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POSITION PAPER ON THE RISKS ASSOCIATED WITH THE USE OF HERBAL PRODUCTS CONTAINING *ARISTOLOCHIA SPECIES*

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Position Paper on the Risks Associated with the Use of Herbal Products containing *Aristolochia* species

1. *Aristolochia* is a plant genus of the family *Aristolochiaceae.* A number of *Aristolochia* species have been used in herbal medicines throughout the world as anti-inflammatory agents for gout, arthritis, rheumatism and chronic inflammatory skin diseases. Some North American species (eg. *Aristolochia serpentaria*) have been used for the treatment of snakebites.

2. Use of *Aristolochia* species in herbal medicines is no longer permitted in many countries due to the toxicity of the aristolochic acid constituents which have been shown to be nephrotoxic, carcinogenic and mutagenic. Some countries, including some Member States of the EU permit homoeopathic products containing *Aristolochia* species provided that high dilutions are used which are not considered to pose a health hazard.

3. *Aristolochia* species, however, continue to be used particularly in traditional chinese medicines (TCM). Since 1993 cases of nephrotoxicity and carcinogenicity have been reported in Belgium, France and UK as a result of inadvertent exposure to *Aristolochia* species in unlicensed herbal medicines.

4. The Chinese Pharmacopoeia includes the following *Aristolochia* species:

Aristolochia species	**Part Used**	**Pin Yin Name**	**Uses**
Aristolochia fangchi Y.C. Wu ex L.D. Chou et S. M. Hwang	root	Guangfangji	anti-rheumatic and diuretic
Aristolochia manshuriensis Kom.	stem	Guanmutong	diuretic, anti-inflammatory, for the treatment of oedema and rheumatic pain.
Aristolochia contorta Bge. *Aristolochia debilis* Sieb. et Zucc.	fruit fruit	Madouling	treatment of respiratory diseases as an anti-tussive and anti-asthmatic.
Aristolochia contorta Bge. *Aristolochia debilis* Sieb. et Zucc	herb herb	Tianxianteng	diuretic for oedema and as an anti-rheumatic.
Aristolochia debilis Sieb. et Zucc	root	Qingmuxiang	relief of pain and to counteract toxicity and cause subsidence of swelling

5. In TCM, *Aristolochia* species are also considered to be interchangeable with other commonly used herbal ingredients and substitution of one plant species for another is established practice. Herbal ingredients are traded using their common Chinese Pin Yin name and this can lead to confusion. For example, the name Fang ji can be used to describe the roots of *Aristolochia fangchi, Stephania tetrandra* or *Cocculus* species.

Plant species supplied as 'Fang Ji'

Pin Yin Name	Botanical Name	Part Used	Latin Pharmaceutical Name
Han fang ji	*Stephania tetrandra*	root	Radix **Stephania tetrandra*
Guang fang ji	*Aristolochia fangchi*	root	Radix *Aristolochia fangchi*
	Cocculus trilobus	root	Radix *Cocculus trilobus*
	Cocculus orbiculatus	root	Radix *Cocculus orbiculatus*

Radix*= root

Similarly, the name Mu Tong is used to describe *Aristolochia manshuriensis*, *Clematis* species or *Akebia* species.

Plant Species Supplied as 'Mu Tong'

Pin Yin Name	Botanical Name	Part Used	Latin Pharmaceutical Name
Mu Tong			
Chuan Mu Tong	*Clematis armandii*	stem	Caulis* *Clematis armandii*
" "	*Clematis montana*	stem	Caulis *Clematis montana*
Bai Mu Tong	*Akebia quinata*	stem	Caulis *Akebia quinata*
" "	*Akebia trifoliata*	stem	Caulis *Akebia trifoliata*
Guan Mu Tong	*Aristolochia manshuriensis*	stem	Caulis *Aristolochia manshuriensis*

Caulis* ≡ stem

Note:*Another *Aristolochia* species, *Aristolochia moupinensis,* has also been reported to be used as Mu Tong

There are reports in some Chinese literature that substitution can occur with 'Ma Dou Ling'.

Plant species supplied as 'Ma Dou Ling'

Pin Yin Name	Botanical Name	Part Used
Ma dou ling	*Aristolochia contorta* Bge. *Aristolochia debilis* Sieb. et Zucc.	Fruit fruit
Gua lou	*Trichosanthis kirilowii*	fruit

The Pin Yin name 'Mu Xiang' is applied to a number of species; there is no evidence of substitution between the species but the common names have the potential for confusion in both Chinese and Japanese.

Plant species supplied as 'Mu Xiang'

Pin Yin Name	Botanical Name	Part Used	Other names
Qing mu xiang	*Aristolochia debilis* Sieb. et Zucc	root	Japanese: Sei-mokkou
Mu xiang	*Aucklandii lappa**	root	
Guang mu xiang	Saussurea lappa*	root	Japanese: Mokkou
Tu mu xiang	*Inula helenium, Inula racemosa*	root	
Chuan mu xiang	*Vladimiria souliei* *Vladimiria souliei var cinerea*	root	Japanese : Sen- Mokkou

* *Aucklandii* and *Saussurea* are the same plant genus.

6. Aristolochic Acids
   The toxic components of *Aristolochia* species are known as aristolochic acids. These are a series of substituted nitrophenanthrene carboxylic acids. The main constituents are 3,4-methylenedioxy-8-methoxy-10-nitrophenanthrene-1-carboxylic acid (aristolochic acid I) and its demethoxylated-derivative, aristolochic acid II . The aristolochic acids appear to occur throughout the plants and have been found in the roots, stem, herb and fruit.
   Aristolochia species also contain the related aristolactams which are phenanthrene cyclic amides.
   Aristolochic acids are not reported to occur outside the *Aristolochiaceae* family. Low levels have been reported in *Asarum* species, another member of the *Aristolochiaceae* which is used in some herbal and homoeopathic products.

7. Aristolochic acids are reported to stimulate defence mechanisms against infections and inflammation in several mammalian species, including humans. Aristolochic acid stimulates the phagocytic activity of peripheral granulocytes in healthy volunteers at oral doses of 3 times 0.3 mg/person and day given for 10 days. This is a daily dose of approximately 0.015 mg/kg bw for a 60 kg person. Anti-inflammatory effects have been attributed to *Aristolochia* drugs and have been demonstrated for aristolochic acids in the respective animal models.

8. Pharmacokinetic data, including metabolism, are available only for a few constituents of *Aristolochia*. Pharmacokinetics of aristolochic acid I and II have been studied in rats, mice, guinea pigs, dogs and humans after oral treatment. The doses studied were in the range of 0.6 to 85 mg/kg bw. Most of the results relate to rats. Following oral administration, aristolochic acid I was readily absorbed from the gastrointestinal tract. After oral administration of aristolochic acid I to rats, about 91% of the dose was recovered in the excreta, equally divided in urine and faeces. Parent compound or conjugates of parent compounds were not detected in rat excreta. More than 80% of the radioactivity was

identified as aristolactam Ia or conjugates thereof. Minor urinary and faecal metabolites of aristolochic acid I were aristolactam I, aristolochic acid Ia, aristolic acid I lacking the nitro-group and 3,4-methylenedioxy-8-hydroxy-1-phenanthrene carboxylic acid. Following oral administration of aristolochic acid II, 13.5% of the dose was recovered in excreta, most of it in faeces. Aristolactam II was the main single component (4.6% in urine and 8.9% via faeces). Additionally, aristolactam Ia and 3,4-methylenedioxy-1-phenanthrene carboxylic acid (aristolic acid II, lacking the nitro group) were detected. However, the main proportion of the dose could not be accounted for. Metabolites, which have lost the nitro group (aristolic acid derivatives) are, probably, formed by gut bacteria, since they have not been observed after intravenous application.

In other laboratory animal species, the metabolic pattern was not reported in detail. It appeared that in mice, metabolism was similar to that observed in the rat. Urine samples of guinea pigs, rabbits, dogs and man were reported to yield not all of the metabolites found in the rat. In rabbits and dogs, small amounts of parent compounds were detected in urine.

In human volunteers treated with daily doses of 0.9 mg aristolochic acids/person for three days, only aristolactam I and II were identified in urine on day 3. Faecal excretion was not examined. Further, aristolochic acids-like substances of not exactly defined identity were detected at relatively high concentrations in bile, urine, cerebrospinal fluid and saliva of human volunteers given aristolochic acids in capsules at a daily dose of 1.35 mg/person for three days. Three nursing mothers treated with oral doses of 1.35 mg aristolochic acids for three days were reported to excrete 2.5-5% of the dose into the breast milk.

No data on the distribution of aristolochic acids or metabolites in the tissues of laboratory animals are available.

9. Data on the acute toxicity of the plant drug *Aristolochia* are not available. In rats, the oral LD_{50} of aqueous solutions of the active ingredient aristolochic acids is 203.4 mg/kg bw in males and 183.9 mg/kg bw in females. The LD_{50} after intravenous administration is reported as 82.5 mg/kg bw and 74.0 mg/kg bw in males and females respectively. In mice, the oral LD_{50} of aristolochic acids is 55.9 in males and 106.1 mg/kg bw in females. The LD_{50}, after intravenous administration, is reported as 38.4 mg/kg bw and 70.1 mg/kg bw in males and females respectively. Rabbits are reported to be more sensitive to the toxic action of aristolochic acids, deaths being observed already after single intravenous doses of 1-5 mg/kg bw. Intragastric and intravenous administration of lethal doses caused sedation, piloerection, abnormalities of co-ordination, dyspnoea, kyphotic posture and occasionally tremor, followed by prone posture and apathy. The animals died within 15 days. The toxic mechanisms have not been examined in detail, but nephropathies and capillary toxicity are normally encountered after single acutely toxic doses in all mammalian species examined.

10. In a non-GLP subacute toxicity study using only 14 male Wistar rats per dose level, animals were treated daily by gavage with doses of 0, 0.2, 1.0, 5.0, 25.0 mg aristolochic acids/kg bw as an aqueous solution. Treatment was for four weeks. The body weights of the animals receiving the highest dose decreased and 2 animals died. Seven animals per dose group were examined for haematological and clinical chemical parameters. At the highest dose of 25 mg/kg bw, mean corpuscular volume and reticulocytes decreased, total serum protein and glucose decreased and urine protein, as well as glucose, increased in comparison to control. Atrophy of thymus and spleen, hepatocellular basophilia, forestomach inflammation, erosion, and hyperplasia as well as nephrosis and degeneration of testes were observed. In the bladder, moderate urothelial hyperplasia and mild cystitis were found. Similar changes, but less severe, were observed in animals receiving a daily dose of 5 mg/kg bw. Mild changes only were found at 1 mg/kg bw.

11. No GLP conform subchronic toxicity studies are available for *Aristolochia* or its main constituents. However, in a pilot carcinogenicity study in rats, in which aristolochic acids were administered as sodium salt orally per stomach tube at dose levels of 0, 0.1, 1, and 10 mg/kg bw, haematological and clinical-chemical changes were not reported in the animals during the first 3 months, until sacrifice. At this time point, animals receiving the highest dose of 10 mg/kg bw already bore papillomas at the site of the forestomach. About half of the animals of this group exhibited hyperplasia of renal pelvis and nearly all showed hyperplasia of urinary bladder. In the lower dose group, nearly all animals exhibited papillomas and a few animals showed hyperplasias of the forestomach or of the urinary bladder. The animals of the lowest dose group showed no corresponding pre-carcinogenic organ changes. An inadequate number of rats (n=9 to 10 per dose) was studied. An exact study design was not given and detailed data were not reported.

12. Specific studies on the effects of *Aristolochia* on male and female fertility have not been submitted. *Aristolochia indica*, containing several aristolochic acids together with other ingredients, was reported to exert antifertility effects in male mice, when given orally at 75 mg of the water soluble part of a chloroform extract/kg bw at three day intervals, 7 times in 19 days. Severe degenerative damage has been observed in the testes of 8-week old male rats given aristolochic acids as sodium salt at a single oral dose of 200 mg/kg bw. Fifteen male rats per dose group received aristolochic acids as sodium salt at daily doses of 1 or 25 mg/kg bw for 4 weeks. At the higher dose level, animals showed reduced weight of testes compared to controls. Degenerative changes were seen in nearly all seminiferous tubules of the testes, the severity of damage, however, being less than after the single dose treatment with 200 mg/kg bw. At the dose of 1 mg/kg bw no changes could be detected in testes.

    Emmenagogue and abortefacient properties of *Aristolochia* species have been demonstrated in animals and man. It could not be clarified which of the ingredients of *Aristolochia* may be responsible for the respective effects.

13. Mutagenic activity of several constituents of Aristolochia was reported. Not all of the constituents of Aristolochia were examined, but all of the aristolochic acids tested so far in Ames tests, tests examining sister chromatid exchange and structural chromosome aberration in human lymphocytes, a mouse bone marrow micronucleus test, a granuloma pouch assay in rats, examining gene mutations at the HGPRT-locus, and in several mutagenicty tests in Drospohila melanogaster, have shown direct mutagenic properties. The addition of S9-mix in the Ames test either led to similar results or decreased the mutagenic effect.

    DNA-adduct formation by several aristolochic acids or their sodium salts has been demonstrated *in vitro* and *in vivo*. For aristolochic acid I and II, it has been shown that adducts are formed via an activated aristolactam-species. The adduct formation occurs exclusively by binding of the aristolochic acid to exocyclic amino groups of purine bases, predominantly deoxyadenosine. The respective adducts may persist lifelong.

14. Aristolochic acids given orally were found to be carcinogenic in rats and mice.

    In male and female rats, the time interval to tumour development decreased dose dependently. Daily doses of 0.1 to 10 mg/kg bw given for three months led to tumours like neoplasms of the forestomach, bladder and kidney in most of the animals. At the highest dose, tumours were already observed after 6 months. Animals receiving the lower or medium dose exhibited similar neoplastic changes after a longer latency period (6 to 12 months for the 1 and 0.1 mg/kg bw group). Four of four male rats and one of five female rats receiving the lowest dose of 0.1 mg/kg bw for 12 months had developed cancer of the

forestomach at 16 months, the last time point of sacrifice. In another study, using the same protocol but aristolochic acid I instead of aristolochic acids, a dose of 10 mg/kg bw given for 3 months induced tumours of the forestomach, the small intestine and the ear duct within 6 months.

A dose of 5 mg/kg bw aristolochic acids given orally per stomach tube for 3 weeks to female mice elicited tumours in all treated animals within 1 year.

15. In 8 of 10 human cancer patients receiving intravenous doses of 1 mg aristolochic acids/kg bw daily for 3 days or longer, elevated blood urea nitrogen levels were reported persisting for 2 months or more. The glomerular filtration rate decreased after 2 days on therapy. At the same time, haematological parameters did not change significantly. Several patients died with acute toxic nephrosis.

16. No NOEL could be established from any pharmacological or toxicological data available and no ADI can be derived

17. Belgian cases

Since 1993 over 100 cases of irreversible nephropathy have been reported in Belgium, in young women attending a slimming clinic. The nephrotoxicity of the treatment has been traced to the inadvertant use of *Aristolochia fangchi* in the formulations as a substitute for *Stephania tetrandra*. One third of the Belgian patients are reported to have received a renal transplant. The distinctive renal fibrosis has been designated as Chinese Herb Nephropathy (CHN). The ingested doses of aristolochic acids have been estimated to be in the range of a few µg per kg bw daily.
One group of investigators (Nortier et al) have reported the detection of DNA-adducts of deoxyadenosine-aristolochic acid in renal tissue from transplant patients. A number of the transplanted patients have also been found to have transitional cell carcinoma in the renal pelvis, ureter and bladder. Among 39 patients who agreed to undergo prophylactic surgery, there were 18 cases of urothelial carcinoma: 17 cases of carcinoma of the ureter, renal pelvis, or both and 1 papillary bladder tumour. Nineteen of the remaining patients had mild-to-moderate urothelial dysplasia and two had normal urothelium. All tissue samples analysed contained aristolochic acid-related DNA adducts. The cumulative dose of Aristolochia was a significant risk factor for urothelial carcinoma, with total doses of more than 200g associated with a higher risk of urothelial carcinoma.

Other workers have hypothesised that "Balkan nephropathy" (BEN), progressing to kidney tumours in humans, may be caused by ingestion of flour containing aristolochic acids as impurities in these regions. However, this has been contested by De Broe who has commented that "Balkan nephropathy" is characterised by small symmetric kidneys with a smooth surface compared with Chinese Herb Nephropathy (CHN) in which the kidneys are shrunken and have irregular contours. Furthermore, BEN progresses very slowly (over 20 years) whereas end-stage renal failure in CHN can be reached 6 to 24 months after ingestion of Aristolochia.

18. French cases

Since 1994 a total of seven cases of nephropathy have been reported in France in patients who took a preparation containing *Aristolochia fangchi* instead of *Stephania tetrandra*.

19. UK cases

In 1999, two cases of end stage renal failure were reported in the UK in women taking unlicensed Chinese remedies for eczema. One of the patient has undergone renal transplant and the other is awaiting transplant. In both cases, the patients had been exposed to aristolochic acids as a result of ingesting *Aristolochia manshuriensis*. The *Aristolochia manshuriensis* had been used as a substitute for Mu Tong in the formulations in place of *Clematis* or *Akebia* species.

Subsequent testing of raw herbal drugs and products labelled as containing Fang ji or Mu Tong on the UK market showed over 40% of samples tested to contain aristolochic acids thus confirming widespread substitution with *Aristolochia* species.

20. Spanish case

A case has been reported in Spain of a patient with end stage renal failure due to chronic intake of an infusion made with a mixture of herbs containing *Aristolochia pistolochia*. This Aristolochia species is native to the Catalonia area.

21. Chinese cases

Reports from China include seventeen cases of renal failure associated with the use of *Aristolochia manshuriensis* as Mu Tong in herbal medicines; twelve of the seventeen patients died as a result of the renal failure.

22. Japanese cases

Ten cases of nephropathy were reported in Japan in 1995; five of the cases were attributed to a Chinese herbal medicine in which *Aristolochia manshuriensis* had been substituted for *Akebia quinata.*

23. Action by EU Member States

Following the reports of *Aristolochia*-related nephrotoxicity in Belgian patients, most EU Member States have taken regulatory action to protect the public from unlicensed medicines containing toxic *Aristolochia* species. In many Member States, *Stephania* species have also been restricted because of the risk of substitution.

24. The recent evidence concerning Mu Tong substitution raises concern and highlights the need for further precautions to exclude *Aristolochia* species from the supply chain. Unless appropriate quality controls procedures are in place the prohibition of species at risk of being confused with *Aristolochia* species needs to be considered. These include:

Akebia quinata, Akebia trifoliata, Clematis armandii, Clematis montana, Cocculus orbiculatus, Cocculus laurifolius, Cocculus trilobus, Stephania tetrandra.

25. The need to control other plant species of the *Aristolochiaceae* family ie. *Asarum* species that may contain aristolochic acids has also to be considered.

Furthermore, recent evidence from Taiwan (Yang et al) of cases of rapidly progressive fibrosing interstitial nephritis associated with Chinese herbal drugs where *Aristolochia* species do not appear to be implicated, raises additional concerns about the use of such products and highlights the need for further investigation and surveillance for other possible toxins.

Conclusions and recommendations

- *Aristolochia* species contain constituents known as aristolochic acids, which are severely nephrotoxic in humans at µg per kg doses.
- The aristolochic acids have also been shown to exert strong mutagenic effects in bacterial and mammalian cell systems and have been shown to be potent carcinogens in rats and mice at low dose levels and also in humans at µg per kg bw levels.
- Exposure to *Aristolochia species* in unlicensed herbal products has resulted in large numbers of patients having renal failure which in some cases has proved fatal. Some patients have also subsequently developed urothelial cancers as a result of exposure to aristolochic acids.
- There are concerns that several herbal ingredients in unlicensed herbal products may be substituted by *Aristolochia* species.
- Member States need to take steps to ensure that the public are protected from exposure to aristolochic acids arising from the deliberate use of *Aristolochia* species or as a result of confusion with other herbal ingredients.

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