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ORIGINAL PAPER

Enforcement of the ban on aristolochic acids in Chinese traditional herbal preparations on the Dutch market

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Abstract In traditional chinese medicine several *Aristolochia* species are used. *Aristolochia* spp. contain a mixture of aristolochic acids (AAs), mainly AA I and AA II which are nephrotoxicants and carcinogens. After AA-related nephropathy (AAN) and urothelial cancer were described in female patients in Belgium following intake of AA-contaminated herbal preparations, herbs with AAs were prohibited worldwide. Confusing nomenclature can cause AA contamination of certain Chinese traditional herbal preparations (THPs). Here we report the results of investigations by the Dutch Food and Consumer Product Safety Authority (VWA) into the presence of AAs in THPs sampled on the Dutch market using a liquid-chromatography–mass spectrometry method. Between 2002 and 2006 we sampled 190 Chinese THPs using recent information on Chinese THPs potentially containing AAs. AA I was found in 25 samples up to a concentration of 1,676 mg/kg. AA II was also found in 13 of these samples up to 444 mg/kg. All 25 positive samples including Mu Tong, Fang Ji, Tian Xian Teng and Xi Xin were part of a group of 68 THPs identified as possibly containing AAs. In a worst-case scenario, use of a sample of Mu Tong with the highest AA content over a 7-day period would result in the same intake levels of AAs which significantly raised the cancer risk in the Belgian AAN cases. Our results show that contaminated THPs still can be found on the market following worldwide publicity. Therefore, it can be concluded that testing of possibly AA-contaminated THPs is still essential.

Keywords Chinese herb nephropathy · Aristolochic acid · Mu Tong · Traditional chinese medicine · Herbal remedies

Abbreviations

AA	Aristolochic acid
AAN	Aristolochic acid nephropathy
BEN	Balkan endemic nephropathy
CHN	Chinese herb nephropathy
CYP	Cytochrome P450
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
QC	Quality control
SCM	Standardized control material
TCM	Traditional chinese medicine
THP	Traditional herbal preparation
VWA	Dutch Food and Consumer Product Safety Authority

Introduction

In traditional chinese medicine (TCM) *Aristolochia* species such as *A. fangchi* and *A. manshuriensis* and others are used to treat snake and insect bites, promote lactation or urination and reduce edema [1]. *Aristolochia* spp. are used for medicinal purposes worldwide. Many herbs from the genus *Aristolochia* and several species of the genus

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Asarum, both belonging to the family of the Aristolochaceae, contain several aristolochic acids (AAs) often accompanied by aristolactams [2–4]. Literature on the toxicity of the Aristolochiaceae and related analytical papers mostly focus on a naturally occurring mixture of AAs mainly consisting of AA I and AA II (Fig. 1) [5].

AAs were shown to be nephrotoxic and carcinogenic in animal studies with rodents [5, 6]. It has been shown in rat studies that the renal proximal tubule is an important target of AA toxicity which can result in renal failure [7, 8]. AAs have been suggested to play a role in the Balkan endemic nephropathy (BEN) characterized by renal interstitial fibrosis. Seeds of *A. clematitis*, which is ubiquitous in BEN-affected areas, may have contaminated grain [9]. The International Agency for Research on Cancer concluded that herbal preparations with *Aristolochia* spp. are carcinogenic to humans and that naturally occurring mixtures of AAs are probably human carcinogens as well [10]. In humans the hepatic and renal activation of AAs is attributed to reductive metabolic activation by cytochrome P450 (CYP) 1A1, CYP1A2, NAD(P)H:quinone oxidoreductase and others. The resulting ultimate carcinogenic species is able to form adducts with DNA, which in turn can cause mutations and neoplastic changes [5, 11]. We recently reviewed the toxic action of AA in some detail [12].

TCM is gaining popularity in Western countries but certain safety issues of Chinese traditional herbal preparations (THPs) such as the deliberate use of high amounts of heavy metals [13] and the presence of AAs invariably require attention. In the USA and the Netherlands Chinese THPs are regarded as foods. In Dutch food law THPs are regulated as herbal preparations in the Commodities Act "Herbal preparations." Since it came into force in early 2001, this Act has prohibited the presence of AAs and their derivatives in herbal preparations with *Aristolochia* spp. This ban was recently extended to all herbal preparations irrespective of the plant species present. Several other countries, such as the UK, the USA, Canada, Australia and New Zeeland, have since 2000 significantly limited or prohibited the sale of AA-containing herbs and issued warnings [14–18]. These measures were inspired by a steadily expanding insight into the nature of the causative agent of poisonings with Chinese THPs in Belgium in the early 1990s. In 1992 a cluster of nine similar cases of renal interstitial fibrosis in female patients was identified in Brussels. All these patients were treated between 1990 and 1992 in a slimming clinic with a regimen consisting of a diet, injections and capsules containing pharmaceuticals such as fenfluramine, herbal preparations and a pancreas extract. In early 1990 the clinic had altered the THP formulation by introducing the Chinese herbs *Magnolia officinalis* and *Stephania tetrandra* to the capsules, replacing other herbs. It was suggested that *S. tetrandra* was inadvertently replaced by *A. fangchi*. Thin-layer chromatography detection of AAs in these herbal preparations failed however [19]. Afterwards AAs were found in 11 of the 12 batches of *S. tetrandra* powder delivered to Belgian pharmacies during the treatment period [20]. The disease became known as Chinese herb nephropathy (CHN). After the initial report more than 100 cases of rapidly progressing renal fibrosis associated with exposure to AAs were identified in Belgium and approximately 170 cases of AA-associated CHN were described in other European countries, the USA and in Asia [5]. In renal tissue of 39 patients who were treated with the Belgian slimming regimen and who were followed for CHN-related end-stage renal failure 18 cases of urothelial cancer were identified. All tissue samples examined contained AA-related DNA adducts [21]. It was found that a total intake of more than 200 g *S. tetrandra* (probably mostly replaced by *A. fangchi*) was associated with a higher risk of urothelial carcinoma [21]. A statistical analysis of the prescriptions and medical files of 71 CHN patients showed that of all administered drugs only the cumulative dose of the contaminated *S. tetrandra* preparation could predict the renal failure progression rate [22]. The typical chronic interstitial lesions of CHN were reproduced in rats injected with 10 mg/kg/day of a mixture of 40% AA I and 60% AA II for 35 days [7]. Nowadays many authors prefer to use the more accurate term "aristolochic acid nephropathy" (AAN) over the term CHN.

As a result of the Belgian CHN cases it became better known why certain Chinese THPs are contaminated with AAs and what the effects of exposure to AAs can amount to. Confusion over the Chinese common name "Fang Ji," which can refer both to the roots of *S. tetrandra* and *A. fangchi*, could have caused the contamination with AAs [19, 20]. In the trade of Chinese herbs the substitution of one plant species for another is established practice [23]. Besides the THPs known to contain *Aristolochia* species some THPs derived from certain plant species can be identified which can be replaced by *Aristolochia* species in practice. Several international food and medicine authorities have published lists of THPs suspected to contain AA. The Dutch Food and Consumer Product Safety Authority

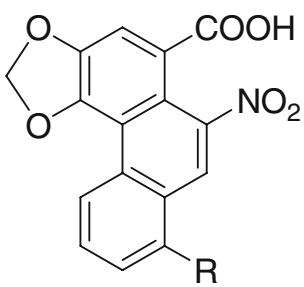

Fig. 1 Structure of aristolochic acid I (R is OCH_3) and aristolochic acid II (R is H)

(VWA) has implemented these lists in its sampling strategies of Chinese THPs and analyzed AAs in commercial samples. Reports providing quantitative data on AA levels of commercial THPs possibly containing AAs are scarce. Such data could, however, be useful to validate the existing lists of suspected THPs and could help authorities to pinpoint their efforts to protect the consumer against exposure to AAs. In the present paper our results in this field over the past 4 years are presented and compared with recent scientific and regulatory data.

Materials and methods

Sampling

VWA inspectors sampled 190 Chinese THPs on the Dutch market in the period from November 2002 to June 2006. Samples were selected by using a list of single-herb THPs and multi-ingredient THPs probably and possibly containing AAs which was based on a list compiled by the FDA in 2001 [24]. The FDA list was supplemented and regularly updated in-house with data on the nomenclature of AA-containing herbal material from books on TCM, commercial TCM databases on the Internet, warnings of other inspection agencies and literature [1, 15, 23–28, 38, 40, 42]. Table 1 presents an extract of our sampling list defining the most pertinent *Aristolochia* species in use in TCM and several plant species with which they could be exchanged. The Chinese common names in the table refer to plants and the parts used and are given in Pin Yin, which is a phonetic representation of Chinese characters. The corresponding Latin pharmaceutical name also presented is a combination of the part of the plant and often its binomial botanical name. Both types of nomenclature are seen in the market. Table 2 presents examples of formulas which can be potentially contaminated with AAs through the presence of Mu Tong, Fang Ji or Xi Xin. For sampling, products were selected by formula name or by screening the ingredient list for suspected herbs. Along with THPs known or suspected to contain AAs, THPs were sampled at random as well. THP names were copied as labeled and are presented in this paper without alterations. Generally no authentication of the herb was performed.

THPs were collected in TCM stores, oriental food stores, wholesale dealers, importers or TCM practitioners throughout the Netherlands. Sampling inspections were held at least each year. Locations were selected from the VWA-inspection database. During inspections of these locations, new suppliers and stores were also identified and visited. THPs were sampled in prepackaged form in capsules or tablets or in many cases in the form of coarse herbal material from glass containers sometimes with limited or no labeling. Samples were taken on the basis of quantities supplied to consumers, which is one unit (e.g., bottle, package or container) or in case of coarse herbal material in amounts higher than 10 g. Mixtures of the coarse materials are assembled in TCM shops according to a formula prescribed by an in-house TCM practitioner. This THP mixture is then prepared at home as a decoction for which

Table 1 Examples of single-herb traditional herbal preparations (*THPs*) possibly containing aristolochic acids

Pin Yin name	Part used	Botanical name	Latin pharmaceutical name, including alternative names
THPs with *Aristolochia* species			
Guang Fang Ji	Root	*Aristolochia fangchi*	Radix Aristolochia fangchi/Aristolochiae Fangchi
Ma Dou Ling	Fruit	*A. contorta* and *A. debilis*	Fructus Aristolochiae
Tian Xian Teng	Herb	*A. contorta* and *A. debilis*	Herba Aristolochiae/Caulis Aristolochiae
Guan Mu Tong	Stem	*A. manshuriensis*	Caulis Aristolochia manshuriensis/Aristolochiae Manshuriensis
Qing Mu Xiang	Root	*A. debilis*	Radix Aristolochiae
Xun Gu Feng, Bai Mao Teng	Herb	*A. mollissima*	Herba Aristolochiae Mollissimae
THPs possibly contaminated with *Aristolochia* species			
Han Fang Ji	Root	*Stephania tetrandra*	Radix Stephania tetrandra
Mu Fang Ji	Root	*Cocculus trilobus* and *C. orbiculatus*	Radix Cocculi Trilobi/Radix Cocculus trilobus/Radix Cocculi
Chuan Mu Tong	Stem	*Clematis armandii*	Caulis Clematis armandii/Clematidis armandii
Chuan Mu Tong	Stem	*Clematis montana*	Caulis Clematidis/Clematis montana/Clematis armandii
Bai Mu Tong	Stem	*Akebia quinata*	Caulis Akebia quinata
Bai Mu Tong	Stem	*Akebia trifoliata*	Caulis Akebia trifoliata
Bai Mao Teng, Bai Ying	Herb	*Solanum lyratum*	Herba Solani Lyrati

From [1, 23, 24, 27, 42]

Table 2 Examples of multi-ingredient THPs possibly containing AAs

Formula name in Pin Yin, in alphabetical order		
Formulas with Mu Tong or Fang Ji		
Anyang Jingzhi Gao	Fang Ji Huang Qi Tang	Quell Fire
Ba Zheng Wan	Fenqing Wulin Wan	Shang Zhong Xia Tong Yong Tong Feng Fang
Chi Kuan Yen Wan	Fu Ke Fen Qing Wan	Shi Xiang Fan Shen Wan
Chu Shi Wei Ling Tang	Gan Lu Xiao Du Dan	Shu Feng Huo Xue Tang
Chun Yang Zheng Ji Wan	Guan Xin Su He Wan	Shu Jing Huo Xue Tang
Da Huang Qing Wei Wan	Guo Qi Yin	Tienchi Hugu Wan
Da Qiang Huo Tang	Ji Jiao Li Huang Wan	Xiao'er Jindan tablets
Dang Gui Si Ni Tang	Ji Sheng Ju He Wan	Xiao Feng San
Dang Gui Si Ni Wan	Jia Wei Wu Lin San	Xiao Huo Luo Dan
Dao Chi San	Ju He Wan	Xiao Xu Ming Tang
Dao Chi Wan	Kat Kit Wan	Xin Yi Wan
Dieda Wan	Kuanhsin Suhowan	Xuan Bi Tang
Er Jia Jian Zheng Qi San	Long Dan Xie Gan Tang	Zhisou Huatan Wan
Ershiwuwei Songshi Wan	Long Dan Xie Gan Wan	Zhu Ling Tang
Fang Ji Fu Ling Tang	Mu Fang Ji Tang	
Formulas with Xi Xin		
Chuan Xiong Cha Tiao San	Du Huo Ji Sheng Tang	San Bi Tang
Chuan Xiong Cha Tiao Wan	Jiu Wei Qiang Hou Tang	She Gan Ma Huang Tang
Da Huang Fu Zi Tang	Ling Gan Wu Wei Jiang Xin Tang	Tong Guan San
Da Qin Jiao Tang	Ma Huang Fu Zi Xi Xin Tang	Wu Mei Wan
Dang Gui Si Ni Tang	Qu Feng Zhi Bao Dan	Xiao Qing Long Tang

From [15, 24, 25, 27, 38, 40, 42]

the herbs are boiled in water or other liquids. The strained liquid is then consumed [1].

Experimental

The method used to quantify AA I and AA II was based on the method described by Flurer et al. [29]. The entire sample was homogenized or in the case of capsules the contents of all the capsules were taken and homogenized, and from this a laboratory sample was taken for further analysis. After homogenization 25 ml of extraction solution was added to 1 g of sample. The extraction solution existed of 80% methanol, 18% water and 2% formic acid. The samples were shaken for 90 min in a shaking machine (Gerhardt LS-20, position 9). Then they were allowed to precipitate for about 1 h. An aliquot of 1 ml was then centrifuged at a minimum of 10,000*g*. The supernatant was transferred to a vial and hermetically sealed for liquid chromatography (LC)–mass spectrometry (MS) analysis. No concentration step was needed.

An ion-trap LC-MS system from Thermofinnigan (LCQ Advantage) equipped with a quaternary pump, an autosampler with a column oven, a photodiode array detector and an integration system together with LC-MS software was used for analysis. The separation was performed on an Alltima C-18 column (150 mm×3.2-mm inner diameter—5-μm particle size) with an Alltima C-18 precolumn (7.5 mm×3.0-mm inner diameter—5-μm particle size) using gradient elution. LC conditions are listed in Table 3. The MS detection was performed by electrospray ionization using the positive mode. The MS conditions are listed in

Table 3 Liquid chromatography (*LC*) conditions for the determination of AA I and AA II

Parameters	Conditions		
Analytical column	Alltima C-18 column (150 mm×3.2-mm inner diameter—5-μm particle size)		
Precolumn	Alltima C-18 (7.5 mm×3.0-mm inner diameter—5-μm particle size)		
Column temperature	30°C		
Injection volume	20 μl (full-loop injection)		
Flow rate	0.30 ml/min		
Mobile phase	Eluent A: 10 mmol ammonium formate in 1% formic acid		
	Eluent B: Methanol		
LC gradient	Time (min)	Eluent A (%)	Eluent B (%)
	0.00	50	50
	10.00	20	80
	21.00	20	80
	22.00	0	100
	25.00	0	100
	26.00	50	50
	34.00	50	50

Table 4. Before injection of samples, the system was equilibrated using 50% eluent A and 50% eluent B. Quantification of AA I and AA II was based on a standard mixture obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands) containing 43% AA I and 54% AA II.

For every series of samples several quality checks were performed, including a check of the validity of calibration, a check on the ratio of the first to the second daughter ion and the analysis of quality control (QC) samples (two standardized control materials, SCMs, containing AA I or AA II). For this QC material the mean and a 95% confidence interval were established. Each measurement of this QC material had to comply with this 95% confidence interval. AA was only quantified when all quality checks for a series of samples were in line with the desired performance characteristics.

Results and discussion

Characteristics of the analytical method

For the in-house validation of the method for quantifying AA I and AA II, the limit of detection (LOD) and the limit of quantification (LOQ) were determined. The LOD was defined as the concentration which is 3 times higher than the range of the chromatographic background of the second daughter ion. The LOD determined in this way was 1.0 mg/kg for AA I and 1.6 mg/kg for AA II. The LOQ was defined as twice the LOD and was 2.0 mg/kg for AA I and 3.2 mg/kg for AA II. Two calibration curves were used, both of which were linear with correlation coefficients of at least 0.97. The linearity for the low-level AA ranges was determined between 86 and 430 ng/ml for AA I and between 108 and 540 ng/ml for AA II. The range of application for preparations containing high levels of AAs was determined as 430–6,450 ng/ml for AA I and as 540–8,100 ng/ml for AA II. The calibration curves for the high concentration range were established by quadratic regression. Preparations containing even higher amounts of AAs were diluted with the extraction solution. The validity of the calibration curve, retention times and LOD were checked with each series of samples. Quantification of both AA I and AA II was based on the first daughter ions; however, amounts were only quantified when the relative intensity of the second daughter ion in proportion to the first daughter ion was within specific limits. For this the peak surface of the second daughter ion was calculated as a percentage of the peak surface of the first daughter ion. For AA I the relative intensity of the second daughter ion (*m*/*z* 341.7) had to be 44.0±11.0% of the first daughter ion (*m*/*z* 298.0). Similarly for AA II the relative intensity of the second daughter ion (*m*/*z* 294.0) had to be 53.5±10.7% of the first daughter ion (*m*/*z* 267.9).

Table 4 Mass spectrometry (MS) conditions for the determination of AA I and AA II

Parameters	Conditions
Capillary temperature	250°C
Sheath gas	45%
Aux/sweep	10
Source voltage	5.00 kV
Source current	80.00 μA
Capillary voltage	9.00 V
Tube lens offset	15.00 V
Scan	*m*/*z* 100.00–400.00
Parent ion AA I	*m*/*z* 359.0: first daughter ion *m*/*z* 298.0; second daughter ion *m*/*z* 341.7
Parent ion AA II	*m*/*z* 329.1: first daughter ion *m*/*z* 267.9; second daughter ion *m*/*z* 294.0

For the recovery studies a blank THP sampled from the market (labeled as Mu Tong and *Clematis armandii* and analytically shown to be free of AA I and AA II) was spiked at levels between 2 and 11 mg/kg. As Mu Tong samples were expected to be frequently contaminated, this blank Mu Tong sample without AAs was considered to be a relevant model for our research and the recovery studies were therefore performed on this sample. The recoveries determined from six replicate measurements under repeatability conditions and in the range 2–11 mg/kg were 87 and 82%, respectively, for AAI and AAII with relative standard deviations of 3.7 and 3.5%, respectively. The reproducibility was defined as 2.8 times the standard deviation obtained from results determined by different operators and at different times using SCMs. For AA I a SCM, a Xi Xin THP sampled from the market containing 4.75 mg/kg AA I, was used to determine the reproducibility, which amounted to 0.94 mg/kg. For AA II a SCM was prepared from a mixture of a Guan Mu Tong THP sampled from the market (an *Aristolochia* sp. with a high level of AA II) and the blank Mu Tong sample without any AAs which was used for the recovery studies. This mixture contained 3.76 mg/kg AA II and the reproducibility was 0.47 mg/kg. Both SCMs were analyzed in each series. These performance characteristics of the method are in agreement with results reported by Trujillo et al. [30].

Confusion of herbs in TCM and sampling of AA-containing THPs

Besides a sensitive analytical method, an effective sampling protocol needed to be developed for enforcement of the ban on AAs. Central to this protocol was information on which Chinese herbs can possibly be replaced by herbs that

contain AAs. In TCM confusion of herbs occurs frequently and can result from similarities in appearance, mistakes in (ancient) textbooks, counterfeits and in many cases ambiguous nomenclature [31]. Contamination of THPs with AAs can often be traced back to confusion over nomenclature. Common or vernacular names of plants are, as opposed to binomial botanical names, not very reliable for unambiguous identification of the particular species as, for instance, the interpretation of common names can even differ between geographical regions. In TCM several plant species share a Chinese common name with an *Aristolochia* sp. and this common name could be seen as a group name for the species concerned. When a prefix is added to the group name the common name refers to only one or two plant species of the group; in many cases, however, only the group name is used. The prefix can point at a region where the plant is grown; for example, the prefix "Chuan" refers to the Sichuan province [1]. The common name Fang Ji refers to at least four plant species but in combination with the prefix "Guang" it is exclusively used for the root of *A. fangchi*. There is also the possibility that a herb has more than one common name, which can lead to confusion as well. For instance, it was recently reported that *A. mollissima* is not only called Xun Gu Feng but Bai Mao Teng as well. This last common name is also used for *Solanum lyratum*, which confusingly has an alternative name as well, namely, Bai Ying [32]. Substitution of *S. lyratum* by *A. mollissima* can occur when only the common name Bai Mao Teng is used when the THP is prescribed, self-medicated, traded, etc. Such a case has been reported in Hong Kong recently where a 60-year-old man was diagnosed with renal failure and urethral cancer after he had erroneously been using Herba Aristolochiae Mollissimae instead of the desired *Solanum* species [31]. The authors indicated that confusion keeps recurring between the names Xun Gu Feng, Bai Mao Teng and Bai Ying [31]. Although *A. mollissima* is entered in the FDA list, the plant's common name is not mentioned nor is *S. lyratum* [24]. Information of this nature might prove valuable when sampling THPs for AA analysis. Tables 1 and 2 present an extract of our sampling list defining the most pertinent single-herb THPs derived from *Aristolochia* spp. in use in TCM, several plant species which could be replaced by *Aristolochia* spp. and a list of possibly contaminated formulas. This list proved useful for sampling 190 Chinese THPs on the Dutch market in the period from November 2002 to June 2006.

Analytical results

The Chinese common names and Latin pharmaceutical names of all 190 THPs were examined for indications that AA-containing herbs might be present. We identified 68 THPs as products which could possibly contain AAs and this subgroup contained all 25 positive samples. The analytical results of the 68 potentially AA-containing THPs are presented in Table 5 grouped by the Chinese common name. AA I was found in all 25 samples positive for AA, the AA I level of four THPs was below the LOQ and AA I contents of the remaining samples ranged between 2 and 1,676 mg/kg. Together with AA I, AA II was detected in 13 samples, with the AA II content of one sample below the LOQ and that of the other samples between 4 and 444 mg/kg. When THPs contained more than the relevant LOQ action was taken to remove the products from the market.

In three of five samples of single-herb THPs (Guan Mu Tong $^{(2x)}$, Qing Mu Xian, Guang Fang Ji and Tian Xian Teng) labeled with names exclusively referring to *Aristolochia* species, significant amounts of AA I were found ranging between 74 and more than 1,000 mg/kg, implying that these samples indeed contained *Aristolochia* spp. The remaining two of these *Aristolochia* samples surprisingly contained no detectable levels of AAs. The identity of the samples was generally not authenticated however. In 11 of 12 samples of THPs with herbs from the genus *Asarum*, which also belongs to the Aristolochiaceae and in which AAs can be expected, low levels of AAs were detected. The remaining 11 positive samples were THPs that contained herbs which can be substituted by *Aristolochia* spp. and belonged to the Fang Ji and Mu Tong groups of Table 5. Of these, four products were incorrectly labeled with names that identified the herbs as AA-free counterparts of *Aristolochia* species. This shows that the problem in TCM of substitution of innocuous herbs with *Aristolochia* spp. is not yet resolved. The problem of substitution of Chinese herbs with toxic counterparts is not limited to *Aristolochia* spp. In 2001 the Dutch Health authorities, including the VWA, were faced with more than 60 poisonings with symptoms including epileptic seizures owing to consumption of a herbal tea where the spice Chinese star anise (*Illicium verum*) was replaced by an unidentified *Illicium* sp. imported from China and which was shown to contain the neurotoxin anisatin [33].

We found no AAs in THPs which to our knowledge were not likely to be contaminated with AAs and could therefore be considered as randomly sampled. These THPs are presented in Table 6. Though AAs were absent, some of these products could pose a health risk to the user because they may contain other natural toxins. For example, we sampled two THPs labeled as Chuan Wu and Radix Aconiti Carmichaeli, which is the root of *Aconitum carmichaeli*. This plant and the related *A. kusnezoffii* (Cao Wu) are used in TCM for the treatment of musculoskeletal disorders and contain the potent neurotoxin and cardiotoxin aconitine. In Hong Kong cases of herb-induced aconitine poisonings are treated almost every year [34]. Also several herbs were sampled that are known to contain pyrrolizidine alkaloids (PAs), which are mutagenic and carcinogenic hepatotox-

Table 5 LC-MS detection of AAs in Chinese THPs potentially containing AAs sampled on the Dutch market

Chinese common name		Latin pharmaceutical name	*N*		AA (mg/kg) in positive samples			
Group	Specific names, when labeled	When labeled	Without AA	With AA	AA I	Mean AA I	AA II	Mean AA II
Mu Tong	Guang Mu Tong	–		2	919; >1,000		82; > 100	
	Guang Mu tong	Caulis Clematidis Armandii	1		ND		ND	
	Mu Tong	–		1	1,453		303	
	Mu Tong (2x)/– (3x)	Caulis Clematidis Armandii—Akebiae/Caulis Clemat(id)is Armandii (4x)	5		ND		ND	
	Mu Tong	Caulis Akebiae		1	1,281		394	
	Chung Mu Tong/ Chuan Mu Tong(2x)/ Chuang Mu Tong	Caulis Akebiae(4x)	1	3	19; 1,113; 1,676	936	4; 190; 444	212
Mu tong (in formula)	Lon Dan Xie Gan (Pian)/ Long Dan Xie Gan Tang	–/Gentiana form	4	3	41; 49; 59	50	11; 9; 14	11
	–	Quell Fire	1		ND		ND	
	Xao Feng San	Tangkuei & Arctium Formula	1		ND		ND	
	Dao Chi San	Rehm. Clematis Armandi Form.	1		ND		ND	
	Xiao Feng San	Tangkuei & Arctium Formula	1		ND		ND	
Mu Xiang	Qing Mu Xiang	Radix Aristolochiae, "Duitse pijp"	1		ND		ND	
	Mu Xian	–	1		ND		ND	
	Mu Xiang	Vladimiria	1		ND		ND	
	Guang Mu Xiang	–	1		ND		ND	
Tian Xian Teng	Tian Xian Teng	Caulis Aristolochiae		1	74		33	
Fang Ji	Niu Ru Shi	Cocculi Sarm. Rad.		1	<LOQ		ND	
	Guang Fang Ji	–	1		ND		ND	
	Fang Ji	–	1		ND		ND	
	Fanji	Sclerotium Poriae Cocos		1	12		<LOQ	
	Fang Ji/–	Radix Stefaniae Tetrandiae (2x)	2		ND		ND	
Fang Ji (in formula)	Fang Ji Qi Tang	Wutian & Astrag. Comb	1		ND		ND	
	Mu Fang Ji Tang	Gypsum, Cinn & Ginseng Comb		1	524		21	
Xi Xin	Xi Xin (Bei) (1x)/Xi Xin	Herba Asari (North) (2x)/Herba Asari/Herba Asari Cum Radice	1	10	<LOQ–31	9	ND	
Xi Xin (in formula)	Dang Gui Si Ni Tang	Tangkuei & Jujube		1	<LOQ		ND	
Wei Ling Xian	Wei Ling Xian	Clematis/ Radix Clematidis/ Radix Clematidis Chinensis	10		ND		ND	
	–/–	Clematidis/Radix Clematidis	5		ND		ND	
Wei Ling Xian (in formula)	Shu Jing Huo Xue Tang	Clematis & Peony Comb	1		ND		ND	
Ba Yue Zha	Ba Yue Zha	Fructus Akebiae	2		ND		ND	

The combinations of Chinese common names and Latin pharmaceutical names were copied from the label or provided by the vendor. No translations of Chinese names into Latin pharmaceutical names were added by the authors. *Dashes* indicate that a name was not present on the label or that the vendor could not specify the name. Chinese common names with small differences which were deemed alternative names were grouped

LOQ limit of quantification, *ND* not detected

icants [12]. The pyrrolizidine alkaloid containing herbs are Zi Cao (*Arnebia euchroma* and *Lithospermum erythrorhizon*), Kuang Dong Hua (*Tussilago farfara*) and Pei Lan (*Eupatorium fortunei*) [35]. Altogether, based on our results it can be concluded that especially single herb THPs under the Chinese common names of Mu Tong, Fang Ji, Tian Xian

Table 6 Randomly sampled Chinese THPs negative for AAs as determined with LC-MS

Chinese common name, as labeled	Latin pharmaceutical name, as labeled
Ba Zhen Wan	–
Ba Zheng Ke Li	Octo Form granules
Bai Hua She She Cao	Herba Hedyoti Diffusae
Bai Qui Feng	–
Bao He Wan	–
Bi Xie	Rhizoma Dioscorea Hypoglaucae
Bian Dou	Semen Lablab Album
Bing Lang	–
Cang Zhu	Rhizoma Atracttylodi
Che Qian Cao (2x)	Plantago Asiatica/Herba Plantagaginis
Chuan Wu/Chuang Mu/–	R. A. Carmichaeli (2x)/Aconit Carmichaeli Preparata
Da Huang (2x)	–
Dang Gui	Chinese Angelica
Dao Chi Pian	Scarlet Form
Ding Chuan Wan	–
Du Zhong Bu Yao Granules	–
Fan Xie Ye	Folium Sennae
Fang Feng	Radix Sapashnikovae S
Fu Ling (3x)/– (2x)	Sclerotium Poriae Cocos (5x)
Fu Ping	–
Gui Fu Di Huang Wan	–
Guo Teng	Rumulus Unicare Cum Uncis
He Shou Wu (2x)/–	Radix Polygoni Multiflori (3x)
Hong Hua	Flos Carthami Tinctorii
Huai Hua	Flos Sophorae
Huang Jing	Rhizoma Polygonati, Polygonum officinale
Huang Lian Tang Granules	–
Huo Xiang Zheng Qi Wan	–
Je Yiao Teng	Caulis Polygoni Multiflori
Jian Pi Wan	–
Jinkuishenqiwan	–
Ku Shen Pian	–
Kuan Dong Hua (3x)/ – (2x) / Dong Hua	Flos Tussilagi Farfarae (6x)
Lei Gong Teng	Herba Polygoni Perfoliali
Li Zhong Ke Li	Midrif Form granules
Ma Chi Xian	Herba Portulacae
Mai Wei Di Huang Wan	–
Mi Niao Ning Ke Li	–
Mi niao ning ke li	–
Qing Fei Ping Chuan Tang Granules	–
Qing Qi Hua Tan Pian	–
Quang Huo Rhizoma	Radix Notop Tergii
Ren Shen Ye	Folium Ginseng
Sang Ju Yin Ke Li	Chrysanth Form
Sang Zhi	–
Shenzhi Jiaonang	–
Shu Gan Wan	–
Shugawan	–
Su Mu	Lignum Sappan
Te Xiao Yao Tong Ling	–
Tiang Huang	–
Wu ji bai fe	–
Wu ji bai feng wan	–
Wu Yao	R. L. Strychnifoliae
Xi Zhi Ren	Black Cardamom
Xia Sang Ju Chong Ji	–

Table 6 (continued)

Chinese common name, as labeled	Latin pharmaceutical name, as labeled
Xiang Yuan	Flos C. Mediae
Xiao Feng Ke Li	Lay Wind Form
Xiao Ji	–
Xiao Yao San	Tangkuei & Bupleurum Formula
Ya Dan Zi	Fructus Brucae Jav
Yan Fu Mu	–
Yang Xue Sheng Fa Jiao Nang	–
Ye Jiae Tang	–
Yin Chen	Herba Artemisiae Scopariae (2x)
Yu Mi Xu	Stigma Maydis
Yu Zhu (2x)/–	Rhizoma Polygonati/ Polygonati Odorati (2x)
Ze Lan/–	Herba Lycopi (2x)
Zi Cao	Radix Arnebiae S. Lithosspermi (2x)
Zi Cao (2x)	Radix Arnebiae (2x)
Zi Hua Di Ding	Herba Violae
Zuo Gui Wan	–
–	Aconite Ginseng & Ginger Combination
–	Artemisia Scoparia
–	Astragalus extract tablets
–	Beautifying and slimming tea
–	Bupleurum & Dragon Bone Combination
–	Flos Chrysanthemi (3x)
–	Herba Artemisiae Annuae
–	Herba Eupatorii Fortunei (2x)
–	Herba Lobeliae Chinensis Cum Radice (2x)
–	Plantaginia Semen extract
–	Radix Phytolaccae
–	Radix Pulsatilae Chinenses
–	Radix Rubiae
–	Rehmannia Eight Formulas
–	Rhizoma Dryopteria Crassihizomae (2x)
–	Taraxaci Herbs

Teng and Xi Xin could be contaminated with AAs. Besides single-herb THPs, certain multi-ingredient THPs can be at risk of adulteration as well (Table 5).

In the following paragraphs we will expand on the possible reasons for contamination of these particular THPs with AAs.

Mu Tong

We found AAs in seven out of 14 single-herb THPs with the pharmaceutical names Caulis Akebia or Caulis Clematis armandii or Mu Tong with or without the prefixes "Guan" or "Chuan." The common Chinese name Guan Mu Tong exclusively refers to the stem of *A. manshuriensis* [23] and two samples exclusively labeled as Guang Mu Tong [sic] contained an AA I level of 919 and 1,000 mg/kg or higher and the AA II contents were 82 and more than 100 mg/kg, respectively. Hashimoto et al. [3] reported AA I contents of *A. manshuriensis* (*Kan-mokutsu* in Japanese) ranging between 0.169 and 0.882%, which is more than 1.5–8.8 times higher than the levels we found. Trujillo et al. [30] reported an AA content of 2,830 mg/kg in a sample of *A. manshuriensis* stem. In our study another sample, verbally indicated to be "Guang Mu Tong" [sic] but labeled as Caulis Clematidis Armandii contained no AAs. The stem of *C. armandii* is called Chuan Mu Tong, however. We therefore conclude that the prefix "Guang" was mistaken for "Chuan," which would explain the absence of AAs. A further five samples labeled as Caulis Clematidis Armandii with or without Mu Tong did, as expected, not contain AAs. Of the positive samples, three were labeled as a Chuan Mu Tong and Caulis Akebia (stem of *A. quinata* or *A. trifoliata*), which to our knowledge is not a common combination. The common Chinese name of Caulis Akebia is Bai Mu Tong and not Chuan Mu Tong, which refers to the stem of *C. armandii* and *C. montana* [1, 23].

Furthermore, two THPs labeled as Mu Tong, one of which also labeled as Caulis Akebiae, contained high levels of both AA I and AA II. These findings strongly underline the fact that the nomenclature of THPs cannot to be relied upon in some cases. Also the fact that AAs were found in half of the Mu Tong samples indicates that this group of THPs needs constant monitoring. Bensky and Gamble [1] noted that in premodern China *Akebia* was used as Mu Tong but that at present *A. manshuriensis* is used most often.

The formula Long Dan Xie Gan Wan is included in the FDA list of potential AA-containing herbs and formulas [24]. The suffix "Wan" in the formula name refers to pill in Pin Yin [1]. The formula Long Dan Xie Gan Tang is reported to contain ten ingredients among which is Caulis Mu Tong [25], for which either *A. manshuriensis* or the known *Akebia* spp. and *Clematis* spp. can be used [1]. In our study we sampled Long Dan Xie Gan and related products with the suffixes "Pian" (tablet) and "Tang" (decoction) [1, 25] and found 41–59 mg/kg AA I in three of eight related samples. Health authorities such as Health Canada and the MHRA in the UK issued warnings against the use of this product in 2002 and 2003 [15, 16]. Alternative names are "Quell fire" and "Lung Tan Xie Gan pills" [36]. Quell fire tablets were taken of the market in 2000 and reformulated as requested by the FDA after the detection of AAs [37]. We found no AAs in one sample of Quell fire which had a different lot number and expiry date than the earlier recalled lots [37]. A case of end-stage renal failure and recurrent carcinomas in the bladder due to the 5-year use of Longdan Xieganwan manufactured in China was reported in the UK [38]. The formulas Dao Chi San and Xiao Feng San were each sampled once and were found to be negative for AAs in our study. Both formulas contain Caulis Mu Tong but some of the classical sources describe Xiao Feng San without this herb however [25]. Xiao Feng San has been found to contain AAs in Australia [18]. The FDA included the formula Dao Chi Wan in the listing of THPs suspected to contain AAs [24]. This formula has probably the same composition as Dao Chi San except that the latter is a powder (San) instead of a pill (Wan) [25]. It is prudent to include the formulas Long Dan Xie Gan, Dao Chi San and Xiao Feng San in a sampling protocol because of the possible inclusion of *A. manshuriensis*.

Mu Xiang and Tian Xian Teng

No AAs were detected in four related Mu Xiang samples. This common name can refer to the roots of *A. debilis, Aucklandii lappa, Saussurea lappa, Inula helenium, I. racemosa* and *Vladimiria souliei* [23]. Qing Mu Xian exclusively refers to the root of *A. debilis* (Table 1) but a sample of this THP contained contrary to expectations no AAs. Although our samples of Mu Xian, Guang Mu Xian (the root of *S. lappa*) and Vladimiria (Chuan Mu Xian is the root of *V. souliei* [1]) could potentially be substituted by *A. debilis* we did not detect AAs. This is in agreement with information from the European Agency for the Evaluation of Medicinal Products [23]. More research is needed however to evaluate the likelihood of this particular substitution. Another single-herb THP originating from an *Aristolochia* sp. is Tian Xian Teng, which according to the *Chinese Pharmacopoeia* is Herba Aristolochiae derived from *A. contorta* and *A. debilis*. A sample of this THP was found to contain 74 mg/kg of AA I and 33 mg/kg of AA II. Although Tian Xian Teng was sampled once and might not be very common on the market it should be included in sampling protocols.

Fang Ji

In TCM the common name Fang Ji generally refers to several different herbs, namely, *Cocculus trilobus*, *Cocculus orbiculatus*, *S. tetrandra* and *A. fangchi*. Guang Fang Ji refers exclusively to *A. fangchi* and is the only of these species to contain AAs [1, 23]. We sampled one THP verbally indicated as Guang Fang Ji which contained no AA however. A low concentration of AA was detected in a sample labeled as Fanji and Sclerotium Poriae Cocos. In a THP called Niu Ru Shiu, unknown to us, but also labeled as Cocculi Sarm. Rad. traces of AA I were detected. Obviously "Rad." stands for "Radix" and in the FDA list the herbs *Cocculus sarmentosus* and *C. trilobus* are included as alternative names for *C. orbiculatus* [24]. The root of *C. orbiculatus* as well as that of *C. trilobus* are called Mu Fang Ji in several sources [1, 39] and might therefore be substituted with Guang Fang Ji (*A. fangchi)* (Table 1) [24]. We found a relatively high AA I concentration of 523 mg/kg and 21 mg/kg of AA II in the multi-ingredient THP Mu Fang Ji Tang, which contains Radix Cocculi Trilobi (Mu Fang Ji) or Radix Stephania tetrandra (Han Fang Ji) according to some sources [40, 41] or Radix *A. fangchi* according to another source [42]. We found no AAs in three single-herb THP samples labeled as Radix Stephania tetrandra and/or Fang Ji. This does not indicate however that Fang Ji requires less attention. An AA-contaminated THP labeled as *Stephania tetrandra* was the cause of the Belgian AAN incident [20–22]. In a Swiss survey of AA I in commercial samples of slimming regimens consisting of Chinese plant mixtures four out of 42 tested positive. AA I was found in a sample Han Fang Ji declared to be *S. tetrandra radix* and traces were found in Han Fang Ji derived from *Sinomenium acutum* [28]. Both species are listed in the FDA list [24]. Another multi-ingredient THP analyzed by the Swiss researchers called Fang Ji Huang Qi Tang contained traces of AA I but a second sample did not [28]. We could not detect AAs either in Fang Ji Qi Tang, which could be a related formula. More attention should be

focused on multi-ingredient THPs and the lists of these THPs potentially containing herbs with AAs should be expanded.

Xi Xin

We found low levels of AA I ranging between the LOQ and 31 mg/kg in ten out of 11 samples labeled as Xi Xin, Xi Xin (Bei) and Herba Asarum or Herba Asarum (North), Herba Asarum cum Radice samples. No AA II was found. In the multi-ingredient THP Dang Gui Si Ni Tang we found a small amount of AA I below the LOQ. This formula contains Xi Xin (Table 2) [25]. Xi Xin refers to *Asarum sieboldii*; the genus *Asarum* belongs to the Aristolochiaceae and could be expected to contain AAs. Hashimoto et al. [3] analyzed the Chinese *Asarum* spp. *A. heterotropoides*, *A. sieboldii*, *A. splendens* and *A. himalaicum* and *A. forbesii* and found only traces of AA I in *A. splendens* and *A. himalaicum*. Schaneberg et al. [4] found up to 370 mg/kg AA I but no AA II in the North American *Asarum* species *A. canadense*. Although the AA levels found in this study are low, batches of THPs containing Xi Xin should be routinely screened for AAs before they are brought on the market.

Wei Ling Xian and Ba Yue Zha: likelihood of substitution by Aristolochia spp.

In the FDA list Wei Ling Xian or the root of *Clematis chinensis* is included as a THP which may be adulterated with AA [24]. The Latin pharmaceutical name is Radix Clematidis and also refers to the roots of *C. hexapetala* and *C. uncinata*. Substitution of the roots of these herbs by, or contamination with, the stem of *C. armandii* or *C. montana* (Caulis Clematidis armandii or Chuan Mu Tong), which in turn can be replaced by the stem of *A. manshuriensis* (Guan Mu Tong), seems not likely because these THPs differ in appearance and moreover the roots of these last *Clematis* spp. are not reported to be in use for Wei Ling Xian [1, 43], which decreases the likelihood of this particular substitution as well. Another hypothetical option is confusion between Wei Ling Xian and Qing Mu Xian (the root of *A. debilis*) but we found no reports of this substitution. We analyzed ten samples of Wei Ling Xian/Radix Clematidis (Chinensis), five samples of Radix Clematidis and a formula containing this herb [25] but no AAs were found. Our results might indicate that Radix Clematidis or Wei Ling Xian is not likely to be replaced by *Aristolochia* spp. Nevertheless the FDA reported in 2001 the contamination of a *C. chinensis* extract with AAs [44]. More research into this substitution is therefore warranted.

Another herb where substitution by *Aristolochia* spp. seems unlikely is Fructus Akebiae or Ba Yue Zha. According to Bensky and Gamble [1] both names refer to the fruit of *Akebia quinata* and *A. trifoliata*. The fruit of these plants has also been reported to be referred to as Yu Zhi Zhi in the Pharmacopoeia of the People's Republic of China *and is entered as such in the FDA list* [24, 45]. We found no AAs in two samples labeled as Fructus Akebiae and Ba Yue Zha. As the common Chinese name of Fructus Akebiae bears no resemblance to a common name of any *Aristolochia* sp., confusion would not seem likely. This has to be supported by additional results, however, before this specific THP may be considered for removal from the list of suspected materials. It might be possible that the fruits of *A. debilis* or *A. contorta* (Ma Dou Ling) and Fructus Akebiae are used interchangeably but we are not aware of reports of such a substitution.

Exposure data

The batches of *S. tetrandra* powder which were replaced by *Aristolochia* spp. in the Belgian AAN incident were reported to contain AA levels up to 1.56 g/kg with a mean of 0.65 g/kg [20]. Interestingly only in two of the 12 batches investigated was tetrandrine, the characteristic alkaloid of *S. tetrandra*, found and in one of these in combination with AAs. This would indicate that the rest of the batches consisted of 100% *Aristolochia* spp. replacing *S. tetrandra* [20]. It was estimated that the cumulative consumption of more than 200 g of these powders raised the risk of urothelial carcinoma [21]. This corresponds to a cumulative chronic intake of 130 mg of AAs when using the mean AA content reported by Vanhaelen et al. [20]. We sampled a THP labeled as Chuan Mu Tong and C. Akebia on the Dutch market with an AA content of 2.1 g/kg consisting of a natural mixture of 1.7 g/kg of AA I and 0.44 g/kg of AA II (Table 5). In a worst-case scenario 62 g of this THP with the highest AA content would supply more than 130 mg of AAs, which, considering the Belgian data, could significantly raise the risk for cancer. According to Bensky and Gamble [1] the recommended dose of Mu Tong is 3–9 g, probably per day. The authors warn against overdose with reference to a case of acute renal failure following a dose of 60 g. No limitation in the duration of use is given. When in our worst-case scenario the preparation with the highest AA content would be used following the highest dosing regimen, exposure to more than 130 mg of AAs would be achieved in 7 days assuming that all AA is released from the matrix. In the Belgian cases of urothelial carcinoma the mean exposure duration was 15 months and generally end-stage renal failure occurred 3–85 months after cessation of the herbal regimen [21]. In Belgium the Fang Ji was consumed as a powder, which might have increased the exposure to AAs. In China, however, Chinese THPs are mostly used as decoctions, which might reduce the toxicity of *Aristolochia* spp. However, in Chinese literature two cases of acute renal failure after consumption of a decoction made of 70 and 175 g of Mu Tong, probably Guan Mu Tong, and four deaths of renal failure after consumption of decoctions of 50–120 g

of Mu Tong were reported [46]. In the UK two cases were reported of end-stage renal failure following the use of Mu Tong containing AA I and AA II. Mu Tong was consumed by one patient as a tea for 6 years and the other patient used a preparation for 2 years in a undisclosed way [47].

Conclusion

Our finding that AAs were detected in 25 of 68 THP samples which could include AA-containing herbs indicates that several years after the ban in the Netherlands the risk of inadvertent exposure to AAs remains significant for those who use these particular THPs. In 1999 the MCA, now the MHRA, found AAs in 40% of the samples with Fang Ji and Mu Tong on the UK market [23]. Although the number of samples is relatively small, we found a similar percentage of samples containing AAs, namely, 37% of the suspected samples, which indicates that the situation has not improved since. In the UK the use of Mu Tong, Fang Ji, Ma Dou Ling or Qing Mu Xiang has been prohibited since 2001. Amongst these are also species such as *S. tetrandra*, *Clematis* spp. and *Akebia* spp. which do not belong to the Aristolochiaceae and in themselves do not contain AAs [15]. The Dutch Commodities Act "Herbal preparations" used to prohibit the sale of *Magnolia officinalis* and *S. tetrandra* as well. After a reevaluation of the literature it was concluded that these herbs in themselves pose little risk and subsequently the prohibition of these herbs was discontinued but it was recognized that a risk of substitution of *S. tetrandra* by *A. fangchi* remained [48].

Internationally the problem of AA-contaminated THPs still requires attention several years after measures by regulatory authorities in countries such as Great Britain, New Zeeland, Canada and Australia and the publicity generated by this [14–18]. The Belgian AAN tragedy clearly illustrates that contamination of THPs with AAs can have very serious consequences. Continued enforcement of the ban of AAs in the Netherlands will show if the problem of AA contamination of Chinese THPs is addressed more actively in the field of trade and if stricter regulatory measures are warranted. When identified, contaminated products will be removed from the Dutch market. The VWA will also in collaboration with customs direct its enforcement at the import of herbal material in order to prevent AA-containing THPs from entering the market. More research into possible contamination of THPs will help to safeguard the quality of Chinese THPs. As contamination is unnecessary TCM practitioners, manufacturers, vendors and importers of Chinese THPs should structurally direct efforts to the avoidance of AAs in THPs known to have the potential for contamination with AAs. Certification of THPs might aid to prevent the import of AA-contaminated products but such a system needs close monitoring. It can be concluded that testing of the imported herbs for AA contamination is still essential.

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