use the animal model that best represents humans. If it is unknown which animal is the best model to represent humans, then the most sensitive species should be used following the general rules applied by health Agencies in the world (Barnes, Dourson, 1988; U.S. EPA, 2002). A similar approach was used here extrapolating from animal LD_{50} data. This approach has the advantage of being health-protective, although it may not be very accurate. A first approximate on in assessing accuracy of any risk extrapolated from experimental animal data to humans is that if experimental results indicate similar toxicities among different animal species, it is likely that the toxicity will be more similar for humans. Therefore, comparison of the mouse and rat LD_{50} values may provide some insight as to the accuracy of the estimate for humans.

The first example here reported is related to products obtained from *Rodhiola rosea* L. for oral administration. The phytochemistry of this species have been well characterized and it has a very low toxicity (Assessment report on *Rhodiola rosea* L., rhizoma et radix). Current toxicity studies indicate its LD_{50} as about 28.6 ml/kg, or about 3360 mg/kg (Brown et. al. 2002). For perspective, such dosage in a 70 kilograms man would be 235 g or 235.000 mg. Since most recommended doses are about 100 to 400 mg and the total recommended dosage for an entire day rarely exceeds 600 to 1000 mg, you can see there is a very large margin of safety, much larger than with apparent.

Table 1

Example of calculation of the different toxicological parameters for the plant with low toxicity *Rhodiola rosea* L.

***Rhodiola rosea* L.**	**Compound**	**LHRD**	**TTC**	**PDE**	**$NOEL_{rat}$**	**$NOEL_{Human}$**
OS	*Rosavin*	200 mg (as dry extract)	540 µg/day	0,672mg/die	6,72 mg/Kg*bw/day	100,8 mg/Kg*bw/day

BW= body weight
OS = Oral Somministration
$NOEL_{rat}$ = 3360 *0,4Kg/200
$NOEL_{human}$= 3360*60Kg/2000
PDE= 6,72*50/5*10*10

This plant belonging to the *Crassulaceae* family and its main component is rosavin. This species is used for temporary relief of symptoms associated with stress, fatigue, exhaustion and mild anxiety states and it was authorized in EU as medicinal product. As shown in the table 1 is possible to assess the safety of products containing herbal preparation of *Rhodiola rosea* L. using different approaches based on different parameters; in this case it is preferable to choose the LHRD approach considering that it is on the market in EU as medicinal product and toxicological data (LD_{50}) shown a trifling toxicity profile. I want to underline that, as is showed in the table, the calculation of TTC, PDE and NOEL value is related to the compound rosavin only whereas, the LHRD is relates to the dry extract (extraction solvent ethanol 60% V/V). Given the extensive use of the product and the time on the market as well the same route of administration the LHRD as it is, is enough to assess the safety. Sometimes this approach needs some adjustments and one this example is the value LHRD/100, this kind of approach is suggested in the in the art.14 of the Directive 2001/83/EC in the contest of the regulation of the homeopathic medicinal products.

The second example concerns a high toxic herbal drug largely used in popular medicine such as *Viscum album* L., a species of the Santalaceae, family commonly known as European mistletoe, common mistletoe or simply mistletoe. The toxicity data used to calculate the parameters in the table 2 are available in the document "Assessment report on *Viscum album* L., herba"[29]

Table 2

Example of calculation of the different toxicological parameters for the plant with high toxicity *Viscum album* L.

***Viscum album* L.**	**Compound**	**LHRD**	**TTC**	**PDE**	**$NOEL_{mice}$**	**$NOEL_{Human}$**
IV	Viscum extract	/	0,15 µg/day	$5{,}83\ 10^{-4}$ mg/die	0,14 mg/Kg*bw/day	17,5 mg/Kg*bw/day
IP	Viscum juice	/	0,15 µg/day	$3{,}73\ 10^{-5}$ mg/die	0,00896 mg/Kg*bw/day	1,12 mg/Kg*bw/day
IP	Viscotoxins	/	0,15 µg/day	$2{,}91\ 10^{-7}$ mg/die	0,00007 mg/Kg*bw/day	0,0175 mg/kg*bw/day
IV	Viscotoxins	/	0,15 µg/day	$1{,}16\ 10^{-9}$ mg/die	0,000014 mg/Kg*bw/day	0,035 mg/kg*bw/day

Bw = Body weight
IP = Intraperitoneally
IV = Intravenously

[29] EMA/HMPC/246778/2009

The *Viscum album* L. juice contains a mixture of different compounds, one of which is toxic. The Viscumin (Olsnes et al. 1982) is a cytotoxic protein (ribosome inactivating protein, or RIP) that binds to galactose residues of cell surface glycoproteins and may be internalized by endocytosis (Olsnes et al. 1982). Viscumin strongly inhibits protein synthesis by inactivating the 60S ribosomal subunit. The structure of this protein is very similar to other RIPs, showing the most resemblance to ricin and abrin. As is shown in the table 2 is possible to assess the safety using different approaches each of them is correct but take into account of different parameters; in this case, rating the well-known toxicity of this specie, it should be chosen the PDE's most restrictive value (1,16 10^{-9} mg/die). Otherwise, if the finish product is made with the juice or the extract, their own PDE's value could be used always taking into account that the final concentration of viscumin must be under its own PDE otherwise, an *ad hoc* toxicity study, concerning the product for which the authorization is required, should be provided. Remembering that, the PDE approach is suggested in the ICH guidelines on solvents and impurities so it's something that we want to avoid in the finished product (like residues and impurities).

Another example of herbal drug widespread used in medicine is the *Aesculus hippocastanum* L. extract and its isolated compound aescin.

Table 3

Example of calculation of the different toxicological parameters for the *Aesculus hippocastanum* L.

***Aesculus hippocastanum* L.**	**Compound**	**LHRD**	**TTC**	**PDE**	**$NOEL_{mouse}$**	**$NOEL_{human}$**
IP	Esculetin^	/	0,15 µg/day (IP)	0.017 mg/die	0,406 mg/Kg*bw/day	50,75 mg/Kg*bw/day
OS	Esculetin^	/	540 µg/day	0.023 mg/die	0,56 mg/Kg*bw/day	70 mg/Kg*bw/day
IP	Esculin^^	/	0,15 µg/day (IP)	0.022 mg/die	0,532 mg/Kg*bw/day	66,5 mg/Kg*bw/day
OS	Aescin	40 mg	540 µg/day	0.001925 mg/die	0,0462 mg/Kg*bw/day	5,775 mg/Kg*bw/day
IV	Aescin	1 mg/ml	0,15 µg/day	7.81 10^{-5} mg/die	0,00186 mg/Kg*bw/day	0,2345 mg/Kg*bw/day
OS	*Aesculus hippocastanum* L., extract	400 mg	540 µg/day	0.01238 mg/die	0,297 mg/Kg*bw/day	29,7 mg/Kg*bw/day
IP	*Aesculus hippocastanum* L., extract	/	540 µg/day	0.6875 10^{-3} mg/die	0,0165 mg/Kg*bw/day	1,375 mg/Kg*bw/day

BW = Body weight
IP = Intraperitoneally
OS = Oral somministration
IV = Intravenously
^= Esculetin (6,7-dihydroxycoumarin)
^^= Esculin (6-(β-D-glucopyranosyloxy)-7-hydroxy-2H-1-benzopyran-2-one, or 6,7 dihydroxycoumarin 6-glucosid)

Aesculus hippocastanum L. belongs to the Hippocastanaceae family and its common name is horse chestnut. Native to Western India, today the horse chestnut is widely distributed all over the world and it grows in Iran, Northern India, Asia Minor, Europe and USA (Bombardelli et al. 1996). It is a 25-30 mt. high tree (Bézanger-Beauquesne et al. 1980). The most used part is the bark that is obtained from the young branches and dried. The composition of horse chestnut bark is complex. The main compounds are coumarin derivate (up to 7%) (Wichtl et al. 2003) Glucosides: Esculin, a glucoside of esculetin, Fraxin a glucoside of fraxetin; Scopolin, a glucoside of scopoletin. Other constituents are: tannins (up to 2 %) (P. Fournier 1948; M. Paris, H. Moyse 1981), flavonoids, anthocyanins (Bombardelli et al. 1996), catechins derivatives (Bombardelli et al. 1996; Wichtl et al. 2003), traces of aescin (cortex) (Wichtl et al. 2003; Schneider 1978). Aescin, the major active principle from aesculus has shown satisfactory evidence for a clinically significant activity in chronic venous insufficiency (CVI), haemorrhoids and post-operative oedema (Sirtori 2001). This shown and help us to understand that many studies have been carried out on this pool of substances and they are still extensively used in therapy until today. For all those reasons, the safety could be assessed on the LHRD approach respectively in the dose of 40mg for the oral administration and 1 mg/ml for the slow intravenous administration. It is important to note that, for regulatory purposes and not only, it is always important to provide the rationale underlying the choice of value rather than another. In addition, it is necessary to specify that if the product in safety assessment is a plant extract and therefore, depending on whether it is an alcoholic, water or hydroalcholic extract we may have more or less all phytocomplex and not just aescine, it is more appropriate to take NOEL human as it results from a more representative datum. However, on the French market is authorized as “traditional use” a product made with the cortex dry extract (solvent water, DER 5-6: 1) of *Aesculus hippocastanum* L. with a posology 200 mg of extract two times daily, that could be used as LHRD for a water extract.

The last example concerns a product containing *Avena sativa* L. commonly named oat used both for medicine and as food. The characterization of the phytochemistry is reported in the document EMEA/HMPC/202967/2007, based on it we could say that all the parts of the plant contain the active principles but the main potentially toxic compounds are avenanthramide and saponins avenacoside A and B. However, avenacoside A oat saponins was 0.04% of dry matter and avenacoside B was 1% of dry substance as determined by thin layer chromatography (TLC) and the toxic effects of many saponins are neutralized in the mouth of animals such as sheep intestinal

bacteria and rumen bacteria (by saliva). Cooking or heat processing can also detoxify saponins. They are highly toxic when given intravenously (Harsha K. et al 2012). In addition, in the document "Scientific Opinion on the substantiation of a health claim related to oat beta-glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006", we could find two methanalisys from which is possible to know that "The estimated daily consumption of oat beta-glucan amounted to 1.1-7.6 g/day, with a mean dosage of 3.7 g/day. The sources of oat beta-glucan included oat bran, oat meal and rolled oats, which were consumed as breakfast cereals, biscuits, bread, muesli, muffins, and powders. The intervention periods of the studies varied from 2.5 to 12 weeks, with a mean intervention period of 5.5 weeks."; from this is clearly possible to understand that 3,7 g/day could be assumed as ADI and 1,1 g/day as LHRD. Concerning the saponins, of course, the intravenous route of administration must be excluded.

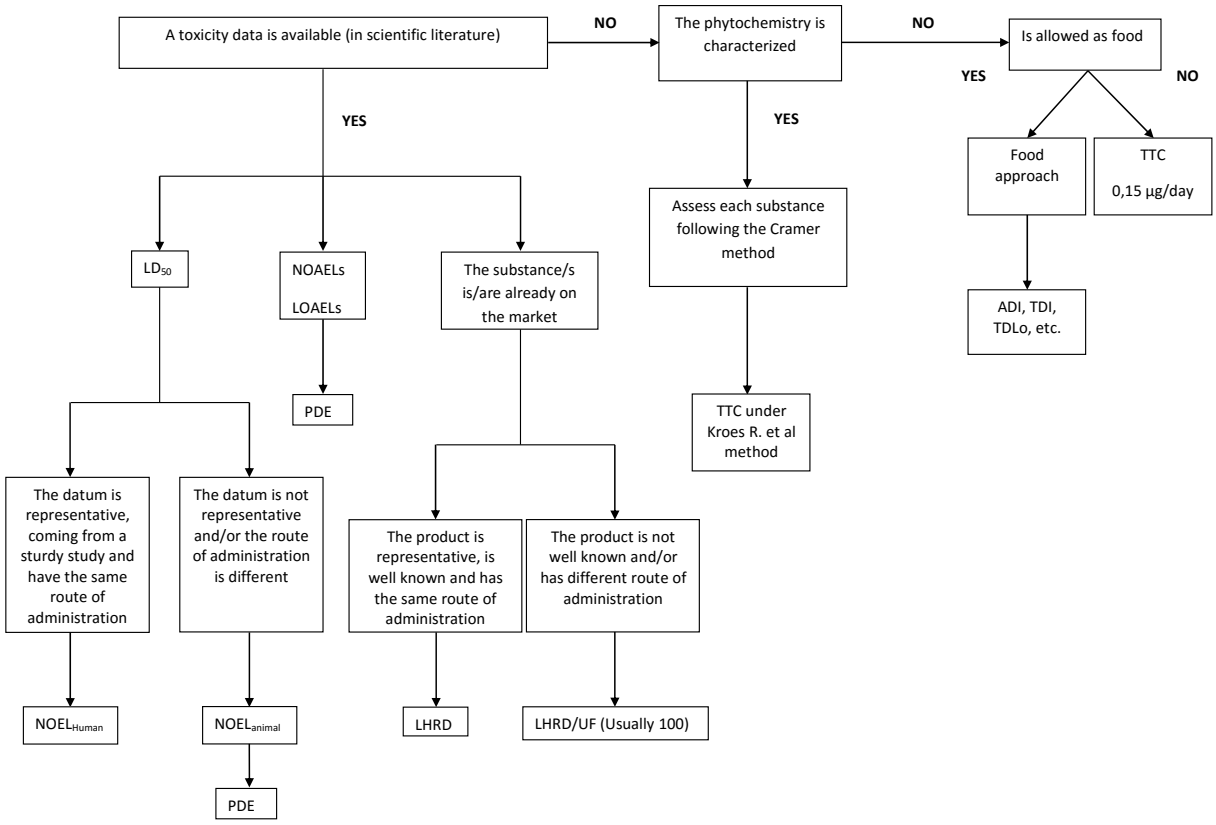
CONCLUSION

The growing use of plant medicines in Europe needs a shared methodology to determine the toxicity and daily exposure levels to these drugs. For this reason, the regulatory agencies of the various countries have undertaken a study that could meet popular uses and toxicological research in the various countries of the union. Fit for purpose of what has been analyzed so far, it is clear that it is possible to make a good assessment of the risk of exposure to a substance in the absence of an *ad hoc* toxicological study without neglecting safety. Indeed, it should be emphasized that often the statistical approach and the application of empirical uncertain factors leads to safety values below those that would result from an *ad hoc* toxicological study by virtue of its approximation. Moreover, the last direction of the European authorities and the scientific community is to reduce animal testing when predicted exposures to a chemical/herbal substance are below a level that would be associated with potential human health concerns, and the proposed approach, summarized for convenience in the decisions tree shown in Figure 2, could be helpful according this line of thoughts and no less important according to the principles of the "green chemistry" too (Anastas, Paul et al. (1998). It is highlight that the decision tree in the figure 2 is designed in the prospective to have a harmonized choice between parameters that are often used in different areas (food, chemicals, etc), in agreement with the view of a European harmonization of the evaluation for products of herbal origin, which lead to an easier circulation of the latter on the market.

In addiction, it is stressed that the authorities can always benefit form special warnings, precautions for use and limitation of use, such as excluding a special population (children, elderly, etc.), to ensure maximum protection for individuals.

Last remark is that the proposed method in not a way to skip the safety evaluation but it should be intended as a tool in the critical situation, a way to have a starting point for a risk assessment; is strongly supported that the reliability of a toxicological data is given by the risk assessment on itself and not merely on the data. Comes to mind when a generic drug has to show that is equivalent to the originator (branded) although most of time is easy to understand this, the equivalence must be demonstrated and dicussed.

Fig 2: decision tree which help to choose the best and safer approach for the assessment.



- Aithal BK, Sunil Kumar MR, Rao BN, Upadhya R, Prabhu V, Shavi G, Arumugam K, Sajankila SP, Udupa N, Satyamoorthy K, Satish Rao BS. Evaluation of pharmacokinetic, biodistribution, pharmacodynamic, and toxicity profile of free juglone and its sterically stabilized liposomes. J Pharm Sci 2011, 100:3517- 3528.
- Akdogan M, Ozguner M, Kocat A, Oncu M, Cicek E., Effects of peppermint teas on plasma testosterone, folliclestimulating hormone and luteinizing hormone levels and testicular tissues in rats. Urology, 2004c, 64:394-398.
- Akdogan M., Gultekin F., Yontem M. Effect of Mentha piperitae (Labiatae) and Mentha spicata (Labiatae) on iron absorption in rats. Toxicol. Health, 2004a, 20:119-122
- Akdogan M., Klinic I., Oncu M., Karaoz E, Delibas N. Investigation of biochemical and histopathological effects of Mentha piperitae L., and Mentha spicata L., on kidney tissue in rats. Hum Exp Toxicol 2003, 22: 213-219.
- Alonso D et al., Effects of topical Arnica gel on post-laser treatment bruises. Dermatol Surg, 2002, 28, 686-688
- American Herbal Association's Botanical Safety Handbook. CRC Press. Boca Raton (Florida, US), 329-331.
- Anastas, Paul T, Warner, John C. 1998. Green chemistry: theory and practice. Oxford [England]; New York: Oxford University Press. ISBN 9780198502340.
- Andro MC, Riffaud JP. Pygeum africanum extract for the treatment of patients with benign prostatic hyperplasia: a review of 25 years of published experience. Curr Ther Res 1995, 56:796–817
- Assessment report on Aesculus hippocastanum L., cortex, EMA/HMPC/354157/2011
- Assessment report on Avena sativa L., herba and Avena sativa L., fructus, EMEA/HMPC/202967/2007
- Assessment report on Rhodiola rosea L., rhizoma et radix, EMA/HMPC/232100/2011
- Assessment report on Viscum album L., herba, EMA/HMPC/246778/2009
- Attaluri A, Donahoe R, Valestin J, Brown K, Rao SSC. Randomised clinical trial: dried plums (prunes) vs. psyllium for constipation. Aliment Pharmacol Ther 2011, 33:822-828
- Babula P, Adam V, Havel L, Kizek R. Noteworthy Secondary Metabolites Naphthoquinones – their Occurrence, Pharmacological Properties and Analysis. Curr Pharm Anal 2009a, 5:47-68.
- Barlow SM, Kozianowski G, Wurtzen G and Schlatter J, 2001. Threshold of toxicological concern for chemical substances present in the diet. Food Chem. Toxicol. 39, 893–905.
- Barnaulov OD, Kumkov AV, Khalikova NA, Kozhina IS, Shukhobodskij BA. [Chemical composition and primary evaluation of the properties of preparations from Filipendula ulmaria (L.) Maxim flowers.] Rastit Resur 1977, 13:661-669
- Barnes D G, Durson M L. 1988. Reference dose (RfD): Description and use in health risk assessment. Regul. Toxicol. Pharmacol. 8:471-486
- Barnes J, Anderson AA, Phillipson JD. Herbal medicines. 3rd ed. Pharmaceutical Press London, Chicago2007, 273-275
- Barnes J, Anderson L, Philipson JD. Shepherd's Purse. Herbal Medicines, 3rd edition, Pharmaceutical Press, London 2007, 541-542.
- Barnes J, Anderson LA, Philipson JD. Herbal medicines. 3rd edition. Pharmaceutical press 2007, 436-46. Available at the EMA-library
- Barnes J, Anderson LA, Philipson JD. Herbal medicines. 3rd edition. Pharmaceutical press 2007, 436-46. Available at the EMA-library
- Barnes J, Anderson LA, Phillipson JD. Chamomile, Roman. In: Barnes J, Anderson LA, Phillipson JD. Herbal Medicines. 3rd Edition. Pharmaceutical Press, London, Chicago 2007, 157
- Barnes J, Anderson LA, Phillipson JD. Herbal Medicines, 3rd ed. Pharmaceutical Press, London 2007; 386-388.
- Battistelli M, De Sanctis R, De Bellis R, Cucchiarini L, Dacha M, Gobbi P. Rhodiola rosea as antioxidant in red blood cells: ultrastructural and haemolytic behaviour. Eur J Histochem 2005, 49(3):243-54.
- Beaux D, Fleurentin J, Mortier F. Effect of extracts of Orthosiphon stamineus Benth, Hieracium pilosella L., Sambucus nigra L. and Arctostaphylos uva-ursi (L.) Spreng. in rats. Phytother Res 1999, 13(3):222-225
- Bebbington A, Kulkarni R, Roberts P. Persistent bleeding after total hip arthroplasty caused by herbal self-medication. The Journal of Arthroplasty 2005, 20(1):125-126
- Belitz H.-D., Grosch W, Schieberle P. 2009. Food Chemistry 4th revised and extended ed.:469-470
- Benbassat N, Kostova B, Nikolova I, Rachev D. Development and evaluation of novel lozenges containing marshmallow root extract. Pak J Pharm Sci 2013, 26(6):1103-1107

- Bergonzi MC et al., Evaluation of skin permeability of sesquiterpenees of an innovative supercritical carbon dioxide arnica extract by HPLC/DAD/MS. Pharmazie, 2005, 60, 36-38
- Bernard P, Balansard P, Balansard G, Bovis A. Valeur pharmacodynamique toniveineuse des préparations galéniques à base de feuilles d'hamamélis. [Venitonic pharmacodynamic value of galenic preparations with a base of Hamamelis leaves]. J Pharm Belg 1972, 27(4): 505-512.
- Bernauer U, Heinemeyer G, Heinrich-Hirsch B, Ulbrich B and Gundert-Remy U, 2008. Exposure-triggered reproductive toxicity testing under the REACH legislation: a proposal to define significant/relevant exposure. Toxicol. Lett. 176, 68–76
- Bernd, A., Simon, S., Ramirez Bosca, A., Kippenberger, S., Diaz Alperi, J., Miquel, J., Villalba Garcia, J.F., Pamies Mira, D., Kaufmann R., Phototoxic effects of Hypericum extract in cultures of human keratinocytes compared with those of psoralen. Photochem Photobiol. 69, 218-221. 1999
- Bézanger-Beauquesne L, Pinkas M, Torck M and Trotin F. Aesculus hippocastanum L. Plantes médicinales des régions tempérées. Paris: Maloine, 1980, 2è ed., 164-165
- Bézanger-Beauquesne L, Pinkas M, Torck M. 1975. Les plantes dans la thérapeutique moderne. Paris: Maloine, 1975, 55-58
- Bickers D, Calow P, Greim h, Hanifin JM, Rogers AE, Saurat JH, Sipes IG, Smith RL, Tagami H. A toxicological and dermatological assessment of linalool and related esters when used as fragrance ingredients. Food and Chemical Toxicology 2003; 41: 919-942
- Blackburn K, Stickney JA, Carlson-Lynch HL, McGinnis PM, Chappell L and Felter SP, 2005. Application of the threshold of toxicological concern approach to ingredients in personal and household care products. Regulatory Toxicology and Pharmacology 43, 249–259.
- Blumenthal M, Busse WR, Goldberg A, Gruenwald J, et al., editors. The Complete German Commission E Monographs. American Botanical Council, Austin Texas 1998, 213
- Bolle P, Faccendini P, Bello U, Panzironi C, Tita B. Ononis spinosa L. Pharmacological effect of ethanol extract. Phamacol Res 1993, Vol. 27, (suppl 1):27-28
- Bombardelli E, Morazzoni P and Griffini A. Aesculus hippocastanum L. Fitoterapia 1996, 67:483–511
- Bombardelli E, Morazzoni P. Prunus africana (Hook f.) Kalkm. Fitoterapia 1997, 68:205-218
- Bradley PR editor. British Herbal Compendium. Vol 1. Fumitory - Fumariae herba. British Herbal Medicine Association, Bournemouth 1992, 102-104.
- Bradley RR, Cunniff PJ, Pereira BJG, Jaber BL. Hematopoietic effect of Radix angelicae sinensis in a hemodialysis patient. American Journal of Kidney Diseases 1999, 34:349-354
- Brendler T, Gruenwald J, Ulbricht C, Basch E. Devil's Claw (Harpagophytum procumbens DC): an evidence-based systematic review by the Natural Standard Research Collaboration. J Herb Pharmacother 2006, 6:89–126
- British Herbal Compendium. Vol. 1. British Herbal Medicine Association, Dorset 1992, 37-39
- British Herbal Pharmacopoeia. 4th ed. British Herbal Medicine Association, Exeter 1996; 117-118
- British Herbal Pharmacopoeia. Part 2. 1st ed. British Herbal Medicine Association, Exeter 1979, 141-143
- British Herbal Pharmacopoeia. Scientific Committee, British Herbal Medicine Association, Bournemouth 1983, 89–90
- Brown R P, Gerbarg P L , Ramazanov Z. 2002 Rhodiola rosea: A Phytomedicinal Overview 2002; 56:40-52
- Brown R, Carter J, Dewhurst I, Stephenson C and Tessier S, 2009. Applicability of thresholds of toxicological concern in the dietary risk assessment of metabolites, degradation and reaction products of pesticides SCIENTIFIC/TECHNICAL REPORT submitted to EFSA. http://www.efsa.europa.eu/en/supporting/pub/44e.htm
- Bruneton J. Farmacognosia: fitoquimica, plantas medicinales. 2nd ed. Acribia, Zaragoza 1998
- Bruneton J. Pharmacognosie: Phytochimie Plantes Médicinales. Ed. Tec & Doc, Paris 1999; 529-530
- Bruneton J. Phytothérapie. Les données de l'évaluation Ed. TEC & DOC- EM Inter, 2002, 101-107
- Buchbauer G, Jäger W, Jirovetz L, Limberger J, Dietrich H. Therapeutic properties of essential oils and fragrances. In: Teranishi R, Buttery RG, Sugisama H, editors. Bioactive Volatile Compounds from Plants. AC Symposium Series 525. Washington DC: American Chemical Society, 1993b
- Burdock GA, Carabin IG, Crincoli CM. Safety assessment of kola nut extract as a food ingredient. Food Chem Toxicol 2009; 47: 1725-32
- Burgos R, Hancke J. Long-term toxicity test. 3 months subchronic toxicity test for Sedum rosea. Swedish Herbal Institute, Gothenburg, Sweden, 1991 unpublished results.
- Capasso F, Mascolo N, Morrica P, Ramundo E Phytotherapeutic profile of some plants used in folk medicine. Boll Soc Ital Biol Sper 1983, 59:1398-1404 (source: ESCOP Monograph)
- Carthew P, Clapp C and Gutsell S, 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47, 1287–1295

- Chandler RF. Herbal medicine: horse chestnut. Can. Pharm. J., 1993, 126, 297, 300-306
- Cheeseman MA, Machuga EJ and Bailey AB, 1999. A tiered approach to threshold of regulation. Food Chem. Toxicol. 3, 387–412.
- Chen SW, Min L, Li WJ, Kong WX, Li JF, Zhang Yj. The effects of angelica essential oil in three murine tests of anxiety. Pharmacology Biochemistry and Behavior 2004, 79:377-382
- Chin Jin Han1, Abas Hj Hussin, Sabariah Ismail - Toxicity study of Orthosiphon stamineus Benth (Misai Kucing) on Sprague Dawley rats, Tropical Biomedicine 25(1): 9–16 (2008)
- Chouinard LE. The role of psyllium fibre supplementation in treating irritable bowel syndrome. Can J Diet Pract Res 2011, 72(1): e107-114
- Chow SY, Liao JF, Yang HY, Chen CF. Pharmacological effects of Orthosiphonis herba. J Formosan Med Assoc, 1979. 78: 953-960.
- Chudnicka A, Matysik G. Research of enzymatic activities of fresh juice and water infusions from dry herbs. J Ethnopharmacol 2005; 99(2):281-286
- CIR Expert Panel (2001) Final report on the safety assessment of Arnica montana extract and Arnica montana. Int J Toxicol 20 (Suppl 2):1-11
- Commission E Monographs. Enzianwurzel - Gentianae radix. Bundesanzeiger 223, 1985. Translated in Blumenthal M., Busse W.R., Goldberg A., Gruenwald J., Hall T., Riggins W., Rister R., Klein S. The Complete German Commission E Monographs.
- Commission E monographs. Orthosiphonis folium (Orthosiphonblätter). 1986, revised in 1990.
- Corazza M et al., Use of topical herbal remedies and cosmetics: a questionnaire based investigation in dermatology out patients. Journal compilation European Academy of Dermatology and Venerology, 2009, 10.1111, 1468-3083
- Cramer GM, Ford R A & Hall R L. 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16, 255-276.
- De Smet PAGM, Keller K, Hänsel R, Chandler RF. Adverse Effects of Herbal Drugs Vol. 2. Springer-Verlag, Berlin / Heidelberg 1993; 217-228.
- DeFeudis FV. Ginkgo biloba extract EGb 761: from chemistry to the clinic. Ullstein Medical, Wiesbaden 1998
- Delaney EJ, 2007. An impact analysis of the application of the threshold of toxicological concern concept to pharmaceuticals. Regulatory Toxicology and Pharmacology 49, 107124
- Deng S, Chen S-N, Yao P Et al. Serotonergic activity-guided phytochemical investigation of the roots of Angelica sinensis. J Nat Prod 2006, 69:536-541
- Deters A, Ziffel J, Hellenbrand N, Pappai D, Possemeyer C, Hensel A. Aqueous extract and polysaccharides from Marshmallow roots (Althaea officinalis L.): Cellular internalisation and stimulation of cell physiology of human epithelial cells in vitro. J Ethnopharmacology 2010, 127:62-69
- Dewhurst I, and Renwick AG, 2013. Evaluation of the Threshold of Toxicological Concern (TTC) –Challenges and Approaches. Regulatory Toxicology and Pharmacology 65, 168–177
- Doehmer J, Eisenbraun J. Assessment of Extracts from Mistletoe (Viscum album) for Herb–Drug Interaction by Inhibition and Induction of Cytochrome P450 Activities. Phytother Res 2012, 26(1):11- 17, doi 10.1002/ptr.3473
- Doehmer J, Eisenbraun J. Assessment of Extracts from Mistletoe (Viscum album) for Herb–Drug Interaction by Inhibition and Induction of Cytochrome P450 Activities. Phytother Res 2012, 26(1):11- 17, doi 10.1002/ptr.3473
- Donatus AA, Sardjoko, Vermeulen NPE. Cytotoxic and cytoprotective activities of curcumin. Biochem Pharmacol 1990, 39(12):1869-1875
- Dos Santos JG Jr, Do Monte FH, Blanco MM, Lanziotti VM, Maia FD, Leal LK. Cognitive enhancement in aged rats after chronic administration of Equisetum arvense L. with demonstrated antioxidant properties in vitro. Pharmacology Biochemistry and Behaviour 2005b, 81(3):593-600
- Draft Scientific Opinion on Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC) EFSA. (2011). Available at: http://www.efsa.europa.eu/en/consultations/call/110712a.htm
- Duband F, Carnat AP, Carnat A, PetitJean-Freytet C, Clair G, Lamaison JL. Composition aromatique et polyphénolique de l'infusé de Menthe, Mentha x piperita L. Ann. Pharm. Franç. 1992; 50(3):146-155.
- Edenharder R and Tang X. Inhibition of the mutagenicity of 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene by flavonoids, coumarins, quinones and other phenolic compounds. Food Chem
- EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific Opinion on the reevaluation of curcumin (E 100) as a food additive. EFSA Journal 2010, 8(9):1679 doi: 10.2903/j.efsa.2010.1679
- EFSA, 2009.Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal 1150, 1-72.

- EFSA, 2011. EFSA external scientific report on Applicability of physicochemical data, QSARs and read-across in Threshold of Toxicological Concern assessment. Prepared by Arianna Bassan, Elena Fioravanzo, Manuela Pavan and Matteo Stocchero.
- EFSA, 2012a. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA Journal 10(7), 2750 [103 pp.]
- EFSA, 2013. Scientific Opinion on the hazard assessment of endocrine disruptors: scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. EFSA Journal 11(3): 3132 [84 pp.]
- EFSA. Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern. European Food Safety Authority. EFSA Journal 2009, 7:281
- EFSA. Tolerable upper intake levels of vitamins and minerals. European Food Safety Authority, 2006 135-150
- EMA/HMPC/143659/2015
- EMA/HMPC/198794/2012
- EMA/HMPC/232100/2011
- EMA/HMPC/246778/2009
- EMA/HMPC/246799/2009
- EMA/HMPC/436680/2015
- EMA/HMPC/578322/2008
- EMA/HMPC/614586/2012
- EMA/HMPC/680615/2013
- EMA/HMPC/712510/2014
- EMA/HMPC/712650/2014
- EMA/HMPC/722365/2010
- EMA/HMPC/748221/2011
- EMEA/HMPC/107079/07
- EMEA/HMPC/138386/2005
- EMEA/HMPC/202967/2007
- Englert J and Harnischfeger G. Diuretic action of aqueous Orthosiphon extract in rats. Planta Med, 1992. 58 :237-8.
- Escher SE, Tluczkiewicz I, Batke M, Bitsch A, Melber C, Kroese ED, Buist HE and Mangelsdorf I, 2010. Evaluation of inhalation TTC values with the database RepDose. Regul. Toxicol. Pharmacol. 58 (2), 259–274
- ESCOP Monograph. HYPERICI HERBA (St. John's Wort). ESCOP Monographs Second edition. ESCOP 2003
- ESCOP Monographs 2nd ed. Althaeae radix–Marshmallow root. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 32-35
- ESCOP Monographs 2nd ed. Carvi fructus – caraway fruit. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 64-69
- ESCOP monographs 2nd ed. Centaurii herba - Centaury. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 70
- ESCOP Monographs 2nd ed. Filipendulae ulmariae herba – Meadowsweet. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 157-161
- ESCOP Monographs 2nd ed. Gentianae Radix – Gentian Root. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart-New York 2003, 174-177.
- ESCOP Monographs 2nd ed. Ginkgo folium – Ginkgo leaf. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 178-210
- ESCOP Monographs 2nd ed. Juniperi pseudo-fructus – Juniper. European Scientific Cooperation on Phytotherapy, editor. Thieme Verlag, Stuttgart 2003; 282-285.
- ESCOP Monographs 2nd ed. Lavandulae Flos, Lavandulae Aetheroleum. European Scientific Cooperative on Phytotherapy, editor. Supplement 2009. Georg Thieme Verlag, Stuttgart 2009; 147-156
- ESCOP Monographs 2nd ed. Ononidis radix - Restharrow root. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003, 351-353
- ESCOP Monographs 2nd ed. Pruni africanae cortex – Pygeum bark. European Scientific Cooperative on Phytotherapy, editor. Thieme Verlag, Stuttgart Supplement 2009, 206
- ESCOP Monographs 2nd ed. Supplement 2009. Centellae asiaticae herba. European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2009
- ESCOP Monographs. 2nd ed. Grindeliae Herba monograph. European Scientific Cooperative on Phytotherapy, editor. Georg Thieme Verlag, Stuttgart, Supplement 2009, 131-134

- ESCOP, ESCOP Monographs (2nd edition completely revised and expanded) European Scientific Cooperative on Phytotherapy, editor. Thieme, Stuttgart 2003.
- ESCOP. Harpagophyti radix (Devil's Claw root). In ESCOP Monographs. 2nd ed. Supplement 2009, 135- 147
- Esposito M, Carotenuto M. Ginkgolide B complex efficacy for brief prophylaxis of migraine in school- aged children: an open-label study. Neurological Sciences 2011, 32(1):79-81
- Eteng MU, Eyong EU, Akpanyung EO, Agiang MA, Aremu CY. Recent advances in caffeine and theobromine toxicities: a review. Plant Foods Hum Nutr 1997; 51: 231-43
- European Food Safety Authority (EFSA). Scientific opinion on the safety assessment of carvone, considering all sourse of exposure. EFSA Journal 2014, 12 (7): 3806 74pp
- European Pharmacopoeia 6.0 (2008a) Lavender flower oil. Council of Europe. 1/2008: 1534
- European Pharmacopoeia 6.0. Tormentil tincture – Tormentillae tinctura. Council of Europe. 01/2008: 1895
- European Pharmacopoeia 6th ed. Centaury – Centaurii herba. Council of Europe. 01/2008:
- European Pharmacopoeia 6th ed. Fumariae herba. Council of Europe. 1/2008: 1952.
- European Pharmacopoeia 6th ed. Meadowsweet – Filipendulae ulmariae herba. Council of Europe. 01/2008:1868, 2344-2345
- European Pharmacopoeia 7.0. Restharrow root – Ononidis radix. Directorate for the Quality of Medicines & Healthcare of the Council of Europe 2010, 01/2008:1879
- European Pharmacopoeia 7th ed. Colae semen. Council of Europe. 01/2008:1504 corrected 6.0
- European Pharmacopoeia 7th ed. Council of Europe. 1/2010: 2104
- European Pharmacopoeia 7th ed., Aniseed - Anisi fructus. Council of Europe. 1/2012: 0262; 3858- 3859.
- European Pharmacopoeia 8th ed. Equisetum Stem - Equiseti herba.
- European Pharmacopoeia 8th ed. Knotgrass – Polygoni avicularis herba. Council of Europe. 07/2013: 1885
- European Pharmacopoeia Online 8.2. Caraway fruit – Carvi fructus. Council of Europe. 01/2008:1080
- European Pharmacopoeia Online 8.2. Caraway oil – Carvi aetheroleum. Council of Europe. 01/2008:1817
- European Pharmacopoeia. 8th ed. Pygeum africanum bark - Pruni africanae cortex. Council of Europe. 1/2015:1886
- European Pharmacopoeia. Ph. Eur. 6.0, 1380 (01/2008)
- Evaluation of certain food additives. Fifty-first report of the Joint FAO/WHO Expert Committee on the Food Additives; WHO Geneve 2000.
- Everett DJ, Greenough RJ, Perry CJ, et al. Chronic toxicity studies of Efamol evening primrose oil in rats and dogs. Med Sci Res 1988a; 16: 863-864
- Everett DJ, Perry CJ, Bayliss P. Carcinogenicity studies of Efamol evening primrose oil in rats and mice. Med Sci Res 1988b; 16: 865-866
- FDA. Listing of Food Additive. http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm [accessed Nov. 2011]
- Federal Register. The Daily Journal of the United States Government. Rule. Laxative Drug Products for Over-the-Counter Human Use; Psyllium Ingredients in Granular Forms. A Rule by the Food and Drug Administration on 03/29/2007
- Felter S, Lane RW, Latulippe ME, Llewellyn GC, Olin SS, Scimeca JA and Trautman TD, 2009. Refining the Threshold of Toxicological Concern for risk prioritization of trace chemicals in food. Food Chem. Toxicol. 47, 2236–2245.
- Forgacs P, et al. Alcaloides des Papavéracées II: Composition chimique de dix-sept espèces de Fumaria. Plantes Med Phytother 1986, 20: 64-81.
- Fournier P. Marronnier d'Inde – Aesculus hippocastanum L. Le livre des plantes médicinales et vénéneuses de France. Paris: P. Lechevalier Éditeur, 1948, T.2, 475-479
- Fournier P. Marronnier d'Inde – Aesculus hippocastanum L. Le livre des plantes médicinales et vénéneuses de France. Paris: P. Lechevalier Éditeur, 1948, T.2, 475-479
- Frank C. Lu, Kacew S. 2002. Fundamentals, target organs and risk assessment, first published 2002 by Taylor & Francis:81-84
- Gaedcke F. Fraxinus. In: Hänsel R, Keller K, Rimpler H, Schneider G, editors. Hagers Handbuch der Pharmazeutischen Praxis. Drogen E-O, Vol 5. 5th ed. Springer-Verlag, Berlin, 1993, 188-200
- Galloway SM, Vijayaraj Reddy MA, McGettigan K, Gealy R and Bercu J, 2013. Potentially mutagenic impurities: Analysis of structural classes and carcinogenic potencies of chemical intermediates in pharmaceutical syntheses supports alternative methods to the default TTC for calculating safe levels of impurities. Regulatory Toxicology and Pharmacology 66, 326–335
- Gardner Z. and McGuffin M., 2013. Eleutherococcus senticosus (Rupr. & Maxim.) Maxim., Araliaceae.

- Gawrońska-Grzywacz M, Krzaczek T. Identification and determination of triterpenoids in Hieracium pilosella L. J Sep Sci 2007, 30(5):746-750
- Glisic SB, Milojevic SZ, Dimitrijevic SI, Orlovic AM, Skala DU. Antimicrobial activity of the essential oil and different fractions of Juniperus communis L. and comparison with some commercial antibiotics. J Serb Chem Soc 2007, 72:311-320.
- Göbel H, Heize A, Ingewersen M, Niederberger U, Gerber D. Harpagophytum-Extrakt LI 174 (Teufelskralle) bei der behandlung unspezifischer rûckenschmerzen. Schmerz 2001, 15:10-18
- Goetz P. Plantes médicinales d'Amérique du Nord: apport à la phytothérapie française du traitement des affections des voies respiratoires. Phytothérapie 2005, 1 :19-27
- Göggelmann W, Schimmer O, Mutagenic activity of phytotherapeutic drugs. Prog Clin Biol Res, 1986,206, 63-72
- Gold LS, Manley NB, Slone TH, Rohrbach L and Garfinkel GB., 2005. Supplement to the Carcinogenic Potency Database (CPDB): results of animal bioassays published in the general literature through 1997 and by the National Toxicology Program in 1997–1998. Toxicol. Sci. 85 (2), 747–808
- Greeson, J.M., Sanford, B., Monti, D.A. St. John's wort (Hypericum perforatum): a review of the current pharmacological, toxicological, and clinical literature. Psychopharmacology. 153, 402-414. 2001
- Grigorescu EM, Ciulei I, Stanescu U.- Index Fitoterapeutic, Edit. Medicală, Bucureşti 1986, 199-200
- Gruenwald J, Brendler T, Jaenicke C, LaGow B, editors. PDR for Herbal Medicines. 4th ed. Thomson PDR, Montvale 2007, 429-430
- Gruenwald J., Brendler T., Jaenike C. PDR for Herbal Medicines. 2nd ed. Montvale, NJ: Medical Economics Company, Inc, 2004, 408-414
- Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, (CDER), 2005.
- Guideline for residual solvents, EMA/CHMP/ICH/82260/2006 (ICH guideline Q3C (R6) on impurities).
- Guideline on medicinal products for the treatment of Alzheimer's disease and other dementias from Committee for medicinal products for human use (CHMP). Doc. Ref. CPMP/EWP/553/95 Rev. 1. European Medicines Agency, London 2009
- Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities, EMA/CHMP/CVMP/SWP/169430/2012.
- Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products, EMA/HMPC/162241/2005 Rev. 2.
- Guideline on the Limits of Genotoxic on the Impurities, EMEA/CHMP/QWP/251344/2006.
- Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM et al. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet Toxicol 1967, 5: 141- 157.
- Hall, R.L. and Oser, B.L. 1970. Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. 4. GRAS substances. Food Technol. 24(5): 25-34.
- Hamamelidis aqua (Hamamelis water). European Scientific Cooperative on Phytotherapy, Exeter 2003. In: ESCOP Monographs, 2nd edition, 2003.
- Hamamelidis folium (Hamamelis leaf). European Scientific Cooperative on Phytotherapy, Exeter 2003. In: ESCOP Monographs, 2nd edition, 2003.
- Hamamelis. In: WHO monographs on selected medicinal plants. WHO, Geneva 2002, 124-136.
- Hänsel R, Keller K, Rimpler H, G. Schneider. Hagers Handbuch der Pharmazeutischen Praxis. Drogen E- O, Springer Verlag, Berlin 1993; 561-579.
- Hänsel R, Keller K, Rimpler H, Scheidner G. Hagers Handbuch der pharmazeutischer Praxis, Springer- Verlag, Berlin 1993, 929-936
- Hänsel R, Keller K, Rimpler H, Schneider G. Hager's Handbuch der Pharmazeutischen Praxis. Spinger Verlag, Berlin 1993; 630-644
- Hänsel R., Keller K., Rimpler H., Schneider G. Hager's Handbuch der Pharmazeutischen Praxis. Drogen A-D. Springer-Verlag, Berlin 1992: 437-46.
- Hara Y. Green tea- Health benefits and application, Marcel Dekker Inc, 2001, 18:178
- Harsha K, Preeti P, Manish K P, Harendra K, Amit C K, Devi D J. 2012. Foaming glycosides: a review, Journal of Parmacy Volume 2, Issue 5,. 2012:23-28
- Heinoven M., Ollilainen V., Linkola E., Varo P., Koivistoinen P. Carotenoids and retinoids in Finnish foods: cereal and bakery products. Cereal Chem. 1989; 66 (4): 270-3.
- Hellum BH, Tosse A, Hoybakk K, Thomsen M, Rohloff J, Georg Nilsen O. Potent in vitro inhibition of CYP3A4 and P-glycoprotein by Rhodiola rosea. Planta Med 2010, 76(4):331-8.
- Hellum BH, Tosse A, Hoybakk K, Thomsen M, Rohloff J, Georg Nilsen O. Potent in vitro inhibition of CYP3A4 and P-glycoprotein by Rhodiola rosea. Planta Med 2010, 76(4):331-8.

http://www.who.int/ceh/publications/endocrine_disrupters_child/en/index.html
- Huber R, von Ditfurth A, Amann F, Güthlin C, Rostock M, Tritler R, Kümmerer K, Merfort I. Tormentil for active ulcerative colitis. J Clin Gastroenterol 2007, 41:834-38
- Humfrey CDN, 2007. Recent Developments in the Risk Assessment of Potentially Genotoxic Impurities in Pharmaceutical Drug Substances. Toxicological Sciences 100(1), 24–28.
- JECFA: Joint FAO/WHO Expert Committee on Food Additives, 44th meeting: WHO Food Additives Series 35: Curcumin.
- JECFA: Joint FAO/WHO Expert Committee on Food Additives. Safety evaluation of certain food additives. Trans-anethole. WHO Geneva, 1999.
- Joksić G, Stanković M, Novak A. Antibacterial medicinal plants Equiseti herba and Ononidis radix modulate micronucleus formation in human lymphocytes in vitro. Journal of Environmental Pathology, Toxicology and Oncology 2003, 22(1):41-48
- Joksić G, Stanković M, Novak A. Antibacterial medicinal plants Equiseti herba and Ononidis radix modulate micronucleus formation in human lymphocytes in vitro. J Environ Pathol Toxicol Oncol 2003, 22:41-4
- Kalkhof H, Herzler M, Stahlmann R and Gundert-Remy U, 2012. Threshold of toxicological concern values for non-genotoxic effects in industrial chemicals: re-evaluation of the Cramer classification. Archives of Toxicology 86, 17-25
- Karmally W., Montez MG., Palmas W. Et al. Cholesterol-lowering benefits of oat-containing cereal in Hispanic Americans. J. Am. Diet. Assoc. 2005; 105: 967-70.
- Kazemivash N, Jamali M. Investigation of cytotoxic effects of methanolic total extract of the aerial part of polygonum aviculare L. in vegetative and generative phase on nalm-6 (pre-B-cell leukaemia) cell line. Journal of Biology and Today's World 2013, 11:506-508
- Kerscher MJ, Korting HC. Treatment of atopic eczema with evening primrose oil: rationale and clinical results. Clin Investig 1992; 70: 167-171
- Kienle GS, Kiene H. Die Mistel in der Onkologie - Fakten und konzeptionelle Grundlagen. Schattauer Verlag, Stuttgart, New York, 2003
- Koster S, Boobis AR, Cubberley R, Hollnagel HM, Richling E, Wildemann T, Würtzen G and Galli CL, 2011. Application of the TTC concept to unknownsubstances found in analysis of foods. Food and Chemical Toxicology 49, 1643–1660
- Kostova I, Iossifova T. Chemical components of Fraxinus species. Fitoterapia 2007, 78:85-106
- Kreitmar H. Pharmakologische wirkung des alkaloids aus Peumus boldus Molina. Pharmazie 1952; 7: 07-511.
- Kroes R, Galli C, Munro I, Schilter B, Tran LA, Walker R and Würtzen G, 2000. Threshold of Toxicological Concern for Chemical Substances Present in the Diet: A Practical Tool for Assessing the Need for Toxicity Testing. Food and Chemical Toxicology 38, 255-312
- Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Würtzen G. 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem Toxicol. 2004:65-83
- Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG and Würtzen G, 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Food Chem. Toxicol. 42, 65–83
- Kroes R, Renwick AG, Feron V, Galli CL, Gibney M, Greim H, Guy RH, Lhuguenot JC and van de Sandt JJ, 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45, 2533–2562
- KSRNAM Kiso to Rinsho. Clinical Report. (Yubunsha Co., Ltd., 1-5, Kanda
- Kucinskaite A, Poblocka-Olech L, Krauze-Baranowska M, Sznitowska M, Savickas A, Briedis V. Evaluation of biologically active compounds in roots and rhizomes of Rhodiola rosea L. cultivated in Lithuania. Medicina (Kaunas) 2007, 43(6):487-94.
- Kucinskaite A, Poblocka-Olech L, Krauze-Baranowska M, Sznitowska M, Savickas A, Briedis V. Evaluation of biologically active compounds in roots and rhizomes of Rhodiola rosea L. cultivated in Lithuania. Medicina (Kaunas) 2007, 43(6):487-94.
- Kujawski R, Ozarowski M, Derebecka-Holysz N, Bartkowiak-Wieczorek J, Bogacz A, Karasiewicz M, et al. Effect of Willow herb (Epilobium angustifolium L.) extract on gene expression of selected P450 cytochromes in rat liver – preliminary study. Herba Pol 2009, 55(4):52-64
- Kumarasamy Y, Nahar L, Sarker SD. Bioactivity of gentiopicroside from the aerial parts of Centaurium erythraea. Fitoterapia 2003a, 74(1-2):151-154
- Kuo J, Chen KW, Cheng IS, Tsai PH, Lu YJ and Lee NY, 2010. The effect of eightweeks of supplementation with Eleutherococcus senticosus on endurance capacity andmetabolism in human. Chin J Physiol, 53(2):105-111.

- Kuroda K, Kaku T. Pharmacological and chemical studies on the alcohol extract of Capsella bursapastoris.
- Laerum OD, Iversen OH. Reticuloses and epidermal tumors in hairless mice after topical skin applications of cantharidin and asiaticoside. Cancer Res 1972, 32:1463-69
- Lapenna S and Worth A, 2011. Analysis of the Cramer classification scheme for oral systemic toxicity – implications for its implementation in Toxtree. EUR 24898 EN. Publications Office of the European Union, Luxembour
- Laufersweiler MC, Gadagbui B, Baskerville-Abraham IM, Maier A, Willis A, Scialli AR, Carr GJ, Felter SP, Blackburn K and Daston G, 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regulatory Toxicology and Pharmacology 62, 160-182
- Le Bourhis B. Propriétés biologiques du trans-anethole. Essai de détermination de la dose journalière acceptable. Parfums Cosmet Sav Fr 1973, 3: 450-456.
- Lee D, Park J, Yoon J, Kim MY, Choi HY, Kim H, 2012. Neuroprotective effects of Eleutherococcus senticosus bark on transient global cerebral ischemia in rats. J Ethnopharmacol, 139 (1):6-11.
- Lee YJ, Chung HY, Kwak HK, Yoon S, 2008. The effects of A. senticosus supplementation on serum lipid profiles, biomarkers of oxidative stress, and lymphocyte DNA damage in postmenopausal women. Biochem Biophys Res Commun, 375(1):44-48.
- Leeman WR, Krul L and Houben GF, 2013. Complex mixtures: Relevance of combined exposure to substances at low dose levels. Food and Chemical Toxicology 58, 141–148
- Leistner E, Drewke C. Ginkgo biloba and ginkgotoxin. Journal of Natural Products 2010, 73(1):86-92
- Leslie G.B., Salmon G. Repeated dose toxicity studies and reproductive studies on nine Bio-Strath herbal remedies. Swiss Med 1979, 1: 43-45.
- Leuschner, J. Preclinical toxicological profile of Hypericum extract LI 160. Abstracts of the 2nd International Conference on Phytomedicine, Munich. SL 80, 1996
- Levy-Appert-Collin M-C, Levy J. Sur quelques preparations galeniques de feuilles de Boldo (Peumus boldus, Monimiacees J Pharm Belg 1977; 32: 13-22
- Li ZB, Wang JY, Jiang B, Zhang XL, An LJ, Bao YM. Benzobijuglone, a novel cytotoxic compound from Juglans mandshurica, induced apoptosis in HeLa cervical cancer cells. Phytomedicine 2007, 14:846- 852.
- Life Sciences, 1969, 8(I):151-155.
- Lin FSD. Trans-anethole. In: Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series 28. WHO Geneva, 1991: 135-152.
- Lin SC, Lin CC, Lin YH, Supriyatna S, Teng CW. Protective and therapeutic effects of Curcuma xanthorrhiza on hepathotoxin-induced liver damage. Am J Chin Med 1995, 23:243-254
- Lund K, Rimpler H. Tormentil rhizome: isolation of an ellagitannin and pharmacological screening. Dtsch Apoth Ztg 1985, 125:105-08
- Madaus G. Lehrbuch der biologischen Heilmittel. Georg Thieme Verlag, Leipzig 1938
- Magistretti MJ. Remarks on the pharmacological examination of plant extracts. Fitoterapia 1980; 51:67-79.
- Marlett JA, Kajs TM, Fischer MH. An unfermented gel compound of psyllium husk promotes laxation as a lubricant in humans. Am J Clin Nutr 2000, 72(3):784-789
- Martindale. The Extra Pharmacopoeia. 30th ed. The Pharmaceutical Press, London 1993, 1401
- Martindale: The Complete Drug Reference. Shepherd's Purse.S. C. Sweetman. The Pharmaceutical
- Masamoto Y. [Sensitization and crossreaction of simple coumarins.] Yakugaku Zasshi. 2001, 121:97- 103 [Japanese]
- Mascolo N, Autore MG, Capasso F, Menghini A, Fasulo MP. Biological screening of Italian medical plants for anti-inflammatory activity. Phytother Res 1987, 1:28-31
- Matsushima T., Araki A., Yagame O., Muramatsu M., Koyama K., Ohsawa K. et al. Mutagenicities of xanthone derivatives in Salmonella typhimurium TA100, TA98, TA97 and TA2637. Mutation Res 1985, 150: 141-146.
- Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Van Raaij M and Vickers C, 2011. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regul Toxicol Pharmacol, 60(2 suppl 1), S1-S14
- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA. 2002
- Mohammad Jamal Saharkhiz, Marjan Motamedi, Kamiar Zomorodian, Keyvan Pakshir, Ramin Miri, and Kimia Hemyari Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of Mentha piperita L., doi: 10.5402/2012/718645
- Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. Salmonella Mutagenicity tests: II. Results from the testing of 270 Chemicals. Envir Mutagen 1986, 8(7): 1-27.
- Müller L, Mauthe RJ, Riley CM, Andino MM, DeAntonis D, Beels C, DeGeorge J, DeKnaep, Joseph AGM, Ellison D, Fagerland JA, Fran, R, Fritschel B, Galloway S, Harpur E, Humfrey CDN, Jacks AS, Jagota N, Mackinnon J, Mohan G,

Ness DK, O'Donovan MR, Smith MD, Vudathala G and Yotti L, 2006. A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. Regulatory Toxicology and Pharmacology 44 (2006) 198–211

- Munro IC, Ford R A, Kennepohl E & Sprenger J G. 1996. Correlation of structural class with no-observed- effect levels: a proposal for establishing a threshold of concern. Food and Chemical Toxicology 34, 829-867.
- Munro IC, 1990. Safety assessment procedures for indirect food additives: an overview. Report of a workshop. Regul. Toxicol. Pharmacol. 12, 2–12. threshold of concern. Food Chem. Toxicol. 34, 829–867.
- Munro IC, Renwick AG and Danielewska-Nikiel B, 2008. The Threshold of Toxicological Concern (TTC) in risk assessment. Toxicol. Lett. 180, 151–156.
- Myers JP, Zoeller RT and vom Saal FS, 2009. A Clash of Old and New Scientific Concepts in Toxicity,
- Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. Contact Dermatitis 2008, 58(3): 134-141
- National Toxicology Program (NTP). Toxicology and Carcinogenesis Studies of Turmeric Oleoresin (Cas No. 8024-37-1) in F344/N Rats and B6C3F1 Mice (Feed Studies). Technical Report Series No. 427, NIH Publication No. 93-3158. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC 1993
- natural source for element supplementation. Acta Alimentaria, 2005, 34(2):161-167
- NIIRDN Drugs in Japan (Ethical Drugs). (Yakugyo Jiho Co., Ltd., Tokyo, Japan) Volume (issue)/page/year: 6,APP-2,1982
- Notice to Applicants Medicinal products for human use – volume 2B - Presentation and format of the dossier Common Technical Document (CTD), https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-2/b/update_200805/ctd_05-2008_en.pdf
- NTP technical report on the toxicology and carcinogenesis studies of Ginkgo biloba extract (CAS No. 90045-36-6) in F344/N rats and B6C3F1/N mice (gavage studies). National toxicology program, Research triangle park, North Carolina 2013
- NTP Toxicology and Carcinogenesis Studies of d-Carvone (CAS No. 2244-16-8) in B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser 1990, 381:1-113
- Okpanyi, S.N., Lizba, H., Scholl, B.C., Miltenburger, H.G. Genotoxicity of a standardized Hypericum extract. Arzneimittelforschung. 40, 851-855. 1990
- Olsnes S, Stirpe F, Sandvig K, Pihl A. 1982. Isolation and characterization of viscumin, a toxic lectin from Viscum album L. (mistletoe). 1982 25;257(22):13263-70
- Opdyke DL. Monographs on fragrance raw materials: Boldo oil. Food and Chemical Toxicology 1982; 20 (Suppl.6): 643-644.
- Opdyke DLJ. Peru balsam. Food Cosmet Toxicol 1974, 12: 951-952
- Oser BL & Hall RL (1977). Criteria employed by the expert panel of FEMA for the GRAS evaluation of flavouring substances. Food and Cosmetics Toxicology 15, 457-466.
- Oser, B.L. and Ford, R.A. 1977. Recent progress in the consideration of flavoring ingredients under the Food Additives Amendment. 10. GRAS substances. Food Technol. 31(1): 65-74.
- Paris M, Moyse H. Abrégé de matière médicale, Tome 1. Paris : Masson, 1981, 147-150
- Paris M, Moyse H. Abrégé de matière médicale, Tome 1. Paris: Masson, 1981, 147-150
- Ph. Eur. 6th ed. Ash leaf - Fraxini folium. Council of Europe. 01/2008:1600; 1222-1223
- Pharmacopée Française - French Pharmacopoeia 10 th ed. Grindélia - Grindelia sp. 1998
- Pinalli R, Croera C, Theobald A and Feigenbaum A, 2011. Threshold of toxicological concern approach for the risk assessment of substances used for the manufacture of plastic food contact materials. Trends in Food Science & Technology 22, 523-534
- Porchezhian E, Ansari SH, Shreedharan NK. Antihyperglycemic activity of Euphrasia officinale leaves.Fitoterapia 2000; 71(5):522-526
- Principles for the safety assessment of food additives and contaminants in food, WHO, 1987.
- Rennen MAJ, Koster S, Krul CAM and Houben GF, 2011. Application of the threshold of toxicological concern (TTC) concept to the safety assessment of chemically complex food matrices. Food and Chemical Toxicology 49, 933–940
- Renwick AG, 2004. Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents. Toxicol. Lett. 149, 223–234
- Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Greig JB, Kleiner J, Lambe J, Müller DJ, Smith MR, Tritscher A, Tuijtelaars S, van den Brandt PA, Walker R and Kroes R, 2003. Risk characterisation of chemicals in food and diet. Food and Chemical Toxicology, 41(9), 1211-1271

- Riaz A, Khan RA, Ahmed SP. Assessment of anticoagulant effect of evening primrose oil. Pak J Pharm Sci 2009; 22(4): 355-9
- Roman A, Rusu MA, Puica C, Borsa M. Citotoxic effects of three speies of Epilobium (Onagraceae) herbal extracts in rats. Studia Universitatis "Vasile Goldis", Seria Stiintele Vietii 2010, 20(1):19-23
- Roman I, Rusu MA, Puică C, Borşa M. [Emphasis of some preparate of Polygonum action on the vital organs in laboratory animals]. Annals of the Romanian Society for Cell Biology 2008, 13:183-198
- Rossi PG, Berti L, Panighi J, Luciani A, Maury J, Muselli A. Antibacterial Action of essential oils from Corsica. J Essent Oil Res 2000, 19:176-182.
- Rouhi H, Ganji F. Effect of Althaea officinalis on cough associated with ACE inhibitors. Pakistan J Nutr 2007, 6(3):256-258
- Rowbotham AL and Gibson RM, 2011. Exposure-driven risk assessment: applying exposure-based waiving of toxicity tests under REACH. Food Chem. Toxicol. 49, 1661–1673.
- Salvador RL. Herbal medicine - Ginkgo. Canadian Pharmaceutical Journal 1995, 39-41, 52
- Samojlik I, Mijatović V, Petković S, Skrbić B, Božin B. The influence of essential oil of aniseed (Pimpinella anisum, L.) on drug effects on the central nervous system. Fitoterapia 2012.
- SCCS/SCHER/SCENIHR, 2012. Opinion on: Use of the threshold of toxicological concern (TTC) approach for human safety assessment of chemical substances with focus on cosmetics and consumer products. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf
- Schleich S, Papaioannou M, Baniahmad A, Matusch R. Activity-guided isolation of anantiandrogenic compound of Pygeum africanum. Planta Med 2006a, 72(6):547-551
- Schleich S, Papaioannou M, Baniahmad A, Matusch R. Extracts from Pygeum africanum and other ethnobotanical species with antiandrogenic activity. Planta Med 2006b, 72(9):807-813
- Schmidgall J, Schnetz E, Hensel A. Evidence for bio adhesive effects of polysaccharides and polysaccharide-containing herbs in an ex vivo bioadhesion assay on buccal membranes. Planta Med 2000, 66:48-53
- Schmidt M., Schmitz H.-J., Baumgart A., Guedon D., Netsch M.I., Kreuter M.-H., Schmidlin C.B., Schrenk D. Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. Food Chem Toxicol 2005, 43:307–314
- Schneider E. Des plantes pour votre santé, Dammarie-Les-Lys : Éditions SDT, 1978, 124
- Schneider K, Kubelka W. Zur Hämolytischen Wirkung von Schachtelhalmkraut (Herba Equiseti). Scientia Pharmaceutica 1989, 57(3):214-215
- Schnuch A, Lessmann H, Geier J, Frosch PJ, Uter W. Contact allergy to fragrances: frequencies of sensitization from 1996 to 2002. Results of the IVDK. Contact Dermatitis 2004, 50(2): 65-76
- Scientific Opinion on the substantiation of a health claim related to oat beta-glucan and lowering blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006 - EFSA Journal 2010;8(12):1885
- Shushunov S, Balashov L, Kravtsova A, Krasnogorsky I, Latte KP, Vasiliev A. Determination of acute toxicity of the aqueous extract of Potentilla erecta (tormentil) rhizomes in rats and mice. J Med Food 2009, 12:1173-76
- Siddique YH, Ara G, Beg T, Faisal M, Ahmad M, Afzal M. Antigenotoxic role of Centella asiatica L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. Toxicol In Vitro 2008, 22:10-17
- Sirtori C R. 2001. Aescin: pharmacology, pharmacokinetics and therapeutic profile. Pharmacol Res. 2001;44(3):183-93.
- Snodin DJ and McCrossen SD, 2012. Guidelines and pharmacopoeial standards for pharmaceutical impurities: Overview and critical assessment. Regulatory Toxicology and Pharmacology 63, 298–312.
- Snodin DJ and McCrossen, SD, 2013. Mutagenic impurities in pharmaceuticals: A critique of the derivation of the cancer TTC (Threshold of Toxicological Concern) and recommendations for structural-class-based limits. Regulatory Toxicology and Pharmacology 67, 299–316.
- Spettoli E et al., Contact dermatitis caused by sesquiterpene lactones. Contact Dermatitis, 1998, 9 (1), 49-50
- Springer-Verlag, Berlin 1994, 1160-1179
- Stanojević L, Stanković M, Nikolić V, Nikoli L, Ristić D, Canadanovic-Brunet J, et al. Antioxidant Activity and total Phenolic and Flavonoid Contents of Hieracium pilosella L. extracts. Sensors (Basel) 2009, 9(7):5702-5714
- Stein GM. Toxicology of mistletoe and their components. In B ssing A, editor. Mistletoe. The Genus Viscum. Hardwood Academic Publishers, Amsterdam 2000, 183-194
- Stein GM. Toxicology of mistletoe and their components. In: Büssing A, editor. Mistletoe. The Genus Viscum. Hardwood Academic Publishers, Amsterdam 2000, 183-194
- Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet (Kroes et. al, 2003)

- Study 6114-184, 187, 188. Procter & Gamble. Subchronic toxicity in the rat. Pharmaco-toxicological expert report for Metamucil, January 2001
- Suda-Cho, Chiyoda-ku, KS Bldg., Tokyo 101, Japan) V.1- 1960 Volume (issue)/page/year: 8,118,1974
- Szentmihalyi K, Taba G, Lado C, Fodor J, Then M, Szoke E. Medicinal plant teas recommended as
- Tago Y, Wei M, Ishii N, Kakehashi A, Wanibuchi H. Evaluation of the subchronic toxicity of dietary administered Equisetum arvense in F344 rats. Journal of Toxicologic Pathology 2010, 23(4):245-251
- Tahraoui A, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of a lyophilized aqueous extract of Centaurium erythraea in rodents. J Ethnopharmacol 2010, 132(1):48-55
- Tezuka Y, et al. Constituents of the Vietnamese Medicinal Plant Orthosiphon stamineus. Chem Pharm Bull, 2000. 48 (11): 1711-1719.
- The Merck Index, 11th edition
- Turley SD, Daggy BP, Dietschy JM. Psyllium augments the cholesterol-lowering action of cholestyramine in hamsters by enhancing sterol loss from the liver. Gastroenterology 1994, 107(2):444-452
- U.S. EPA (U.S. Environmental Protection Agency). (2002a) A Review of the Reference Dose and Reference Concentration Processes. Risk Assessment Forum, Washington, DC. December. EPA/630/P-02/002F.
- U.S. EPA (U.S. Environmental Protection Agency). (2002b) Interim Reregistration Eligibility Decision Document - Fenamiphos. Office of Pesticide Programs, Washington, DC. EPA 738-R-02-004.
- Updated on revision chapters 3 and 5 of the GMP guide, EMA/INS/GMP/809387/2.
- US FDA, 1993. United States Food and Drug Administration. Food additives: Threshold of Regulation for Substances Used in Food-Contact Articles. Federal Register 58: 52719–52729, Tuesday October 12
- Van Hellemont J. Compendium de Phytothérapie. 1st ed. Association Pharmaceutique Belge, Brussels 1986
- Verschaeve L, Kestens V, Taylor JLS, Elgorashi EE, Maes A, Van Puyvelde L, et al. Investigation of the antimutagenic effects of selected South African medicinal plant extracts. Toxicology in Vitro 2004, 18(1):29-35
- Vitalone A, Bordi F, Baldazzi C, Mazzanti G, Saso L, Tita B. Anti-proliferative effect on a prostatic epitheliala cell line (PZ-HPV-7) by Epilobium angustifolium L. Il Farmaco 2001, 56:483-489
- Wegner T. Anwendung eines Trockenextraktes aus Gentianae luteae radix bei dyspeptischem Symptomkomplex. Z Phytotherapie 1997, 19: 163-164.
- Weiss RF, Fintelmann V. Lehrbuch der Phytotherapie. 9th ed. Hippokrates Verlag, Stuttgart 1999; 253- 255.
- WHO Monographs on Selected Medicinal Plants. Vol 2. Pygeum africanum. World Health Organization, Geneva 2009, 83-91
- WHO monographs on selected medicinal plants. Vol. 3. Radix Harpagophyti. World Health Organisation. Geneva 2007, 182-194
- WHO, 1997. Evaluation of certain food additives and contaminants (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868
- WHO, 2006. Evaluation of certain food contaminants (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930
- WHO, 2009. Assessment of combined exposures to multiple chemicals: report of a WHO/IPCS international workshop on aggregate/cumulative risk assessment. IPCS harmonization project document no. 7 http://www.who.int/ipcs/methods/harmonization/areas/workshopreportdocument7.pdf?ua=1
- WHO, 2011. Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria Series 240.
- WHO, 2012. Endocrine disrupters and child health: Possible developmental early effects of endocrine disrupters on child health. 84 pp.
- Wichtl M et al. 2003. Plantes thérapeutiques, 2è ed. Paris: Editions Tec et Doc/EM Inter, 2003, 294-296
- Wichtl M et al. Plantes thérapeutiques, 2è ed. Paris: Editions Tec et Doc/EM Inter, 2003, 294-296
- Wichtl M. Herbal drug and Phytopharmaceuticals 3rd ed. MedPharm GmbH Scientific Publishers 2004, 271-273
- Wichtl M. Herbal drugs and Phytopharmaceuticals. 1st ed. Medpharm Scientific Publishers GmbH, Stuttgart 1994, 480-482
- Wichtl M. Herbal drugs and Phytopharmaceuticals. 3rd ed. Medpharm Scientific Publishers GmbH, Stuttgart 2004, 421-423Evaluation of certain food additives. Fifty-first report of the Joint FAO/WHO Expert Committee on the Food Additives; WHO Geneve 2000.
- Williamson E, Driver S, Baxter K. Stockley's Herbal Medicines Interactions. 1st ed. Pharmaceutical
- with Important Implications for Public Health. Environ Health Perspect 117:1652–1655
- Wren RC. Potter's New Cyclopedia of Botanical Drugs and Preparations. The C.W. Daniel Company Limited, Safron Walden, Essex 1998
- Wynne PM, Vine JH, Amiet RG. Protopine alkaloids in horse urine. J Chromatogr B Analyt Technol Biomed Life Sci 2004, 811: 85-91.

- Yen GC, Chen HY, Peng HH. Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food Chem Toxicol 2001, 39:1045-53
- Zaragoza MC, Lopez D, Saiz MP, Poquet M, Pérez J, Puig-Parellada P Et al. Toxicity and antioxidant activity in vitro and in vivo of two Fucus vesiculosus extracts. J Agr Food Chem 2008, 56:7773-7780
- Zarn JA, Engeli BE and Schlatter JR, 2011. Study parameters influencing NOAEL and LOAEL in toxicity feeding studies for pesticides: Exposure duration versus dose decrement, dose spacing, group size and chemical class. Regulatory Toxicology and Pharmacology 61, 243–250
- Zawodsky, L. Klinische Erfahrungen mit dem Amphocholeretikum Oddibil. [Clinical experience with Fumaria officinalis nebulisate, an amphocholinergic agent.] Wiener Medizinische Wochenschrift 1974, 124(46): 677-680.
- Zhu J, Wan X, Zhu Y, Ma X, Zheng Y, Zhang T. Evaluation of salidroside in vitro and in vivo genotoxicity. Drug Chem Toxicol 2010, 33(2):220-6.
- Zhu J, Wan X, Zhu Y, Ma X, Zheng Y, Zhang T. Evaluation of salidroside in vitro and in vivo genotoxicity. Drug Chem Toxicol 2010, 33(2):220-6.
- Zimmermann W. Planzliche Bitterstoffe in der Gastroenterologie. Z Allgemeinmed 1976, 54:1178-1184