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Review

Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment

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ABSTRACT

Although herbal medicinal products (HMP) have been perceived by the public as relatively low risk, there has been more recognition of the potential risks associated with this type of product as the use of HMPs increases. Potential harm can occur via inherent toxicity of herbs, as well as from contamination, adulteration, plant misidentification, and interactions with other herbal products or pharmaceutical drugs. Regulatory safety assessment for HMPs relies on both the assessment of cases of adverse reactions and the review of published toxicity information. However, the conduct of such an integrated investigation has many challenges in terms of the quantity and quality of information. Adverse reactions are under-reported, product quality may be less than ideal, herbs have a complex composition and there is lack of information on the toxicity of medicinal herbs or their constituents. Nevertheless, opportunities exist to capitalise on newer information to increase the current body of scientific evidence. Novel sources of information are reviewed, such as the use of poison control data to augment adverse reaction information from national pharmacovigilance databases, and the use of more recent toxicological assessment techniques such as predictive toxicology and omics. The integration of all available information can reduce the uncertainty in decision making with respect to herbal medicinal products. The example of *Aristolochia* and aristolochic acids is used to highlight the challenges related to safety assessment, and the opportunities that exist to more accurately elucidate the toxicity of herbal medicines.

Contents

Abbreviations: AA, aristolochic acid; AAN, aristolochic acid nephropathy; AR, adverse reaction; DEG, differentially expressed gene; DES, diethylstilbestrol; GMP, good manufacturing practices; cGMP, current good manufacturing practices; HCP, healthcare practitioner; HMP, herbal medicinal product; MAH, market authorisation holder; NTP, National Toxicology Program; PCC, poison control centre; QSAR, quantitative structure activity relationship; TCM, traditional Chinese medicine.

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Introduction

Herbal medicinal products (HMPs) are widely used around the world, increasingly so in Western nations. A survey conducted in 2005 revealed that 71% of Canadians were using natural health products, a term which includes not only HMPs but also vitamins and minerals. In this study, 11% of the persons surveyed used herbal remedies and algal/fungal products (IPSOS Reid, 2005). In the United States, about 19% of the adult population were using HMPs as of 2002 (Kennedy, 2005). Another study has shown about 36% of pregnant women in Norway use herbs (Nordeng and Havnen, 2004).

Although HMPs are widely considered to be of lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effects (De Smet, 2004). High profile issues such as adverse reactions associated with *Ephedra* and *Aristolochia* have shown that HMPs can produce toxicity in humans. While inherent toxicity of certain herbs is well known, adverse effects from the use of HMPs may also result from contamination of products with toxic metals, adulteration with pharmacologically active synthetic compounds, misidentification or substitution of herbal ingredients, or improperly processed or prepared products (Ernst, 2004, Van Breemen et al., 2008, Ankli et al., 2008; Chan, 2009). Interactions may also occur between drugs, foods and other HMPs taken concomitantly (Foster et al., 2005, Fugh-Berman, 2000, Goldman et al., 2008).

Recently, there has been increased discussion on the safety assessment of herbs. Protocols and guidance documents on safety and toxicity testing of HMPs have been issued by the International Life Sciences Institute (summarised by Schilter et al., 2003), the Institute of Medicine / National Research Council (2004), the Union of Pure and Applied Chemistry (Mosihuzzaman and Choudhary, 2008), the European Medicines Agency (EMEA) (e.g. EMEA, 2007, 2009), and most recently by the European Food Safety Authority (EFSA, 2009). These guidance documents discuss the assessment of the safety of herbs for use in both foods and medicines. The types of testing described in these guidance documents represent the ideal type of information that could be obtained in order to adequately characterise the toxicity of a specific herb or a finished herbal product ready for the marketplace.

International regulatory systems for HMPs can be quite variable in terms of safety and toxicity testing requirements. In countries where HMPs are less strictly regulated than synthetic drugs, and where limited toxicity testing is required, or where HMPs are regulated as intermediate products classified separately from foods and drugs, where less stringent requirements exist for certain sub-types of products (e.g. traditionally used herbs where their long-term use is considered evidence for safety), pre-market assessment may be based on limited information.

Even in countries where HMPs are assessed in detail before market authorisation is given, pharmacovigilance is a critical activity to promote the safe use of HMPs throughout their life cycle. As the use of HMPs grows around the world, the identification of safety signals becomes of increased importance. The identification and investigation of safety signals associated with HMPs are subject to the same challenges as signals arising from pharmaceutical drugs. There are, however, challenges unique to HMPs. There are often deficiencies in both the quantity of information (e.g. under-reporting of adverse reactions, general lack of toxicological information on herbs) and the quality of information (e.g. poor quality of adverse reaction case reports or lack of information on the quality of HMPs associated with case reports submitted to regulatory authorities or published in the scientific literature). These factors present challenges when signals of safety concerns arise.

In the regulatory context, safety assessment can have bearing on whether certain products should be restricted, removed from the market, or have augmented safety information placed on labelling. In instances where little toxicity information exists on a specific herbal product or its ingredients, regulatory decisions on risk mitigation activities are likely to take a cautious approach, until further information is obtained which can potentially clarify the toxicity of the product, and reduce uncertainty in the risk assessment of HMPs.

This paper discusses the challenges which are faced in the assessment of safety of HMPs. Also discussed is the need for careful consideration of existing data, and opportunities for increasing both the quantity and quality of knowledge. From the post-market perspective, an integrative approach is necessary to investigate safety signals for any product type. Clinical assessment of adverse reaction reports, either submitted to the regulatory authority, or published in the scientific literature, needs to be considered along with available toxicological and pharmacological information in order to fully characterise potential safety concerns. While challenges exist for the assessment of HMP safety, efforts are being made to add quality information to the herbal safety knowledge base so that judgements on the hazard and risk of HMPs can be made with increased certainty.

Regulation of herbal medicinal products internationally

A regulatory framework for HMPs provides consumers greater assurance that the identities of medicinal ingredients have been verified, that they have been properly quantified per unit dose, that there has been an assessment of the safety and efficacy of the product

prior to granting of market authorisation, and that the product is within tolerance limits for contaminants. Requirements for Good Manufacturing Practices (GMP) provide a framework for assuring continuing quality and the ability to deal appropriately and quickly with problems when they do arise. Adverse reaction reporting requirements facilitate the detection of such problems. Four different national regulatory frameworks are summarised below, to illustrate some similarities and differences in the regulation of HMPs.

In the United States, HMPs are regulated as dietary supplements, a subset of foods. Under the *Dietary Supplement Health and Education Act* of 1994 (DSHEA), the dietary supplement manufacturer is responsible for ensuring that a dietary supplement is safe before it is marketed. The United States Food and Drug Administration (FDA) is responsible for taking action against any unsafe dietary supplement product after it reaches the market. Generally, manufacturers do not need to register their products with the FDA, nor obtain FDA approval, before producing or selling dietary supplements unless it is a New Dietary Ingredient, or to verify the acceptability of a structure–function type of claim. The FDA Final Rule for current good manufacturing practices (cGMPs) for dietary supplements requires that proper controls are in place for dietary supplements so that they are processed in a consistent manner, and meet quality standards (US Department of Health and Human Services, 2007). The cGMPs apply to all domestic and foreign companies that manufacture, package, label or hold dietary supplements, including those involved with the activities of testing, quality control, packaging and labelling, and distribution in the U.S. The Rule establishes cGMPs for industry-wide uses that are necessary to require that dietary supplements are manufactured consistently as to identity, purity, strength, and composition. Manufacturers, packers, or distributors are required to submit all serious adverse event reports associated with use of the dietary supplement in the United States to the FDA, through the adverse reaction reporting program (Med Watch). FDA's post marketing responsibilities include monitoring safety, e.g. voluntary dietary supplement adverse event reporting, and product information, such as labelling, claims, package inserts, and accompanying literature (US FDA 2009a, 2009b).

In Canada, HMPs are regulated under the *Natural Health Products Regulations* (NHPR) which came into force on January 1, 2004. These regulations are distinct from the regulations for food and drugs. This is a mandatory pre-market system where each HMP must receive market authorisation by obtaining a product licence based on evidence that the product is safe under the recommended conditions of use without a prescription, effective for the proposed claims, and of high quality. Each importer, manufacturer, packager and labeller of HMPs requires a site licence issued on the basis of evidence of compliance with GMPs created specifically for natural health products (which includes HMPs). Every licensee must provide an expedited case report for each serious adverse reaction to their product occurring in Canada and each serious unexpected adverse reaction occurring inside or outside Canada. They must also prepare and maintain an annual summary report with analysis of all adverse reactions, to be provided to the Department of Health upon demand (Government of Canada 2009).

In Australia, HMPs are regulated by the Therapeutic Goods Administration (TGA) as medicines under the *Therapeutics Goods Act* 1989, using risk-based pre-market assessment procedures based on toxicity of ingredients, dosage form, serious disease claims, side effects/interactions, and adverse reactions. Listed medicines are considered low risk and are included on the Australian Registry of Therapeutic Goods (ARTG). Listed Medicines may only contain ingredients that have been evaluated by the TGA to be low risk, must be manufactured by licensed manufacturers in accordance with the principles of GMP, and may carry indications only for health maintenance and health enhancement or certain indications for non-serious, self-limiting conditions. Most, but not all, HMPs included on the ARTG are Listed Medicines. Herbal medicines that are assessed to be of higher risk are individually evaluated for quality, safety and efficacy for licensing as Registered Medicines. Each Australian manufacturer of HMPs must hold a manufacturing license and comply with the Australian Code of GMP for Medicinal Products. The Code applies to all medicines manufactured in Australia, including complementary medicines such as HMPs. An adverse reaction reporting system for medicines in Australia is well established. The Australian "Blue Card" scheme covers all medicines and most health professionals. In addition, sponsors of all medicines included in the ARTG are under an obligation to report adverse reactions to the TGA (TGA, 2006).

In the European Union (EU), HMPs are classified as "regular" medicinal products if they claim to treat or prevent illness, or if they are to be administered with a view to restoring, correcting or modifying physiological functions. As such, they are subject to the general regulations for medicines as laid down in the various national medicines laws. A marketing authorisation as a HMP is granted based on a "full" dossier in terms of proof of quality, safety and efficacy in almost all Member States but the Committee on Herbal Medicinal Products (HMPC), part of the EMEA, establishes Community herbal monographs to simplify the authorisation of HMPs. With respect to GMP compliance, the EU follows the Pharmaceutical Inspection Cooperation Scheme (PIC/S). The competent authority may carry out announced or unannounced inspections of active substance manufacturers in order to verify compliance with the principles of GMPs for active substances placed on the Community market. The Marketing Authorisation Holder (MAH) must ensure that they have an appropriate system of pharmacovigilance and risk management in place in order to assure responsibility and liability for its products on the market and to ensure that appropriate action can be taken, when necessary. Specifically, the MAH must have an approved system of reporting adverse reactions. Governmental responsibilities lie with each Member State's agencies (European Union 2004, 2008, 2009). For example, in the United Kingdom, the Medicines and Healthcare products Regulatory Agency (MHRA) provides transitional provisions where an HMP legally on the UK market as an unlicensed herbal remedy in accordance with the *Medicines Act* 1968 can continue to be marketed as an unlicensed herbal remedy until April 30, 2011. At that time all manufactured HMPs will be required to have either a Traditional Herbal Registration or a Marketing Authorisation based on the European Directive. The Traditional Herbal Registration is a simplified UK registration scheme that began in 2005 with specific standards of safety and quality, agreed indications based on traditional usage, and systematic patient information allowing the safe use of the product (MHRA, 2009).

Adverse reactions and causality assessment for HMPs: limitations, challenges and opportunities for improvement

An adverse reaction (AR) is defined as a noxious and unintended response to a marketed health product, which occurs at doses normally used or tested for the diagnosis, treatment or prevention of a disease or the modification of an organic function (Health Canada, 2009a). In the literature, there are few clinical studies with most herbs despite the fact that many have been employed for centuries as traditional medicines; therefore, surveillance for HMP-related ARs consists mainly of voluntary reporting from consumers and Health Care Practitioners (HCP) and published reports which are usually single reports or small case series. Due to the lack of clinical trials for most HMPs, post-market pharmacovigilance is a critical source of safety information; however, the assessment of ARs associated with HMPs offers unique challenges in the quantity and quality of available information (Farah et al., 2000, Gardiner et al., 2008).

Adverse reaction reports: quantity

While the under-reporting of ARs to synthetic drugs is known (Hazell and Shakir, 2006), the number of suspected dietary

supplement ARs that are reported in the United States is estimated to be less than 1% of the total that may actually occur (Woo, 2007). The low incidence of AR reports associated with HMPs is partially due to the fact that consumers generally regard them as safe and therefore believe their symptoms could not be attributable to the use of these products. They often do not report HMP use to a HCP even when asked on a questionnaire (Hensrud et al., 1999) and patients may feel that HMP use may not be accepted by their HCP (Busse et al., 2005). Barnes et al. (1998) noted that, in the United Kingdom, 30% of consumers surveyed would report a serious AR suspected to be related to either conventional or herbal health products, to their physician. However, 26% of respondents would report a serious suspected AR to conventional medications, but would not report a suspected AR to a herbal medication. This under-reporting by patients is exacerbated by the fact that not all ARs reported to physicians are subsequently reported to regulatory authorities. HCPs who are in a position to observe ARs, often pharmacists and physicians, may not report ARs for a variety of reasons. In addition to reasons found previously (the impression the AR is already known, forgetting to report, unwillingness to report on suspicion alone, pressures of clinical practice, and uncertainty about the reporting process) a recent study has also identified the risk perception of the reporter, the seeming remoteness of the regulatory authority and the lack of feedback from the regulator as important variables (Nichols et al., 2009). The quantity of AR reports in a spontaneous reporting system is not reflective of the frequency of ARs in the population of users, due to under-reporting and the lack of information on the sales of marketed products.

Increasing the quantity of AR reports for herbal products

In Canada and the United States, marketers are required to report serious or serious, unexpected ARs. "Serious" is defined in accordance with the International Conference On Harmonisation (ICH, 2003), as life threatening or fatal, causing or prolonging hospital admission, causing persistent incapacity or disability, causing a congenital anomaly or birth defect, or is a medically important event or reaction. "Unexpected" is defined as a frequency/severity not consistent with what is labelled (product monograph, product label). If it is not clear whether the reaction is serious (i.e. medically significant) or unexpected, the AR should be submitted to the regulatory authority (US FDA, 2009c, Health Canada, 2009a). As well, in Canada, market authorisation holders are required to prepare an annual safety summary report on suspected ARs, which is to be submitted to Health Canada on request. Such requirements can aid in the increased reporting of serious ARs associated with HMPs.

Education of potential HCP reporters such as physicians, pharmacists, and complementary medicine providers to make them aware that HMP ARs are reportable to regulatory authorities, and providing information enabling them to do so, may increase the number of reports for this product type. After identifying that pharmacists often do not report suspected ARs related to HMP-drug interactions, a recent educational undertaking in Canada attempts to address this issue (Charrois et al., 2007). As noted below, educational activities may also increase the quality of reports. The issuance of risk communications by regulatory agencies can also serve to identify potential risks to consumers and health care practitioners, as well as to provide contact information for the reporting of ARs associated with the issue at hand. A review of postmarket surveillance of HMPs outlines the initiatives ongoing in Canada to increase the reporting of ARs (Murty, 2007).

Active surveillance for ARs also would be expected to increase the quantity of reports. A study known as Pharmacy SONAR (Study of Natural Health Products Adverse Reactions) was piloted in Canada (Cvijovic et al., 2009). In this pilot study, staff at four pharmacies were directed to ask patients picking up prescriptions about whether they had used a natural health product (including HMPs) in the last 3 months at the same time as a conventional medication, and whether they had experienced an AR. Such studies would be expected to increase reporting of herbal/drug interactions. Along with the educational activities noted above, such efforts may increase herbal product AR reporting in general.

Use of data derived from poison control centres

Poison control centres (PCC) have been investigated as potential novel sources of ARs for HMPs. In a study of adverse drug reactions reported to a PCC in the United States during the period of 2000 to 2007, dietary supplements, herbs and homeopathic products combined was one of the highest groups of products associated with hospitalisation (Vassilev et al., 2009). In 2002, the total exposures to dietary supplements, herbs and homeopathic products reported to PCCs across the United States numbered almost 23,000 (Watson et al., 2003). In 2004, this number was almost 25,000 (Woolf, 2006). On a national basis in the United States, the volume of toxic exposures submitted to PCCs, suspected to be associated specifically with herbal products, climbed steadily from 1993 (639 reports) to 2002 (11,495 reports) (Woolf et al., 2005). A substantial number of the reports noted by Woolf et al. (2005) related to single or multi-ingredient ephedra products; however, the total number of reports for botanicals other than ephedra increased as well.

One of the first efforts to study reports of adverse effects of HMPs using PCC data was conducted by researchers in the United Kingdom (Perharic et al., 1994, Shaw et al., 1997). The study by Perharic et al. (1994) was retrospective in nature, covering 4 years worth of data on traditional remedies and food supplements, including non-herbal products. A further 1 year prospective study was conducted. In all, 1040 enquiries were received for HMPs, plus animal- and enzyme/glandular products. Of these cases, 270 involved symptomatic exposures. Eight cases of heavy-metal poisonings were related to contaminated products, and the causality of 44 other cases was deemed "highly probable" ($n=2$) or "probable" ($n=42$). The authors found that few of the cases identified retrospectively were adequately documented, creating difficulty in assessment. Many cases lacked proper product identification, past medical history of patients, and dose and duration of exposure. Concomitant use of other products also existed.

A search of the California Poison Control System database revealed 600 exposures to dietary supplements and herbal remedies during an 18-month period. Homeopathic and traditional Chinese medicine products were excluded from the search. The seriousness of the reported adverse reactions ranged from "no treatment" (28%) to "severe" (1.3%). The majority of the exposures were classified as "minor" (33%) or "moderate" (27%), while 12% of exposures could not be evaluated for seriousness (Yang et al., 2002). This paper also ranked the causality of the reports based on temporality and de/re-challenge information. Causality was ranked and found to be "probable" or "possible" in the majority (81%) of cases. The remaining exposures were classified as "unknown." In a follow-up study (Dennehy et al., 2005) over a 6-month period, using the same database, a total of 828 reports were included in the study. About 30% of reports were related to the use of ephedra-containing products. The seriousness of effects related to exposures to non-ephedra products was similar to that reported in the initial study, with the exception that exposures classified as "severe," were 13% compared to 1.3% in the initial study. The percentage of cases with "probable" and "doubtful" causalities was 79%, similar to that found in the 2002 study.

Prospective studies have been carried out using PCC data. Shaw et al. (1997) conducted a 5-year study where PCC enquiries related to traditional remedies and food supplements were collected and followed up by questionnaire, analysis of patient samples, and products, and botanical identification of herbal ingredients. HMPs, royal jelly, pollen, enzyme and hormonal products were combined in one group. This group of products was associated with 660 symptomatic cases over the study period; however, causality assessment could be determined in only 349 of these cases. The

causality of the cases (using the WHO criteria) were: certain ($n=1$), probable ($n=27$), possible ($n=316$) and conditional ($n=5$). Sixty-eight cases involved Chinese or Indian herbal products, of which 10 cases were designated as certain causality. It should be noted that not all cases involved proper use of products. This study did show, however, that PCC data can be a useful source of AR reports, and that prospective monitoring and follow up significantly increased the ability to assess the reports for causality.

Palmer et al. (2003) conducted a 1-year prospective study in PCCs in the United States. ARs associated with dietary supplements (including herbal products) were targeted. Eight PCCs provided data for this study. Using a 5-point Likert scale, the authors selected 489 cases for which they were 50% certain of the causal relationship between the product and the adverse effect. Mild and moderate effects comprised 93% of effects, while severe and fatal cases comprised 6 and 1%, respectively. In all, 30% of effects were of moderate or greater seriousness. The authors noted that dietary supplements were associated with adverse events in all classes of seriousness, and included all organ systems and age groups. In a more recent 1-year prospective study conducted within the California Poison Control System (Haller et al., 2008), reports suspected to be associated with dietary supplements (including herbs) were assessed for causality using WHO criteria. Two thirds of the reports were assessed as probably or possibly related to the dietary supplement in question using WHO causality assessment criteria. The majority of the moderate and serious adverse events comprised sympathomimetic events associated with products containing caffeine, yohimbine, bitter orange (*Citrus aurantium* L., Rutaceae) and gentian (*Gentiana lutea* L., Gentianaceae). Events in other categories included blood coagulation disorders associated with vitamin E, fish oil and ginkgo (*Ginkgo biloba* L., Ginkgoaceae). The authors noted that the ability of clinical toxicologists to evaluate the causality in real time, and the ability to analyse the products involved, strengthened the quality of information obtained.

Other studies have been conducted to investigate the adverse events, submitted to PCCs, suspected as being associated with specific herbs, or types of products (Woolf et al., 2005, Robinson et al., 2004). The larger of these studies was conducted by Woolf et al. (2005), to investigate the severity of the toxic reactions to products containing ephedra (*Ephedra sinica* Stapf, Ephedraceae). The number of reports associated with ephedra increased by 150-fold over a 10-year period, and allowed the calculation of hazard rates of 250/1000 exposures and 267/1000 exposures for products containing ephedra only, and multi-ingredient ephedra-containing products, respectively. In contrast, the hazard rate for herbal products that contained no ephedra was 96/1000 exposures. Rate ratios were 1.25 (95% CI 1.11–1.40) and 1.33 (95% CI 1.27–1.40) for single and multi-ingredient ephedra products, respectively, suggesting that other ingredients did not alter the effects of ephedra alone. The authors noted that this investigation supported the findings of a previous case series of ephedra reported to the US FDA. Robinson et al. (2004) studied the reports submitted on herbal stimulant weight-loss products to a single American PCC. It was found that 78% of all reports involving symptomatic exposures involved doses of the products which were higher than recommended on the product labelling, or which were unknown. However, 70% of persons who had consumed the recommended dose experienced symptoms.

Limitations on the use of PCC data

Several limitations exist in the use of PCC data for monitoring adverse reactions to health products. Calls to PCCs do not always reflect exposures to substances, and often are made to obtain information on particular products or chemicals. As with adverse reactions reported to pharmacovigilance systems, details of actual exposure, the specific product involved, and its ingredients, as well as the clinical outcome may not be known without further case follow-up. Without prompt case follow-up, the determination of causality may be compromised. Retrospective data mining of PCC data can be difficult because of the inability to follow up on reports of interest. Even with prospective studies in PCCs, incomplete case follow-up is a potential limitation (Haller et al., 2008). The difficulties in assessing causality in PCC reports are similar to those already discussed in this paper, such as often unknown product/ingredients, and a lack of ability to analyse all products to rule out contamination or adulteration. The inability to quantify the incidence rates of adverse effects associated with a specific product, due to under-reporting is also problematic. Woolf et al. (2005) has outlined these limitations with respect to PCC reporting of herbal products. Deng et al. (1997) has outlined similar challenges in assessing PCC reports of poisoning associated with traditional Chinese medicine in Taiwan, during a 3-year prospective study, where reports included both raw herbs provided by practitioners, and proprietary finished products.

Advantages of using PCC data

Despite limitations, the analysis of PCC data offers unique benefits. The volume of reports related to herbal / dietary supplements, submitted to PCCs is far greater than those reported to national pharmacovigilance systems. When contained in national databases such as the Toxic Exposure Surveillance System in the United States, PCC data could increase the ability to detect rare adverse events involving herbal products (Gryzlak et al., 2007). With adequate case follow-up, especially in prospective studies, PCC data can serve a valuable complementary role to data submitted to pharmacovigilance systems. Such value has been noted in the above described studies on *Ephedra*. The types of exposures captured by PCCs is another distinct advantage. PCC data includes much information on inadvertent exposures to children, and a range of exposures including both use according to labelled directions and overuse/abuse situations (Woolf, 2006). Thus, with careful follow up, PCC data, when used in combination with data from regulatory pharmacovigilance databases, may lead to insights on the adverse effects of health products in vulnerable populations, and on dose-response relationships. This information would be particularly valuable in the case of HMPs where such information is often lacking. Most importantly, with careful analysis and follow up, such as in prospective studies, PCC data can be used as a surveillance tool to monitor the safety of herbal products (Gryzlak et al., 2007). It has been recommended that the US FDA should employ PCC reports as a data source on these types of products (Institute of Medicine, 2004).

Adverse reaction reports: quality

Some issues related to quality of adverse reaction reports have been reviewed by Gardiner et al. (2008). Many HMP ARs are generated by consumers and this can make interpretation of the information supplied difficult. Objective data such as lab or clinical toxicology reports may be lacking or incomplete (Palmer and Rao, 1999). In these cases ruling out of other possible causes of the AR such as coexistent disease and other medication may not be possible. Exposure information is often inadequate in AR case reports, therefore the actual amount of exposure to the suspect product/herb, and whether it was used according to labelled instructions, may be unknown. Outcome and time to recovery, critical in determining dechallenge, are often lacking or unclear. Additional information is often necessary for meaningful analysis but may not be available unless follow up is conducted soon after the AR is reported.

Herbal medicinal products suspected as being associated with an AR can provide significant challenges. Products often consist of multiple herbs or other ingredients. In such cases, it may only be possible to assign a causality to the product as a whole, and not to individual ingredients. Even with single-ingredient products, it is important that information on the plant part used is identified. Among the various species of *Echinacea* in commerce, different plant parts are used in HMPs. For example, both the roots and aerial

parts of *Echinacea purpurea* (L.) Moench are used, and differences occur in the phytochemical content of these plant parts (Upton, 2004, Upton, 2007). Knowing the specific plant part associated with a suspected AR improves the assessment of any reported adverse effects. In addition, various extraction procedures of the same herb, or plant part, will produce finished products of varying chemical composition (Williamson et al., 1996). Knowledge of the nature of the preparation is ideal for proper assessment. Correct identification on the herbal ingredients can be problematic as incomplete Latin binomials on product labels or in AR reports do not reflect specific plants (Farah et al., 2000). The genus *Eucalyptus* contains some 500 species; thus an AR noting the suspect product as "eucalyptus" makes it difficult to relate this AR to other similar ARs. Also, common names may not be a true indication of species. For instance, "ginseng" should only refer to species in the genus *Panax* such as Asian ginseng (*Panax ginseng* C. A. Mey., Araliaceae) and American ginseng (*Panax quinquefolius* L.) but has been applied to other plants in the same family such as Siberian ginseng (*Eleutherococcus senticosus* (Rupr. and Maxim.) Maxim.) and even plants in different families, such as blue ginseng (*Gynostemma pentaphyllum* (Thunb.) Makino, Cucurbitaceae) and lesser or prince ginseng (*Pseudostellaria heterophylla* (Miq.) Pax, Caryophyllaceae) (McGuffin et al., 2000).

Species misidentification/substitution, contamination and adulteration are known to occur in herbal products (see Product quality below), again making the interpretation of specific ARs more difficult. Identification of the plant part is also ideal, and in some cases essential. For example, castor seed oil is used in food flavourings and cosmetics but castor seed meal is the source of the toxic protein ricin (USDA, 2009). In some cases, marketed products identified by trade name may not allow differentiation between similarly named products from different manufacturers, which may have very different ingredients. In an ideal identification the scientific binomial name of all herbal ingredients, the manufacturer and complete product name, lot number or analysis of the product taken would be given. The mechanism of injury for a HMP can be particularly challenging given that many products are multi-herbal combinations. Absolute identification of the species or phytochemical suspected may only be available through analysis of the product.

Causality assessment

Causality assessment, or the linking of the observed adverse event to the suspected product, is a pivotal step in management plans in pharmacovigilance and data sharing between regulatory agencies. Assessment of suspected ARs associated with HMPs is particularly challenging as in addition to the quantity of adverse reports there are significant quality issues which impact on the ability to adequately investigate the association between the AR and the product or ingredient in question. There are many different methods proposed for causality assessment consisting of three main types; algorithmic, probability based and expert analysis. There is no universally accepted method recognised, however the factors important in assessment are: temporality between the exposure to the suspect substance and the AR, including dechallenge and rechallenge, the role of coexistent disease and medication as alternative etiologic possibilities, and the examination of a plausible pathophysiologic mechanism of injury which includes the history and pharmacology of the suspect ingredient (Edwards and Aronson, 2000, Arimone et al., 2007, Agbabiaka et al., 2008).

The World Health Organisation (WHO), Uppsala Monitoring Centre (UMC) in consultation with the national centres who participate in the WHO Programme for International Drug Monitoring have developed a method for causality of case reports (WHO, 2007a). This method takes into account the quality of the data available for analysis as well as expert opinion on pharmacologic and clinical aspects. In this paradigm, causality is divided into six descriptive categories; Certain, Probable/Likely, Possible, Unlikely, Conditional/Unclassified and Unassessible. These categories allow a gradation of association with Certain described as a clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary. The other categories allow for less definitive associations, including the description Unassessible, where incomplete data prevents a causal association to be made for a particular case report.

The quality of AR reports impacts on causality assessment of HMPs particularly, and causality cannot give a quantitative measurement of relationship likelihood. However in postmarket surveillance the linking of product/ingredient use to AR reports will continue to be an important component for risk mitigation, regulatory activity, and data sharing between regulatory authorities. The WHO International Regulatory Cooperation for Herbal Medicines (IRCH) was established in 2005 to protect and promote public health and safety through improved regulation for herbal medicines, and as such is a forum for sharing information about safety signals and regulatory activities between participating countries (WHO, 2009).

Increasing the quality of AR reports for herbal products

The quality of AR reports of HMPs can be improved by increased follow-up where practical, efforts at educating reporters, and use of consistent terminology in reports.

Follow-up with reporters of ARs for any health product is an extremely important method for clarifying details in case reports, and for adding additional quality information which can aid in causality assessment. This follow-up is most likely to be successful if it is done shortly after the AR report is received. Targeted monitoring of products is able to quickly identify cases of interest, and may allow for analysis of the actual product used. This is especially valuable for HMPs, where products can be tested for quality issues such as contamination or adulteration. Targeted monitoring has highlighted species misidentification in a AR of hepatotoxicity associated with a product labelled as containing black cohosh (Jordan et al., 2008, abstract. Paper in preparation).

The quality of ARs depends in part on educating potential reporters of what types of data are valuable for assessment. Such educational efforts can take the form of online educational modules, such as those directed at naturopathic doctors in Canada (Health Canada, 2006). In order to provide consistency in the naming of herbs in AR reports, the WHO Collaborating Centre for International Drug Monitoring has recommended the use of proper scientific binomial names for herbs used in medicine, including the use of such names (where this information is available) in the coding of AR reports (Farah et al., 2006). This would ensure comparability between reports from various international pharmacovigilance databases. It is equally important for the authors of published adverse reaction case reports to identify the specific product(s) involved, including label and manufacturer information, specific ingredients and dose employed. Published case reports would also benefit from analysis of the suspect product used, for contamination and adulteration, or species identification, where possible. A lack of, or incorrect, herbal identification has been noted as a potential source of confusion in published case reports of HMP toxicity (But et al., 1993).

Product quality

A particular challenge in reviewing safety data on HMPs is the quality of products suspected to be linked to ARs, or those used in experimental investigations. Causality assessment of ARs, as well as the results of research on the inherent toxicity of HMPs, can be

confounded by quality issues. Purity of the HMP may be compromised by the presence of weeds, dirt, pesticides and pollutants such as PCBs, toxic metals, radioactivity, bacteria, moulds and mycotoxins, processing impurities and solvent residues. Identity issues such as species substitution, misidentification or adulteration may also occur. Issues related to HMP quality have been reviewed (Elvin-Lewis, 2005, Fu et al., 2009, Smolinske, 2005, Srinivasan, 2006, Van Breeman et al., 2007).

Specifications for the quality of some HMP ingredients are set out in pharmacopoeias such as the United States Pharmacopeia (USP, 2008), the European Pharmacopoeia (EDQM, 2009) and the British Pharmacopoeia (BP, 2008), which are recognised as official in many jurisdictions. The high quality of the finished product requires not only ingredients that meet pharmacopoeial standards but also depend on such factors as manufacturing, packaging, labelling, and importation activities that comply with current GMPs. The WHO has published guidelines on the quality of herbal medicines with reference to contaminants and residues (WHO, 2007b), on Good Manufacturing Practices for herbal medicines (WHO, 2007c), on Good Agricultural and Collection Practices (GACP) for medicinal plants (WHO, 2003), and on quality control methods for medicinal plant materials (WHO, 1998).

Issues related to heavy metal content of HMPs

The reasons for the presence of metals in HMPs are varied. Plants may accumulate heavy metals from the environment during growth (Gasser et al., 2009, Street et al., 2008), heavy metals may be intentionally added to products within specific traditional health paradigms such as Ayurveda (Cooper et al., 2007, Saper et al., 2008), and contamination may occur through inadequate quality control. Even though traditional preparation methods for the addition of metals to herbs are intended to render these metals non-toxic, poisonings related to metal exposure have been reported (Saper et al., 2008). Whatever the route by which heavy metals such as lead, cadmium and arsenic become present in HMPs, the levels of these metals can be extremely high (Cooper et al., 2007, Saper et al., 2008), and poisonings have occurred (Gair, 2008, Health Canada, 2005).

Issues related to purity: microbial contamination

Microbial contamination can occur during the collection and the processing of ingredients or finished products. Some microbial species are common in the environment, while others can be introduced due to poor quality control or hygiene practices (Sagoo et al., 2009). Microbial contamination of HMPs including the health implications has been reviewed (Guédon et al., 2007a). The potential microbial contamination of herbs can be increased by the use of manures in agriculture, including those which may contain toxic strains of *Escherichia coli*. The drying of herbs shortly after harvest lessens the potential for the growth of microorganisms (WHO, 2003, 2007b). Similarly, fungal attack of plants can introduce mycotoxins in HMPs (Roy et al., 1988). This latter issue has been reviewed recently (Guédon et al., 2007b).

Issues related to plant identity

The correct identification of plant material during collection and processing is critical for the quality of HMPs. Several cases of incorrect plant substitution or mis-identification have been reported in the literature.

In 1990, Koren et al. reported a case of maternal and neonatal androgenisation associated with a herbal product. The mother experienced increased hair growth on her head, forehead and pubic area. At birth, the male child had thick hair in the pubic region, and on the forehead, along with red swollen nipples. The mother had been taking a commercial product labelled as containing "Siberian ginseng" during the pregnancy and 2 weeks of breast feeding. In response to this case, Awang (1991) analysed bulk lots of the herbal material used by the manufacturer of the commercial product noted in Koren's original report (Koren et al., 1990). The dates of these lots overlapped the pregnancy of Koren's patient. After analysis, Awang (1991) found that the lots of material used to manufacture the "Siberian ginseng" product were not comprised of authentic Siberian ginseng *(Eleutherococcus senticosus* (Rupr. and Maxim.) Maxim.), but likely contained silk vine (*Periploca sepium* Bunge, Apocynaceae). It had previously been known that some *Periploca* species had been substituted for *Eleutherococcus* in some imported products, possibly due to confusion of the Chinese names of the two plants (Awang, 1991).

A number of *Aristolochia* species have been used in herbal medicines throughout the world as antiinflammatory agents for gout, arthritis, rheumatism and chronic inflammatory skin diseases. Use of *Aristolochia* species in herbal medicines is no longer permitted in many countries due to their content of aristolochic acids which have been shown to be nephrotoxic, carcinogenic and mutagenic. Since 1993, cases of nephrotoxicity ("Chinese herb nephropathy") have been reported in Belgium, France, and the UK as a result of inadvertent exposure to *Aristolochia* species (EMEA, 2002), due to mistaken identity of Chinese medicinal herbs. More detail on the issue of *Aristolochia* and aristolochic acids is provided in the Case Study at the end of this paper.

Another example of misidentification potentially affecting consumer safety is with respect to black cohosh (*Actaea racemosa* L., Ranunculaceae), which has been widely used to relieve symptoms associated with menopause (Mahady, 2005). The use of black cohosh has been linked to cases of hepatotoxicty (Mahady et al., 2008). It has previously been shown that some black cohosh products in the United States do not actually contain authentic black cohosh (Jiang et al., 2006). This raises the question as to whether adverse reactions to products purporting to be associated with black cohosh are actually related to authentic *Actaea racemosa*. In one specific Canadian hepatotoxic AR, assigned a causality of "probable," the product used by the consumer was obtained for analysis. This analysis revealed that despite the product being labelled as containing black cohosh, authentic *Actaea racemosa* was not present (Jordan et al., 2008, abstract. Paper in preparation). Several groups of researchers, in recognition of the black cohosh identity problem with marketed HMPs, have developed analytical methods to ensure the correct identification of *Actaea racemosa* (Avula et al., 2007, He et al., 2006, Jiang et al., 2006, Zerega et al., 2002). For black cohosh, care should be exercised in attributing causality of adverse reactions to this herb, unless the plant species has been adequately identified.

A review by the US Pharmacopeia (USP) Dietary Supplements Information Expert Committee (DSIEC) studied international case reports of hepatotoxicty associated with black cohosh. The USP DSIEC could not identify a strong link between the reported liver reactions and black cohosh, due to limitations in the studied AR reports, as well as a lack of well-defined animal data and a clear mechanism of action. Despite the limitations of the available data, considering the seriousness of the possible adverse reactions and the increase in recent reports, the DSIEC suggested that USP black cohosh monographs carry a warning statement on product labels (Mahady et al., 2008). Adequate species identification in the reported cases of hepatotoxicity suspected to be associated with black cohosh would have enabled a more comprehensive assessment.

Other cases of mistaken identity of herbs involving poisonous substitutions that led to serious adverse reactions in consumers are shown in Table 1.

Issues related to purity: economically motivated adulteration

One of the greatest risks to human health related to HMPs arises from economically motivated adulteration. Such adulteration can

Table 1
Examples of herb misidentification that have causes adverse reactions in consumers (MHRA 2006, Smolinske, 2005).

Intended herb	Toxic herb used due to misidentification
Gentian (*Gentiana luteum* L., Gentianaceae)	Mayapple (*Podophyllum peltatum* L., Berberidaceae)
Skullcap (*Scutellaria lateriflora* L., Lamiaceae)	Germander (*Teucrium polium* L. or *T. chamaedrys* L., Lamiaceae)
Plantago sp., Plantaginaceae	Wooly foxglove (*Digitalis lanata* Ehrh., Plantaginaceae, also placed in Scrophulariaceae)
Chinese star anise (*Illicium verum* Hook. f.)	Japanese star anise (*Illicium anisatum* L., Illiciaceae)

occur through the addition of undeclared pharmaceutical drugs or, in illicit attempts to evade detection of adulteration, their analogues (Elvin-Lewis, 2005, Ernst, 2002, Huang et al., 1997). Examples of HMP types commonly adulterated include sleep aids that have been adulterated with benzodiazepines such as estazolam or clonazepam (Health Canada, 2007) weight loss products adulterated with sibutramine or fenfluramine (Corns and Metcalfe, 2002, Jung et al., 2006, Yuen et al., 2007), erectile dysfunction or sexual enhancement products adulterated with sildenafil, tadalafil, vardenafil, or their analogues (Li et al., 2009, Singh et al., 2009), remedies for diabetes adulterated with glibenclamide (Padinjakara et al., 2009), and body-building products adulterated with androgenic steroids (US FDA, 2009d). Health Canada regularly issues Foreign Product Alerts which identify adulterated and contaminated products found by regulators world-wide (Health Canada, 2009b, 2009c).

Other adulteration can occur through the intentional substitution with cheaper plant material (Yu et al., 1995). More recently, Zhang et al. (2009) recommended that research on pomegranate (*Punica granatum* L., Lythraceae) supplements should only be conducted on well characterised supplements, since their research indicated that some pomegranate products currently on the market are either adulterated or of very poor quality.

Genomic and metabolomic approaches have been applied in the identification of medicinal herbs. A major review by Sucher and Carles (2008) has outlined the methods which have been applied to the identification of medicinal plants, including omics technologies. This paper also lists over 400 plants for which such methods have been used in authentication. Metabolomics has been used to distinguish between individual species within a genus, for example *Ephedra* (Kim et al., 2005) and *Echinacea* (Gilroy et al., 2003), but also to discriminate between the regional origins of particular species such as *Panax ginseng* (Kang et al., 2008). Further discussion on the use of metabolomics in species identification has been written by Heinrich (2008). Omics technologies have been discussed as ways to increase herbal product quality by identifying specific herbs through biological fingerprinting, and aid in the prevention of species misidentification or adulteration with other species (Shyur and Yang, 2008). Difficulties with the use of omics for species identification include the lack of a preferred method for metabolite identification and the lack of reproducibility in different laboratories, or may be due to variation in chemical composition of herbs (for example due to growing conditions) (Shyur and Yang, 2008). The eventual use of omics technologies for plant identification remains to be seen.

The challenges associated with product quality can be illustrated by the product PC-SPES. This product was a commercially produced mixture of 8 herbs, and indicated for use in prostate cancer. It was tested *in vitro*, *in vivo* (Kubota et al., 2000), and in clinical trial studies (Small et al., 2000), and showed efficacy in these studies. At a later date, PC-SPES was found to be adulterated with diethylstilbestrol (DES), ethinyl estradiol, warfarin, and indomethacin (Ko et al., 2003, Sovak et al., 2002). Bonham et al. (2002a, 2002b) studied PC-SPES using microarray technology, and found changes in the expression of a variety of genes, including those involved with microtubule dynamics. The batches of PC-SPES used by Bonham et al. were analysed for DES, since certain batches of the product were already known to be adulterated with this drug. However, no analysis was conducted for other adulterants, including ethinyl estradiol. Importantly, a note at the end of one paper by Bonham et al. (2002b), identified the recently published study by Sovak et al. (2002), and noted the possibility that some of the effects due to PC-SPES administration could have been related to adulterants. In a recent review of the use of omics technologies in the assessment of natural products (Chekir-Ghedira, 2008), the studies by Bonham are discussed, but no mention of the adulteration of the product is noted. PC-SPES was removed from the market in 2002 (Ko, 2004).

A review of 81 randomised clinical trials of HMPs (Wolsko et al., 2005) noted that few studies actually characterised the products employed. Interpretation of published studies on HMPs can be difficult without the description of the specific product under study. Thus, ARs or published efficacy / toxicity studies associated with HMPs can be confounded by product quality issues. In order to fully understand and assess ARs and toxicity information, knowledge of the product or test material quality is ideal. For toxicological research, such knowledge is critical. In reports of ARs, this information is often lacking.

While the studies on PC-SPES related to efficacy, determination of product quality is a critical consideration for both efficacy and safety investigations, and the ability to properly interpret such studies. Studies conducted by the National Toxicology Program involve authentication of the herbs used. The United States Pharmacopeia (USP) publishes standard monographs on herb identification, and has a verification program in which commercial products conforming to USP standards can be labelled with a USP verification mark (Srinivasan, 2006). In studies conducted by the National Toxicology Program (NTP), study material is rigorously selected and authenticated (e.g. Weber et al., 2003).

Herbs as complex mixtures

Assessment of the safety of HMPs or dietary supplements is complicated by the very nature of the ingredients used. Whole herbs and their extracts contain a myriad of phytochemicals. At least 50,000 compounds have been isolated from plants, and one estimate notes the total number of plant metabolites likely exceeds 200,000 (Gomase et al., 2008). Interactions between phytochemicals, and even between different plants used in combination, form the basis of therapeutic use in traditional healing paradigms such as traditional Chinese medicine (TCM) and Ayurveda (Kang, 2008, Williamson, 2001). Against this background, the efficacy and toxicological effects of whole plants or extracts may be different than those of their isolated constituents.

Various authors have reviewed studies showing herbs with evidence for combined or synergistic therapeutic activity of their constituents (Ma et al., 2009, Ulrich-Merzenich et al., 2007a, Williamson, 2001). Wang et al. (2008a) have shown that a butanol subfraction of an ethanolic extract of echinacea (*Echinacea purpurea* (L.) Moench, Asteraceae) significantly stimulated CD83 expression in human immature dendritic cells, whereas an ethyl acetate subfraction inhibited the expression of the same gene. A recent paper (Neto et al., 2008) on the anticancer activity of cranberry (*Vaccinium macrocarpon* Aiton, Ericaceae) has reviewed the activity of whole fruit, specific extracts and individual constituents. This *in vitro* work has shown that the inhibition of tumours by cranberry (and other berries) could be the result of additive or synergistic activity of various compounds such as anthocyanins, proanthocyanidins, ursolic acid, and flavonols. These specific phytochemical constituents are able to inhibit cell proliferation on their own, but in some cases they have less activity than total extracts of cranberry (Neto et al., 2008). It is important to note that due to the abundance of uncharacterised phytochemicals in whole plants and many herb extracts, and the

possibility of many complex interactions, it is not always possible to conclude that true synergy between specific isolated phytochemicals within a herb or herbal extract provides the explanation for the net activity, as other unknown constituents may have a contributory role (Wagner and Ulrich-Merzenich, 2009, Williamson, 2001).

Although less studied than efficacy, the complexity of plant-based medicinal products provides challenges when assessing the toxicity of commercially available herbal products. Despite advances in the development of techniques and theories in mixture toxicology (Borgert, 2007, Yang et al., 2004), a great deal of work remains to be done. Borgert (2007) has outlined that traditional approaches to predicting the toxicity of a mixture have been either to (1) compare the mixture of interest with one for which the toxicity is already defined, or (2) take into account the toxicity of the individual components, allowing for potential interaction. Herbs, and most herbal extracts, are extremely complex mixtures, where their constituents can not be completely defined or even enumerated. Neither of these traditional approaches lend themselves easily to the toxicological assessment of herbs. As McCarty and Borgert (2006) point out, uncertainty is created in making conclusions on the toxicity of mixtures based on the known toxicity of specific individual constituents. This can be illustrated by the examples of basil and licorice.

Jeurissen et al. (2008) have studied the effects of basil (*Ocimum basilicum* L., Lamiaceae) extract on the metabolism of 1′-hydroxyestragole. Basil contains the allylalkoxybenzene estragole which is metabolised to the proximate carcinogen 1′-hydroxyestragole by P450 enzymes (primarily 1A2 and 2A6). A sulfation step then follows producing 1′-sulfooxyestragole, the ultimate carcinogen. Upon loss of the sulfate moiety, a carbocation is formed which is able to form DNA adducts, mainly with deoxyguanosine (Fig. 1). As noted by Jeurissen et al., the European Union (Scientific Committee on Food) has recommended restrictions on the use of estragole in foods, due to the potential carcinogenicity of its metabolites. Although isolated estragole has been shown to be carcinogenic in animal models, basil itself contains a complex array of phytochemicals. Jeurissen et al. (2008) describes earlier studies in which an extract of basil was found to contain inhibitors of P450 1A2, one of the cytochrome isoforms key to the metabolism of estragole to 1′-hydroxyestragole. In their 2008 study, Jeurissen et al. showed that a methanol extract of basil inhibited the formation of deoxyguanosine adducts, in a dose-dependent manner, in both rat and human liver S9 homogenates incubated with 1′-hydroxyestragole. In HepC2 cells, a similar dose-dependent result was obtained. In addition, the sulfotransferase inhibitor pentachlorophenol produced an approximate 90% reduction in the adduct formation, similar to the highest concentration of basil extract used. Basil extract did not inhibit adduct formation when incubated with the direct electrophile 1′-acetoxyestragole, in S9 liver homogenates. The authors concluded that the basil extract modulated the activity of the sulfation step in the metabolism of 1′-hydroxyestragole, and that the potential adverse effects of estragole are likely lower when estragole is consumed along with other components in basil. Confirmatory animal studies were recommended by the authors.

Cantelli-Forti et al. (1994) studied the effects of an aqueous licorice (*Glycyrrhiza glabra* L., Fabaceae) root extract on the pharmacokinetics of the active component glycyrrhizin, in rats and humans. The toxicity of glycyrrhizin has been the subject of many studies, and its effects in humans and experimental animals have been summarised (Isbrucker and Burdock, 2006). In the rat pharmacokinetic study conducted by Cantelli-Forti et al. (1994), where equivalent amounts of glycyrrhizin were administered either as pure compound or as a licorice extract, both the area under the curve and the Cmax for plasma levels were significantly reduced when glycyrrhizin was provided as a component of the licorice extract. The same authors conducted a study in human volunteers. Aside from one volunteer who was administered twice the amount of glycyrrhizin, the plasma levels of glycyrrhizin were below the detection limit. For the other volunteers, the glycyrrhizin metabolite glycyrrhetic acid was used as a surrogate marker. In the volunteers, the plasma levels of glycyrrhizin (in the single person

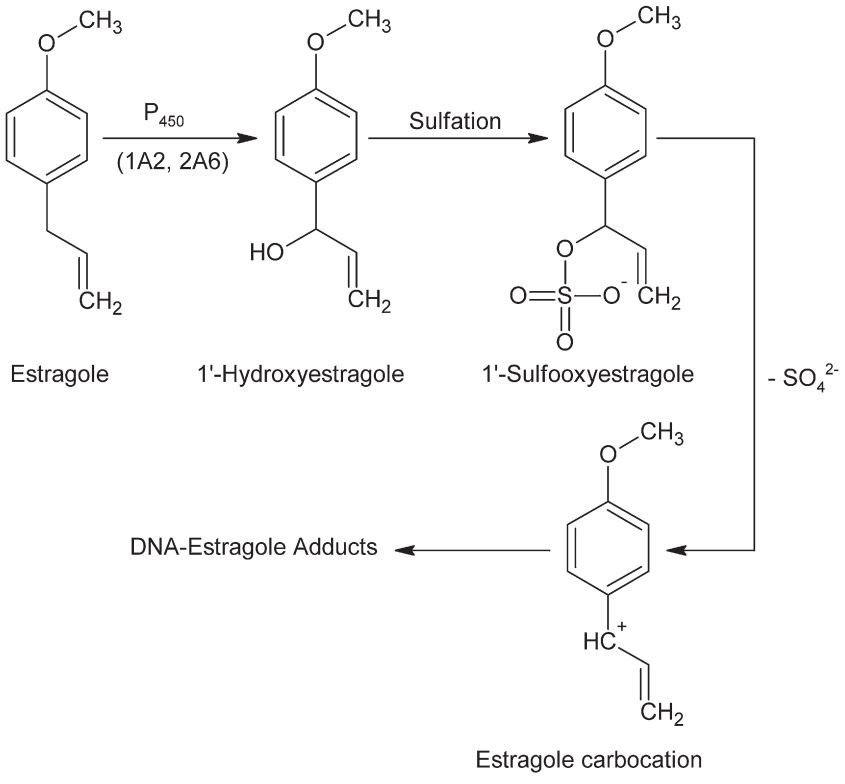

Fig. 1. Bioactivation mechanism for estragole. 1A2, 2A6 = the primary cytochrome P450 isozymes responsible for the bioactivation.

noted above) or glycyrrhetic acid were lower when glycyrrhizin was given as a component of the licorice extract. Despite the considerable variation in results seen in the human volunteers, the results were similar to those noted in rats. The authors concluded that the lower plasma levels of glycyrrhizin/glycyrrhetic acid seen after the administration of the licorice root extract, was due to reduced intestinal absorption caused by other components in the extract, and that clinical studies on aqueous licorice root extracts show that the use of extracts is safer that when glycyrrhizin is administered alone.

Basil and licorice serve as examples illustrating that the toxicity of isolated phytochemicals may not reflect the overall toxicity of the whole plant or plant extracts; however, in some instances the known toxicity of certain phytochemicals accurately reflects the toxic potential of the herb as a whole. An example is provided by the aristolochic acids found in species of the genus *Aristolochia*, as well as in certain other herbs. This particular example is discussed in more detail, later in this paper.

Unless the toxicity of a particular plant and its constituents have been well characterised, the use of data pertaining to isolated phytochemicals needs to be carefully considered. As Schilter et al. (2003) point out: "Interactions between constituents will be extremely important when data for highly purified preparations are used to assess relatively unpurified material." The majority of such interaction data are based on *in vitro* studies. Ideally, such studies should be followed up with *in vivo* investigations. Given the phytochemical complexity of herbs and their extracts, however, this is not always practical (Rietjens et al., 2008). Solutions to the paucity of information on potential matrix effects in medicinal herbs could include assessment of potential interactions between classes of compounds, or the major compounds involved (Rietjens et al., 2008), and the use of omics technologies to determine potential global effects of interactions in mixtures (see Use of omics technologies in the study of herbal safety). Yang et al. (2004) have discussed the possibility of using *in silico* methods for the investigation of simple chemical mixtures; however, at present, it is not possible to use such methods for examining the sorts of complex combinations of chemicals present in plants (see Predictive toxicity of herbal constituents).

The obvious solution to the possibility of matrix effects is to study the whole plants or herbal products to which people are exposed in commerce. In the ideal situation, plants and finished products would be of constant composition, so that any specific study could be compared to the actual commercially available product. As outlined elsewhere in this review, any study conducted on whole plants or extracts needs to use only botanically/chemically authenticated starting material.

Despite challenges associated with the complexity of herbal health products, regulatory decisions on risk mitigation activities need to be based on all available pertinent information on the safety of herbs/herbal products. This information may consist of information on specific products (e.g. the actual product taken by a consumer who has experienced an adverse reaction), or on general information on the toxicity of particular phytochemicals which the herb or product contains. Such an example is provided by plants containing aristolochic acids (see Integrative case study: *Aristolochia* species and aristolochic acids).

Predictive toxicology

Predictive toxicology has been widely used to screen and assess the potential toxicity of environmental chemicals (industrial chemicals and pesticides). It is advantageous when the numbers of individual chemicals to be screened far outweighs the capacity for assessment, and where there is concern for the large scale use of animals in toxicity testing. Predictive toxicity assays can be used to screen large numbers of chemicals with the aim of prioritising further testing, depending on the assumed hazard. In the realm of environmental chemicals, significant activities are being directed to build databases to support the EPA's ToxCast initiative. The aim of ToxCast is to prioritise chemicals for further testing, and to develop toxicity signatures, using a variety of types of data, including predictive toxicology such as high-throughput omics information and leveraging the already existing toxicological information from animal testing (e.g. those data submitted as part of pesticide registration) (Dix et al., 2007). In support of ToxCast, the Aggregated Computational Toxicology Resource (ACToR) has been developed, containing information on chemical structure as well as *in vitro* and *in vivo* testing results from international sources and academia, in a centralised location (Judson et al., 2008). The ToxRef database has also been developed. This database has been developed using animal toxicity data from pesticide registration in the United States. Martin et al. (2009) have shown that ToxRef is a promising tool to classify chemical agents in terms of their chronic toxic effects.

The use of computational toxicology such as quantitative structure–activity relationships (QSAR) to assess environmental chemicals has been employed by regulatory agencies, and regulatory decisions are made on the results of these assessments (Helma, 2005, Valerio, 2009). QSAR models on their own have also been shown, overall, to predict mutagenicity and carcinogenicity of chemicals. In a recent study (Benigni and Bossa, 2008) the best results were obtained using models that distinguished between active and inactive chemicals (70% to 90% accuracy). In this same study, however, genotoxic-based structure alert models did not fare so well. Integration of predictive toxicity paradigms such as that used in ToxCast should contribute greatly to predictive toxicology. As pointed out by Helma (2005), computational toxicology data can augment hazard assessment by filling in missing experimental data (e.g. where mutagenicity or carcinogenicity information is lacking), and its predictive capacity increases when combined with other types of predictive analyses. The benefits and limitations of *in silico* toxicology methods have been reviewed by Valerio (2009).

Predictive toxicity of herbal constituents

In silico modelling

The search for novel therapeutically active phytochemicals has employed various types of *in silico* models (reviewed by Rollinger et al., 2006); however, limited work has been done on analysing possible toxicity of phytochemicals by predictive methods. Such work has been conducted on phytoestrogens, including the development of QSAR models to predict the carcinogenic potential of these compounds (Singh, 2001). The various computational methods used to study the relationship between flavonoid structure and estrogenic activity, including QSAR and docking analyses, have been reviewed by Vaya and Tamir (2004).

In silico methods have been used to investigate prediction of the cytotoxic activity of sesquiterpene lactones (common to plants of the Asteraceae family) (Fernandes et al., 2008, Scotti et al., 2007). Although these studies are aimed at determining potential therapeutic activity through cytotoxicity, they show the promise of using such methods for predicting potential toxicity based on structure. Scotti et al. (2007) employed QSAR to study the cytotoxic activity of 37 sesquiterpene lactones. This study found that a number of specific structural elements and skeletal types are required for the greatest cytotoxic activity. Expanding on these results, Fernandes et al (2008) used an artificial neural network to examine 55 sesquiterpene lactones, with regard to their cytotoxic potential. The resulting data revealed that prediction of active and inactive structural elements can be achieved by this method. The cytotoxic activity was accurately predicted in 89% of the test chemicals. Marles et al. (1995) used QSAR to contrast the cytotoxicity of sesquiterpene lactones with their

desirable effect of blood platelet serotonin release inhibition as a potential mechanism for migraine prophylaxis.

The key papers on the *in silico* prediction of toxicity from phytochemicals are by Valerio et al. (2007) and Arvidson et al. (2008). In a validation study, Valerio et al. (2007) used a QSAR model for rodent carcinogenicity, which had been previously evaluated for pharmaceutical use. Predictions were made for 101 phytochemicals which had not been part of the carcinogenicity database used. The modelling results were compared to experimental studies on carcinogenic risk, which were available on these phytochemicals. This paper showed that there was 97% sensitivity (correctly predicted carcinogens) and 53% specificity (correctly predicted non-carcinogens). The false positive and false negative rates were 47% and 4%, respectively. Overall concordance between the QSAR and experimental assignments of high vs. low risk was 80%. For those phytochemicals tested in chronic rodent carcinogenicity studies, the sensitivity and specificity were 93% and 55%, respectively.

Arvidson et al. (2008) conducted a similar study using a series of *in silico* models, in consensus fashion, to compare the predicted carcinogenicity with experimentally derived conclusions of carcinogenic risk from long-term rodent bioassays. The phytochemicals selected for study were estragole, pulegone, aristolochic acid I, lipoic acid, 1-octacosanol and epicatechin. These chemicals were chosen since they have been the subject of rodent cancer studies, and they have been examined in other toxicological and mechanistic assays, both *in vivo* and *in vitro*. The compounds found by experimental evidence to be carcinogenic and/or genotoxic were estragole, pulegone and aristolochic acid I. Those which were negative in rodent carcinogenicity assays are lipoic acid, 1-octacosanol and epicatechin. Each of the phytochemicals were screened by consensus modelling, employing QSAR modelling software (MDL-QSAR, TOXLITE, OncoLogic, Derek), toxicity database and data mining software (Vitic and Leadscope), and biological systems pharmacology data mining software (MetaDrug). The consensus model used in this study was successful in predicting low carcinogenic potential in those chemicals with experimental evidence of low carcinogenic or genotoxic potential. Two of the 3 chemicals identified experimentally as having high carcinogenic potential through experimental means were also identified as having high potential in the consensus model. The single exception was pulegone, for which no prediction could be made. Pulegone could not be screened by MDL-QSAR and OncoLogic and the results of the other individual software programs were mixed. This investigation illustrated that using several approaches in a consensus model appears to be a more accurate approach than using only one or two computational programs.

In silico methods to predict toxicity of bioactive herbal constituents have shown their potential in contributing to the knowledge base for this group of chemicals. Work so far has mainly centred on the validation of these prediction models. Also, *in silico* estimation of toxicity of phytochemicals has mainly focussed on carcinogenicity, because of the paucity of this sort of data for herbs. While validation studies have mainly been successful, there are some difficulties in extending their use for decision making on the potential toxicity of phytochemicals. Databases of information on phytochemicals and whole plants is not as well developed as for industrial chemicals; however, more data will become available, especially with the advent of omics technologies. At present, computational toxicology is limited in its ability to make predictions of toxicity for mixtures of chemicals, such as those which occur in plants where additive, antagonistic or synergistic interactions may be present (Benz, 2007, Valerio et al., 2007, 2009). It is worthwhile to point out, however, that certain *in silico* approaches have been used to study the toxicity of simple mixtures of chemicals such as industrial solvents (see Yang et al., 2004). As well, Wang et al. (2008b) have used quantitative composition-activity relationships (QCAR) to examine the cytotoxicity of ginsenoside-containing extracts from *Panax ginseng* towards MCF-7 cells.

As for the assessment of any chemical by *in silico* methods such as QSAR based on rodent data can not be readily extrapolated to humans. As pointed out by Valerio et al. (2007), data on the prediction of carcinogenic potential can not be used to estimate the total risk, nor provide information on the potential site of carcinogenic action in the body.

Despite the challenges in the extrapolation of *in silico* data to "real world" use of herbal products, the advantages of such predictive approaches include filling data gaps for herbs which have been studied, and the use in situations where no other information is available for specific herbs, and where time or expense does not allow other information to be produced. *In silico* predictions are currently being used by regulators around the world for industrial and environmental chemicals. There is little doubt that data derived from these predictive methods will be immensely valuable when used to screen phytochemicals for additional testing, and when considered in an integrated fashion with other toxicological information, when conducting hazard assessments of herbal constituents or extracts.

Omics

The development and refinement of "omics" technologies, including genomics/transcriptomics, proteomics and metabolomics has lead to advances in the assessment of both efficacy and safety of health products. The omics technologies as applied to toxicology (toxicogenomics, toxicoproteomics and toxicometabolomics) have direct application to drug discovery and development in that they can be applied to augment the prediction of potential toxicity of new drugs in earlier stages of product development (Amir-Aslani, 2008, Blomme et al., 2009, Lord et al., 2006).

Omics technologies can be used to define global changes in gene or protein expression, or metabolite profiles which can be lead to the classification of test substances with regard to toxicity, the identification of novel biomarkers of toxicity, identify the potential tissue or cellular location of toxic insult, and allow the determination of the adverse effects of chemical mixtures and clues as to the mechanism/mode of toxicity (Cheok et al., 2003, Fielden and Kolaja, 2006, Gatzidou et al., 2007, Lettieri, 2006, Tennant, 2002, Waters and Jackson, 2008). Timelines of injury induction can be determined as well, with early changes documented before overt toxicity is apparent (see the discussion of riddelliine, below). Integration of the various omics technologies can lead to much enhanced understanding of toxicant mechanism of action (Xu et al., 2008, 2009) and forms the basis of the systems toxicology approach (Waters and Fostel, 2004, Waters and Jackson, 2008). The challenges and opportunities of omics technologies in toxicology and risk assessment have been reviewed (Cunningham et al., 2003, Gomase et al., 2008, Robertson, 2005, Ulrich-Merzenich et al., 2009).

Use of omics technologies in the study of herbal safety

The main focus of omics in the assessment of herbal products and phytochemicals has been directed towards the beneficial properties of these substances (Ovesná et al., 2008, Ulrich-Merzenich et al., 2007a). For example, Knasmüller et al. (2008) have reviewed the use of omics-based studies in validating health claims for dietary antioxidants. Proteomics has been specifically employed to study the effect of grape seed extract on the modulation of brain protein profiles in rats (Deshane et al., 2004). The results of this latter study showed that the amount and isoforms of a number of proteins were altered, suggesting a potential neuroprotective role of grape seed extract. A genomic study by Chen and Cheng (2009) determined that feverfew (*Tanacetum parthenium* (L.) Sch. Bip., Asteraceae) extracts altered the expression of 400 genes (involved in cell migration, cytokine production and metabolism) using a human monocytic cell line.

Further review of the use of proteomics and metabolomics in the research of the beneficial effects of dietary supplements has been published by Astle et al. (2007).

Recently, Ulrich-Merzenich et al. (2009) discussed the use of omics in the prediction and assessment of plant extract toxicity, identifying its potential use in predicting toxicity, the development of screening tests, and the development of biomarkers of toxicity. These uses are no different from those proposed for synthetic drugs. One of the original aims of the National Center for Toxicogenomics (originally part of NIEHS) was to establish gene expression signatures for hepatotoxicity in acute, subchronic and chronic exposure scenarios (Tennant, 2002). Phytochemicals including the pyrrolizidine alkaloid riddelliine were among the first hepatotoxicants selected for study (Cunningham et al., 2003). In addition, phytochemicals (pulegone, tannic acid and trans-anethole) have been used along with synthetic compounds, to elucidate a gene expression signature for oxidant stress/reactive metabolites in rat liver (McMillian et al., 2004). The toxicological study of both isolated phytochemicals and herbs in their own right has been growing in recent years, and the use of omics technologies has distinct advantages for studying the potential of these substances for producing toxicity. Chekir-Ghedira (2008) has recently reviewed the use of microarrays in the assessment of various herbs and herbal mixtures.

As noted earlier in this paper, the complex chemistry and interactions between the multitude of phytochemicals in herbs presents a unique challenge for assessing both efficacy and toxicity. The use of omics in the assessment of chemical mixture toxicity in general, has been reviewed by Yang et al. (2004). Omics has reinforced the fact that synergies exist between phytochemical constituents; research on isolated compounds may not necessarily reflect the action of whole plants or even specific plant extracts (reviewed by Ulrich-Merzenich et al., 2007a, and Williamson, 2001). As noted elsewhere in the present review, these interactions should be considered in reviews of the safety of specific herbs, where applicable.

While individual herbs are comprised of large numbers of chemical classes and individual phytochemicals, finished herbal products can be further complicated when they contain multiple herbs. Ulrich-Merzenich et al. (2007b) have investigated the product Phytodolor, which is a combination product containing extracts of three herbs, indicated for rheumatic pain. These researchers investigated the gene expression and protein profiles in human fibroblasts, and noted the modulation of genes involved in immunoregulation, inflammation and apoptosis. The gene expression profiles of the individual single extracts did not predict those of the combination product. Although aimed at investigation of therapeutic effect, this paper shows the promise inherent in omics research directed to complex mixtures.

The unsaturated pyrrolizidine alkaloid riddelliine can be used an example of a phytochemical which has been studied using omics technologies. This compound has been the subject of a number of reports, including publications by the NTP (Chan et al., 1994, 2003). The NTP studies have shown that riddelliine is genotoxic *in vitro* and *in vivo*, and tumourigenic in experimental animals. Mei et al. (2007) studied the hepatic gene expression in female Big Blue rats, following gavage treatment with 1 mg/kg body weight riddelliine for 12 weeks. Whole genome expression microarray analysis of hepatic samples revealed that 919 genes were differentially up- or down-regulated by the riddelliine treatment. Functions altered by the treatment included tissue development, cell death, cell-to-cell signalling, cell cycle and cellular growth and proliferation. Also detected were changes in the expression of genes involved in hepatic endothelial cell injury (e.g. cell death and signalling and leptin receptor expression), compatible with the known effect of riddelliine on this cellular subtype. All of the changes noted in this study occurred in the absence of tumours, thus representing early changes related to the carcinogenic process initiated by this particular phytochemical. In addition, this study supports and further details the information on riddelliine derived from other *in vitro* and *in vivo* work.

The study of Mei et al. (2007) was extended by Guo et al. (2007) to include a toxicogenomic comparison between the effects of riddelliine, as a prototypic carcinogenic pyrrolizidine alkaloid, and a pyrrolizidine alkaloid-containing plant, comfrey (*Symphytum officinale* L., Boraginaceae). Big Blue rats were either gavaged with 1 mg/kg body weight riddelliine, or were fed a diet containing 8% comfrey root, for 12 weeks. At the end of the study period, livers were used for gene expression analysis. Principal component analysis showed that globally, there was a distinct separation between the gene expression profiles of comfrey and riddelliine. In addition, there were 3 times as many differentially expressed genes (DEGs) in the comfrey group, compared with the riddelliine group, with the shared DEGs comprising 12% and 43% of the total DEGs in the comfrey and riddelliine treated groups, respectively. The differences likely reflect the multiple effects related to the complex phytochemical composition of comfrey, as opposed to the those produced by an isolated pyrrolizidine alkaloid. It should be noted, however, that when the expression of genes related to drug metabolism and cancer-related endpoints were considered, a much better correlation between comfrey and riddelliine was observed. In terms of functional processes associated with carcinogenesis, the processes shared between the comfrey and riddelliine treated groups represented 55% and 40% of the total functional processes affected in the comfrey and riddelliine groups, respectively. This study also found an unreported induction of the phase III ATP-binding cassette gene Abcb1 by both comfrey and riddelliine. There is a high degree of variability of pyrrolizidine alkaloid content in different comfrey species, including ones which are non-toxic (having a saturated necine base) (Jaarsma et al., 1989, Muetterlein and Arnold, 1993), nevertheless, the study by Guo et al. (2007) showed that high throughput omics technologies can be employed to confirm results from other types of studies and can identify novel information as part of a global survey of gene expression. The study also shows that the effects of isolated phytochemicals may not always be the same as for a whole plant; however, by studying similarities between the two, valuable inferences can be drawn on the role of individual, or classes of, phytochemicals in the potential toxicity of medicinal herbs.

The investigation of potential adverse effects of specific herbs in humans, through omics methods, can be illustrated by the research of Wang et al. (2005) who looked at the effects of German chamomile (*Matricaria recutita* L. as provided in the paper, more properly *Matricaria chamomilla* L., Asteraceae) using a metabolomic approach. Botanically identified chamomile flowers were prepared as a tea and provided to human volunteers for 2 weeks (200 ml/day). Daily urine samples were obtained during a 2-week baseline, during the dosing period, and again during a 2-week post-dosing phase. The urine samples were analysed by ^{1}H NMR. The treatment with chamomile tea produced an increase in the excretion of glycine, hippurate, and an unknown metabolite (possibly derived from a constituent of chamomile). Urinary creatinine excretion was decreased. By the end of the 2-week post-dosing period, the metabolite alterations had not resolved to baseline (hippurate excretion continued to be elevated). The authors speculated that the chamomile treatment had disrupted the gut microflora, which had not recovered at the end of the 2-week post-treatment period. This study emphasises that omics technologies can be employed in the global assessment of safety of whole herbs versus isolated constituents, and the results can lead to more focussed research on specific areas of interest. This type of global assessment would be especially important when researching those herbs where little safety information is known.

Omics studies have been applied to Traditional Chinese medicine (TCM). As the TCM paradigm includes the use of whole herbs with complex phytochemical profiles and combination therapy with multiple remedies, the safety assessment of TCM products has been

challenging (Kang, 2008, Lao et al., 2009). Concern has been raised that studies showing toxicity of isolated phytochemicals may not apply to the use of whole plants or complex extracts, where interactions between constituents may occur. High throughput global analysis techniques such as genomics, transcriptomics, proteomics and metabolomics have great appeal in the assessment of safety of such complex therapeutic mixtures (Cho, 2007, Kang, 2008, Lao et al., 2009, Li, 2007). Metabolomics has been used to study the toxicity of Hei-Shun-Pian (traditionally processed root of aconite, *Aconitum carmichaelii* Debeaux, Ranunculaceae) in rats (Li et al., 2008). This study showed that the processed root contained no aconitine, but did contain other aconitum alkaloids. A 2-week administration of a decoction of Hei-Shun-Pian produced dose-dependant effects on urinary metabolites, suggestive of cardiac effects.

Omic technologies present a valuable new addition to the research tools used for herbal health products, and are currently being used as part of research being conducted by the National Institutes of Health Botanical Research Centers (Barnes et al., 2008). In some cases, omics has been suggested for the rapid screening of potential toxicity related to complex multi-herb products (Chen et al., 2006).

Integrative case study: *Aristolochia* species and aristolochic acids

In 1992, consumers of an herbal weight-loss preparation in Belgium exhibited severe renal disease manifested by interstitial fibrosis, which rapidly progressed to renal failure (Vanherweghem et al., 1993). The appearance of this disease (at the time termed Chinese Herb Nephropathy, now generally referred to as aristolochic acid nephropathy, AAN) correlated with the change in the herbal formula to include what was intended to be *Stephania tetrandra* S. Moore, Menispermaceae. It became apparent, however, that the product actually contained *Aristolochia* species, as the batches of *S. tetrandra* used to prepare the slimming product did not contain the expected tetrandrine, but rather aristolochic acids (AA, such as AAI or AAII, Fig. 2). This substitution may have occurred due to the similarity of the pin yin names of the plants (Han Fang Ji and Guang Fan Ji for *S. tetrandra* and *Aristolochia fangchi*, respectively). The toxicity of the nitrophenanthrene aristolochic acids became of international concern since cases of renal failure were later confirmed in Europe, Asia, and North America, and public risk communications were issued by various regulatory agencies (Health Canada, 2004, US FDA, 2001). Urothelial cancinomas were found in patients suffering from Chinese Herb Nephropathy, and it was found that patients had aristolochic acid-related DNA adducts in renal tissue (Nortier et al., 2000, Schmeiser et al., 1996). Of importance, Lemy et al. (2008) have noted that patients with aristolochic acid nephropathy who had undergone renal transplantation, are at risk from bladder urothelial cancers even 15 years after cessation of aristolochic acid exposure. The history and evolution of this issue has been reviewed (Arlt et al., 2002, Cosyns, 2003), most recently by Debelle et al. (2008), and will not be reviewed further here. Because of intensive research on plants containing AAs, as well as on the isolated AAs themselves, and toxicological information in both humans and animals, this issue can serve as a model example to illustrate the points made in this paper.

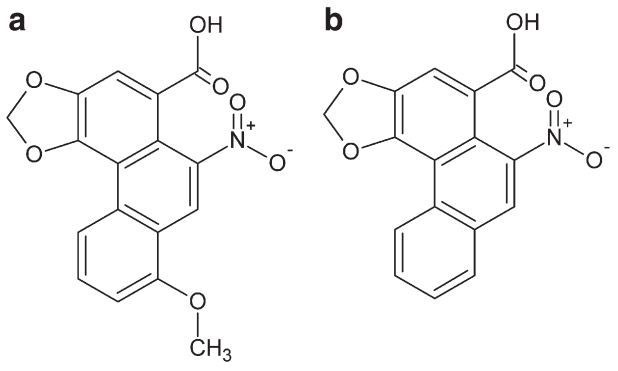

Fig. 2. Chemical structures of (a) aristolochic acid I and (b) aristolochic acid II.

Species identification

A recent report from Saudi Arabia outlines a case of acute renal failure in a woman associated with "Chinese herbs," and discusses the literature on AA (Saad and Shaikh, 2009). Although the paper outlines evidence to suggest the serious nature of the case is related to the herbs ingested, no other information on the herbs is provided, and it is also not clear if the herbs were part of a finished marketed product, or whether they were prescribed by a traditional practitioner. It is unclear from this paper whether the reaction was actually associated with *Aristolochia* species. AA-containing herbal products continue to be sold internationally (Debelle et al., 2008). While the paper by Saad and Shaikh outlines potential toxicity from medicinal herbs in general, further information on the actual product taken (listing of herbal ingredients on any available labelling information, or definitive species identifications) would have contributed to the discussion on the current availability of AA-containing products in the international marketplace.

The plant family Aristolochiaceae has 460 species in 7 genera of which the two commercially traded ones are *Aristolochia* and *Asarum*, both of which contain aristolochic acids. The genus *Aristolochia* has 370 species. The main commercial species are *Aristolochia clematis* L. (birthwort), *A. contorta* Bunge (aristolochia), *A. cymbifera* Mart. and Zucc. (mil homens), *A. debilis* Siebold and Zucc. (aristolochia), *A. fangchi* Y.C. Wu ex L.D. Chow and S.M. Hwang (aristolochia fangchi), *A. indica* L. (Indian birthwort), *A. manshuriensis* Kom. (Manchurian birthwort), *A. pentandra* Jacq. (Marsh's Dutchman's pipe), and *A. serpentaria* L. (Virginia snakeroot) (McGuffin et al. 2000).

The complexities of identifying actual components of herbal medicines (Chinese traditional medicine, TCM, specifically), have been addressed by Wu et al. (2007). This paper highlights the fact that one herbal term used in TCM can actually refer to different species. The primary source of confusion of other plant species with *Aristolochia* is probably the use in commercial trade of the pinyin (phonetic) spelling of their Chinese names, particularly with respect to "Fang Ji," "Mu Tong" or "Mu Xiang." These spellings fail to capture critical aspects of pronunciation that properly distinguish these names in the Chinese language. The trade names may also lack appropriate adjectives that correctly distinguish the species. Other plants with Chinese names similar to *Aristolochia* species are not related to *Aristolochia* and belong to different families (Table 2). There are no reports of aristolochic acid being present in any of these non-*Aristolochia* genera. Because of the non-specificity of the Chinese terms, finished herbal products labelled as containing "Fangji" or "Mu Tong" could potentially contain any of a number of species, including those from the genus *Aristolochia*.

Xue et al. (2008) has further outlined the difficulties in correctly identifying the plants in the fangji group. These plants are distinguishable by leaf shape, but after processing, the identification can become problematic. Chemical analysis of these species may also be made more difficult from the growing conditions of the plants. Joshi et al. (2008) has outlined methods for distinguishing the plants in the fangji category, and Wu et al. (2007) propose using the unique pharmaceutical name of the material used in the preparation of products. In addition, a rapid *in vivo* metabolomic approach using LC-MS for screening TCM herbs, including herbal mixtures, has been proposed by Chen et al. (2006). These suggestions may help in the identification of products which are likely to contain AA, and in the manufacture of safer products.

Table 2
Similarities in pinyin (phonetic) names for various herbs (derived from EMEA, 2002).

Pinyin name	Botanical name	Family
Fang Ji		
Guang fang ji	*Aristolochia fangchi*	Aristolochiaceae
Han fang ji	*Stephania tetrandra*	Menispermaceae
Ripen han fang ji	*Sinomenium acutum*	Menispermaceae
Mu fang ji	*Cocculus orbiculatus*	Menispermaceae
Xiang fang ji	*Diploclisia affinis*	Menispermaceae
Fang ji according to some references but *Shan dou gen, Bai dou gen, Ye dou gen* are more correct	*Menispermum dauricum*	Menispermaceae
Mu Tong		
Guang mu tong	*Aristolochia manshuriensis*	Aristolochiaceae
Bai mu tong	*Akebia quinata, Akebia trifoliata*	Lardizabalaceae
Chuan mu tong	*Clematis armandii, Clematis montana*	Ranunculaceae
Mu Xiang		
Qing mu xiang	*Aristolochia debilis*	Aristolochiaceae
Guang mu xiang	*Saussurea costus*	Asteraceae
Tu mu xiang	*Inula helenium, Inula racemosa*	Asteraceae
Chuang mu xiang	*Dolomiaea souliei*	Asteraceae

Extrapolation of isolated AA to the effects of AA-containing plants and multi-ingredient products

In animal studies, the effects of aristolochic acids have mimicked AAN in humans. Debelle et al. (2002) studied the effects in male Wistar rats injected subcutaneously with a mixture of AAI and II at either 1 or 10 mg/kg bw/day for 35 days. The high dose induced glucosuria, proteinuria and increased serum creatinine, along with tubular necrosis progressing to tubular atrophy with interstitial fibrosis by the 35-day time point. The low dose group had no significant effect on these parameters. Urothelial dysplasia was noted in both groups. Using the same experimental approach, these authors also demonstrated that other potentially toxic ingredients (dexfenfluramine) in the weight loss supplements taken by the AAN patients did not alter the nephrotoxic effects of AA in treated rats. Dexfenfluramine did, however, significantly increase DNA-adduct levels in treated rats (Debelle et al., 2003). Cosyns et al. (2001) developed a model in New Zealand while rabbits which were injected intraperitoneally with 0.1 mg/kg bw of a natural mixture of AAI and AAII, 5 days/week for up to 21 months. This study found that the observed renal pathology was very similar to that noted in patients with Chinese Herb Nephropathy, leading to their conclusion that AAs alone are responsible for this specific nephropathy. Past research (Nortier and Vanherweghem, 2002) had also indicated that tissue samples from patients with Chinese Herb Nephropathy contained AA-related DNA adducts, which had previously been shown to be directly responsible for carcinogenic effects in rodents. Most recently, Schmeiser et al. (2009) have reviewed the literature on the carcinogenicity of plants from the genus *Aristolochia*. These authors have emphasised that the specific DNA adducts formed after the metabolic activation of AA have been detected in animals treated with either isolated AA or to herbal products containing AA. These adducts have also been found in urothelial tissues from patients suffering from AAN.

These studies show that (1) animal models of AA toxicity can mimic the effects in humans, and (2) the administration of isolated aristolochic acid(s) in animals can replicate a syndrome caused by aristolochic acid-containing plants, as well as multi-ingredient products containing these toxins, in humans. In this case, isolated phytochemicals can be used to accurately characterise the toxicity of the whole plant.

In silico data

Stiborová et al. (2008) have reviewed the research leading to the elucidation of the metabolic activation of AAs to the chemical species able to form DNA adducts. This research involved various methods such as human microsomal and cytosolic enzyme studies, and work involving purified enzymes. This research revealed that hepatic and renal NAD(P)H:quinone oxidoreductase (NQO1), hepatic microsomal CYP1A2, renal microsomal NADPH:CYP reductase, and cyclooxigenase contributed to the activation of AAs. Key information included computational molecular modelling which suggests that the binding of AAI to NQO1 is in the same orientation as other substrates of this enzyme.

As outlined in Predictive toxicology, a consensus method using a series of computational software programs has highlighted that this approach is useful in predicting the mutagenicity and carcinogenicity of AAI, with good concordance between the *in silico* results and experimental evidence (Arvidson et al., 2008).

Information derived from omics studies

Aristolochia and AA have been well studied over the last 15 years; however, omics technologies such as genomics and metabolomics have both confirmed data from other studies, and provided valuable new information on the nephrotoxic mechanism of AA and on biomarker identification.

Toxicogenomic studies

Simões et al. (2008) studied AAI-induced changes in gene expression in two human colorectal cell lines, differing in TP53 status. The results, obtained using cDNA microarrays, confirmed the importance of TP53 involvement, but also provided new information: the expression of other genes, such as MYC are altered by AAI, independent of TP53 status. Such studies are able to further elucidate mechanisms of action associated with phytochemicals.

Metabolomic studies

Several studies have aimed to elucidate the changes in the metabolite profile elicited by AA or extracts of *Aristolochia* species. Employing LC-MS analysis, metabolome alterations were studied in Wistar rats exposed by gavage to either AA (single dose of 50 mg/kg bw) or to an aqueous extract of *Aristolochia manchuriensis* for 3 days, providing a dose of 96 mg AA/kg bw/day (Chen et al., 2006). The results showed changes in renal histology and urinary metabolites in the treated groups, distinct from the control group. Furthermore, these alterations were very similar between the groups gavaged with pure AA or the extract of the AA-containing plant. Changes were reflective of increases in homocysteine formation and folate-related metabolism and decreases in arachidonic acid synthesis and homocysteine remethylation. An important finding from this work was the comparability of the results in the groups of animals exposed to the plant extract and purified AA, despite the fact that the extract would have contained many more constituents other than AA. The authors suggested that metabolomics could be used as a rapid screen for nephrotoxicity associated with multi-ingredient herbal supplements which may contain AA. Using the same animal protocol, a study by the same group of researchers (Ni et al., 2007) used GC-MS to examine the metabolomic changes from exposure to AA (no plant extract was used in this latter study). The latter study suggested alterations in the synthesis of free fatty acids, amino acid metabolism and possible alteration of gut microflora. These two studies have highlighted that by employing multiple analytical methods, a more detailed account of metabolic changes can be obtained. Metabolomics have also been used to study the time course of AA nephrotoxicity (Zhang et al.,

2006). Male Wistar rats were injected intraperitoneally with 10 mg AA/kg bw/day for 5 days. Urine was collected and analysed by NMR at various timepoints, up to 224 h after the first injection. The metabolomic analysis showed a time-dependant progression of toxicity primarily reflective of damage to proximal tubules, peaking at about 96–104 h post initial exposure.

Development of biomarkers

Metabolomic studies have been conducted to rapidly determine early biomarkers of AA exposure (Chan and Cai, 2008, Chan et al., 2008, Huang et al., 2009). In an initial study by Chan and Cai (2008), male Sprague–Dawley rats were orally administered 10 mg/kg of a mixture of AAI and AAII per day for 3 days. LC-MS analyses of urine revealed that kynurenic acid and hippuric acid were reduced and increased, respectively, in the urine of treated rats. In a follow-up investigation (Chan et al., 2008), male Sprague–Dawley rats were orally administered 12, 10 or 30 mg/kg, of a mixture of AAI and AAII per day for 3 days, and plasma and urine were collected. Time- and dose-dependant effects on metabolites were noted. Plasma creatinine was elevated in AA-dosed rats. In addition, citric acid concentrations in the urine of treated rats were reduced compared with the control group, and an unknown metabolite, possibly a glucuronide conjugate, was increased. In general, the low dose and control groups clustered together as indicated by principal component analysis, as did the medium and high dose groups. Huang et al. (2009) studied the urinary protein profile in C3H/He mice treated with either AAI or AAII, 1.8 mg/kg/day for 11 days. Proteins were studied using SDS-PAGE and SELDI-TOF MS. The results showed that AAI caused significant proteinuria after 2–3 days while AAII did not. AAI and AAII caused very different changes in the urinary profiles. While more work would need to be conducted to refine the development of biomarkers of AA exposure, high throughput metabolomics shows promise, having identified several candidates, and has distinguished between the effects of AAI and AAII.

The data outlined above on the effects of AA and plants in the genus *Aristolochia*, provides examples of the both the challenges and opportunities in the assessment of HMP safety. The literature on this subject follows the path from initial reporting of human cases (Vanherweghem et al., 1993) to the use of recent technological advances in toxicity assessment. Although the seriousness of the ARs reported in the literature spurred much research, newer technologies such as omics have confirmed older data, but have also produced novel data to clarify the toxicity of AA and those herbs containing this chemical. This example shows the value of integrated approaches to problems in the toxicological assessment of HMPs.

Summary and conclusions

Postmarket safety assessment of herbal medicinal products involves the integration of information from adverse reaction reports and the scientific literature. Challenges associated with the safety assessment of these products relate to both quality and quantity of information. Less than ideal information is often provided in reported cases of adverse reactions to herbal products, both those published in the scientific literature and those submitted to regulatory authorities. There is also a general lack of toxicological information on most herbs. Possible ways to increase the quantity and quality of AR reports are to educate potential reporters of the ideal types of information needed for adequate assessment. The under-reporting of ARs suspected to be linked to HMPs is a more acute problem than for pharmaceutical drugs. Poison control centres have been identified as a potential source of additional adverse reaction data for HMPs, especially since reports to such centres often outnumber those submitted to national pharmacovigilance systems. Targeted or prospective monitoring of AR reports results in higher quality information since follow up is possible with the reporters soon after the suspected reaction occurs.

One of the challenges in toxicological assessment of herbal products is determining the applicability of data on isolated phytochemicals to the "real-life" situation where whole plants or plant extracts are used. While this is a challenge, it has been shown that the toxicity of certain isolated chemicals, for example aristolochic acids, does correlate with the known toxicity of the whole plant or extract. While it may be best to test the whole plant or extract in commerce, this is not always possible or practical. Herbal product quality has been a constant problem, as species misidentification or substitution occurs, and contamination or adulteration may be present. Where cGMPs exist, and where these form part of regulation, quality problems associated with HMPs should be less frequent. Nevertheless, the problem of product quality can present a distinct challenge in the interpretation of published studies of both efficacy and safety of herbal products, unless great care was taken by the authors to identify the test materials in question.

Newer technologies, such as omics and predictive toxicology have more often been applied to therapeutic efficacy or for screening toxicity of chemicals in early drug development. These can, however, be used to add to the knowledge base for herbal product safety. This is especially important in the area of HMP safety, where there is a great paucity of available information. Omics can be used to obtain initial global information which can serve to focus further research, support the findings from other types of studies, and determine potential effects from complex chemical mixtures, such as exist in herbal material. The use of *in silico* methods for the screening of toxicity of phytochemicals could also contribute to hazard assessment, when used with other pertinent information. While further work will clarify the potential use of such newer technologies, they show great promise for use in HMP safety assessment.

Increasing the quantity and quality of clinical and scientific information on HMPs will reduce uncertainty in assessment, and greatly contribute to decision-making related to hazard and risk.

Recommendations

(1) Continued education of reporters of adverse reactions (consumers, health care practitioners, the industry) to pharmacovigilance databases, to increase the quantity and quality of AR reports suspected to be associated with HMPs.
(2) Further exploration of the use of poison control centre data should be done in order to augment available reports suspected to be linked to HMPs.
(3) Authors of scientific publications on HMP adverse reactions or toxicity information should be encouraged to provide as much information as possible when reporting adverse reactions. This includes analysis for contamination/adulteration and herb species identification, where possible.
(4) Continued exploration of omics and predictive toxicology in the toxicological assessment of HMPs in order to provide information that can augment other types of information, allowing an integrated assessment.
(5) Continued exploration of new active surveillance methods such as community-based surveillance (pharmacies, hospitals, points of sale), as potential tools in the detection of safety signals related to HMPs.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.taap.2009.12.005.

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