

PLANTS CONTAINING ARISTOLOCHIC ACID

Plants containing aristolochic acid were considered by a previous IARC Working Group in 2002 ([IARC, 2002](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

For the purpose of this *Monograph*, unless otherwise specified, the term ‘aristolochic acids’ refers to an extract of *Aristolochia* species comprising a mixture of aristolochic acid I and its demethoxylated derivative, aristolochic acid II. *Aristolochia* species also contain the related aristolactams, which are phenanthrene cyclic amides ([EMEA, 2000](#)). In some of the older literature, it is unclear whether individual compounds or mixtures are being discussed when referring to ‘aristolochic acid’.

1.1 Identification of the agent

Aristolochia species refers to several members of the genus (family *Aristolochiaceae*) ([WHO, 1997](#)) that are often found in traditional Chinese medicines, e.g. *Aristolochia debilis*, *A. contorta*, *A. manshuriensis*, and *A. fangchi*. The medicinal parts of each plant (i.e., stem, root, fruit) have distinct Chinese names. Details on these traditional drugs can be found in the *Pharmacopoeia of the People’s Republic of China* ([Commission of the Ministry of Public Health, 2000](#)), except where noted. This Pharmacopoeia includes the *Aristolochia* species presented in [Table 1.1](#).

Table 1.1 *Aristolochia* species included in the *Pharmacopoeia of the People’s Republic of China*

<i>Aristolochia</i> species	Part used	Pin Yin Name
<i>Aristolochia fangchi</i>	Root	Guang Fang Ji
<i>Aristolochia manshuriensis</i>	Stem	Guan Mu Tong
<i>Aristolochia contorta</i>	Fruit	Ma Dou Ling
<i>Aristolochia debilis</i>	Fruit	Ma Dou Ling
<i>Aristolochia contorta</i>	Herb	Tian Xian Teng
<i>Aristolochia debilis</i>	Herb	Tian Xian Teng
<i>Aristolochia debilis</i>	Root	Qing Mu Xiang

In traditional Chinese medicine, *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 ([EMEA, 2000](#)).

Similarly, the name ‘Mu Tong’ is used to describe *Aristolochia manshuriensis*, and certain *Clematis* or *Akebia* species. In some reports in the Chinese literature, ‘Mu Tong’ is substituted with ‘Ma Dou Ling’ (EMEA, 2000).

Table 1.2 lists botanicals known or suspected to contain aristolochic acid.

1.1.1 Aristolochic acid I

Chem. Abstr. Serv. Reg. No.: 313-67-7

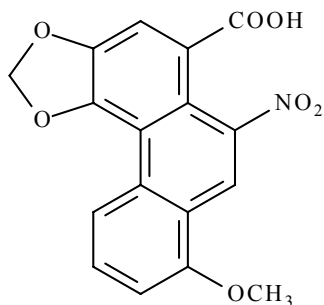
Chem. Abstr. Serv. Name: 8-Methoxy-6-nitrophenanthro[3,4-*d*]-1,3-dioxole-5-carboxylic acid

IUPAC Systematic Name: 8-Methoxy-6-nitronaphtho[2,1-*g*][1,3]benzodioxole-5-carboxylic acid

Synonyms: Aristinic acid; aristolochia yellow; aristolochic acid A; aristolochin; aristolochine; Descressept; isoaristolochic acid; 8-methoxy-3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid; 3,4-methylenedioxy-8-methoxy-10-nitro-1-phenanthrenecarboxylic acid

Description: Shiny brown leaflets (O’Neil, 2006)

(a) Structural and molecular formulae, and relative molecular mass



$C_{17}H_{11}NO_7$

Relative molecular mass: 341.27

1.1.2 Aristolochic acid II

Chem. Abstr. Serv. Reg. No.: 475-80-9

Chem. Abstr. Serv. Name:

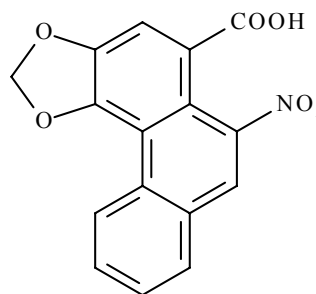
6-Nitrophenanthro[3,4-*d*]-1,3-dioxole-5-carboxylic acid

IUPAC Systematic Name:

6-Nitronaphtho[2,1-*g*][1,3]benzodioxole-5-carboxylic acid

Synonyms: Aristolochic acid B; 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid

(a) Structural and molecular formula, and relative molecular mass



$C_{16}H_9NO_6$

Relative molecular mass: 311.25

1.2 Use of the agent

Several *Aristolochia* species (notably *A. contorta*, *A. debilis*, *A. fangchi*, and *A. manshuriensis*) have been used in traditional Chinese medicine as anti-inflammatory agents, diuretics, and in the treatment of oedema (IARC, 2002). In addition, Chinese herbal remedies labelled ‘Fang Ji’ were sold in Europe as slimming agents. The ingredient *S. tetrandra* was substituted with *A. fangchi* in the preparation distributed in Belgium during the period 1990–92 (Nortier et al., 2000).

The aristolochic acid occurring in *Aristolochia* species used in traditional herbal medicines has been reported to function as a phospholipase A_2 inhibitor, and as an antineoplastic, antiseptic, anti-inflammatory, and bactericidal agent (Buckingham, 2001; Cosyns, 2003).

Table 1.2 Botanicals known or suspected to contain aristolochic acid

Botanical name	Common or other names
<i>Aristolochia</i> spp.	Aristolochia Guan Mu Tong Guang Mu Tong
<i>Aristolochia acuminata</i> Lam. Syn. <i>Aristolochia tagala</i> Champ.	Oval leaf Dutchman's pipe
<i>Aristolochia argentina</i> Griseb.	
<i>Aristolochia baetica</i> Linn. Syn. <i>Aristolochia bracteolata</i> Lam.	
<i>Aristolochia bracteata</i> Retz.	Ukulwe
<i>Aristolochia chilensis</i> Bridges in Lindl.	
<i>Aristolochia cinnabarina</i> C.Y. Cheng & J.L. Wu	
<i>Aristolochia clematitidis</i> L.	Birthwort
<i>Aristolochia contorta</i> Bunge	Ma Dou Ling Tian Xian Teng
<i>Aristolochia cymbifera</i> Mart. & Zucc.	Mil homens
<i>Aristolochia debilis</i> Siebold & Zucc. Syn. <i>Aristolochia longa</i> Thunb. Syn. <i>Aristolochia recurvilabra</i> Hance Syn. <i>Aristolochia sinarum</i> Lindl.	Ma Dou Ling Tian Xian Teng Qing Mu Xiang Sei-mokkou (Japanese) Birthwort Long birthwort
<i>Aristolochia elegans</i> Mast. Syn. <i>Aristolochia hassleriana</i> Chodat	
<i>Aristolochia esperanzae</i> Kuntze	
<i>Aristolochia fangchi</i> Y.C. Wu ex L.D. Chow & S.M. Hwang	Guang Fang Ji Fang Ji Mokuboi (Japanese) Kwangbanggi (Korean) Fang Chi Kou-boui (Japanese)
<i>Aristolochia fimbriata</i> Cham. <i>Aristolochia indica</i> L.	Indian birthwort

Table 1.2 (continued)

Botanical name	Common or other names
<i>Aristolochia kaempferi</i> Willd. Syn. <i>Aristolochia chrysops</i> (Stapf) E.H. Wilson ex Rehder Syn. <i>Aristolochia feddei</i> H. Lévl. Syn. <i>Aristolochia heterophylla</i> Hemsl. Syn. <i>Aristolochia mollis</i> Dunn Syn. <i>Aristolochia setchuensis</i> Franch. Syn. <i>Aristolochia shimadai</i> Hayata Syn. <i>Aristolochia tibetica</i> Franch. Syn. <i>Isotrema chrysops</i> Stapf Syn. <i>Isotrema heterophylla</i> (Hemsl.) Stapf Syn. <i>Isotrema lasiops</i> Stapf	Yellowmouth Dutchman's pipe
<i>Aristolochia kwangsiensis</i> Chun & F.C. How Syn. <i>Aristolochia austroszechuanica</i> C. B. Chien & C. Y. Cheng	
<i>Aristolochia macrophylla</i> Lam. Syn. <i>Aristolochia sipho</i> L'Hét.	Dutchman's pipe
<i>Aristolochia manshuriensis</i> Kom. Syn. <i>Hocquartia manshuriensis</i> (Kom.) Nakai Syn. <i>Isotrema manshuriensis</i> (Kom.) H. Huber	Manchurian birthwort Manchurian Dutchman's pipe Guang Mu Tong Kan-Mokutsu (Japanese) Mokuboi (Japanese) Kwangbanggi (Korean)
<i>Aristolochia maurorum</i> L.	
<i>Aristolochia maxima</i> Jacq. Syn. <i>Aristolochia maxima</i> var. <i>angustifolia</i> Duchartre in DC. Syn. <i>Howardia hoffmannii</i> Klotzsch	
<i>Aristolochia mollissima</i> Hance	
<i>Aristolochia pistolochia</i> L.	
<i>Aristolochia rigida</i> Duch.	
<i>Aristolochia rotunda</i> Linn.	
<i>Aristolochia serpentaria</i> L. Syn. <i>Aristolochia serpentaria</i> var. <i>hastata</i> (Nutt.) Duch.	Virginia snakeroot Serpentaria Virginia serpentry
<i>Aristolochia watsoni</i> Wooton & Standley or <i>Aristolochia watsonii</i> Wooton & Standley Syn. <i>Aristolochia porphyphylla</i> Pfeifer <i>Aristolochia westlandii</i> Hemsl. or <i>Aristolochia westlandi</i> Hemsl.	

Table 1.2 (continued)

Botanical name	Common or other names
<p><i>Aristolochia zollingeriana</i> Miq. Syn. <i>Aristolochia kankauensis</i> Sasaki Syn. <i>Aristolochia roxburghiana</i> subsp. <i>kankauensis</i> (Sasaki) Kitam. Syn. <i>Hocquartia kankauensis</i> (Sasaki) Nakai ex Masam. Syn. <i>Aristolochia tagala</i> var. <i>kankauensis</i> (Sasaki) T. Yamaz.</p>	
<p><i>Asarum canadense</i> Linn. Syn. <i>Asarum acuminatum</i> (Ashe) E.P. Bicknell Syn. <i>Asarum ambiguum</i> (E.P. Bicknell) Daniels Syn. <i>Asarum canadense</i> var. <i>ambiguum</i> (E.P. Bicknell) Farw. Syn. <i>Asarum canadense</i> var. <i>reflexum</i> (E.P. Bicknell) B.L. Rob. Syn. <i>Asarum furcatum</i> Raf. Syn. <i>Asarum medium</i> Raf. Syn. <i>Asarum parvifolium</i> Raf. Syn. <i>Asarum reflexum</i> E.P. Bicknell Syn. <i>Asarum rubroinctum</i> Peattie</p>	<p>Wild ginger Indian ginger Canada snakeroot False coltsfoot Colic root Heart snakeroot Vermont snakeroot Southern snakeroot</p>
<p><i>Asarum himalaicum</i> Hook. f. & Thomson ex Klotzsch or <i>Asarum himalayicum</i> Hook. f. & Thomson ex Klotzsch <i>Asarum splendens</i> (F. Maek.) C.Y. Cheng & C.S. Yang</p>	<p>Tanyou-saishin (Japanese) Do-saishin (Japanese)</p>
<p><i>Bragantia wallichii</i> R.Br. Specimen exists at New York Botanical Gardens. Tropicos does not list this species as a synonym for any <i>Thottea</i> species. Kew Gardens Herbarium does not recognize the genera <i>Bragantia</i>. Until additional information is obtained the name used is as cited in J. Nat. Products 45:657–666 (1982)</p>	

From [FDA \(2001\)](#)

2. Cancer in Humans

The previous evaluation of herbal remedies containing plant species of the genus *Aristolochia* was based on four case reports and two ecological studies ([IARC, 2002](#)). These studies as well as more recent publications are presented below.

2.1 Case reports

Since the early 1990s, several case reports from various countries have raised the possibility of a link between the consumption of Chinese herbal products containing *Aristolochia* species and human nephropathy (Chinese herb nephropathy, subsequently called aristolochic acid nephropathy), and urothelial cancer ([Cosyns et al., 1994](#); [Vanherweghem et al., 1995](#); [Yang et al., 2000](#); [Lord et al., 2001, 2004](#); [Arlt et al., 2004](#)).

2.2 Aristolochic acid nephropathy

[Cosyns et al. \(1999\)](#) examined 19 kidneys and ureters removed prophylactically during and/or after renal transplantation from ten patients treated for aristolochic acid nephropathy in one urology unit in Belgium. [The Working Group noted that no further specification of the formulation of the herbs was given.] The patients were all women with a mean age of 40 years (range 27–59 years). Multifocal high-grade carcinoma *in situ* was observed in four patients (40%).

[Nortier et al. \(2000\)](#) examined 77 kidneys and 78 ureters removed prophylactically from 39 patients treated for aristolochic acid nephropathy in another urology unit in Belgium. The period of use among these patients was closely related to the period of distribution in Belgium of pills containing *A. fangchi* (from 1990–92). Except for a 60-year-old man, all patients were women (aged 54 ± 7 years). Eighteen cases of urothelial carcinoma were found (prevalence, 46%; 95%CI,

29–62%). Except for one case of bladder cancer, all the carcinomas were located in the upper urinary tract and were almost equally distributed between the pelvis and the ureter. Mild-to-moderate dysplasia of the urothelium was found in 19 of the 21 patients without urothelial carcinoma. Among 24 patients who reported a cumulative consumption of less than 200 g of herbs containing *A. fangchi*, eight cases of urothelial cancer were recorded, and among the 15 patients who had ingested more than 200 g, ten cases of urothelial cancer were observed ($P = 0.05$).

[The Working Group noted that there were no control groups in either studies for nephropathy or cancer; however, the use of Chinese herbs by all women, the absence of other common exposure, the presumed low prevalence of malignant disease in this age group compared to the high prevalence observed, and the strong temporal association led the Working Group to the conclusion that there is a causal association between use of the herb and nephropathy/urothelial cancer.]

3. Cancer in Experimental Animals

3.1 Aristolochic acid

Aristolochic acid, tested for carcinogenicity mainly by oral administration in several studies in rats, one study in mice, and by intraperitoneal injection in one study in rabbits, induced tumours at multiple sites. In most studies, the animals were administered a mixture of aristolochic acid I and II (see [Table 3.1](#)). However, carcinogenic effects were also observed with aristolochic acid I alone ([Schmeiser et al., 1990](#); [Cui et al., 2005](#)).

In female NMRI mice, a mixture of aristolochic acid I and II given orally at a dose of 5 mg/kg body weight for 3 weeks increased the incidence of forestomach tumours, kidney adenomas, and lung carcinomas ([Mengs, 1988](#)).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (M, F) up to 9 mo Mengs et al. (1982)	Oral 1.0 mg/kg bw daily for 3 mo 30/sex	Forestomach (squamous cell carcinomas): M–3/11 killed at 6 mo M–0/10 Controls– M 6/9 killed at 6 mo F 2/11 killed at 9 mo Forestomach: M–9/11 killed at 6 mo Controls– M 0/10 killed at 6 mo	[NS] ^a [P = 0.0002]	Mixture (77.2% AAI, 21.2% AAI) No controls at 9 mo
Rat, Wistar (M, F) 12 mo Mengs et al. (1982)	Oral 0.1 mg/kg bw daily for 3 mo 30/sex	Forestomach (squamous cell carcinomas): M–2/7 killed at 12 mo Controls–0/6	[NS] ^a	Mixture (77.2% AAI, 21.2% AAI)
Rat, Wistar (M, F) 16 mo Mengs et al. (1982)	Oral 0.1 mg/kg bw daily for 12 mo 30/sex	Forestomach (squamous cell carcinomas): M–4/4 killed at 16 mo F–1/5 killed at 16 mo Controls– M 0/6 F 0/4	[P = 0.0048] ^a (M); [NS] (F)	Mixture (77.2% AAI, 21.2% AAI)
Rat, Wistar (M) up to 6 mo Mengs (1983)	Oral 10 mg/kg bw daily for up to 6 mo 108 killed sequentially; 37 (controls)	Forestomach (papillomas): 8/8 killed at 1 mo Forestomach (invasive squamous cell carcinomas): 13/18 killed at 6 mo		Mixture (77.2% AAI, 21.2% AAI) Study on histopathogenesis of forestomach carcinoma Controls: tumour data NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (M) up to 7 mo Schmeiser et al. (1990)	Oral 10 mg/kg bw daily for 3 mo 40; 8 (controls)	Forestomach (squamous cell carcinomas): 15/40 Ear duct (squamous cell carcinomas): 7/40 Intestine (adenocarcinomas or sarcomas): 23/40 Kidney (adenocarcinomas): 1/40 Pancreas (squamous cell carcinomas): 3/40 Lung (squamous cell carcinomas metastasis): 1/40 Haematopoietic system (lymphomas): 1/40 Controls—0/8 for each tumour site	[<i>P</i> = 0.04] ^a [NS] [<i>P</i> = 0.003] [NS] [NS] [NS] [NS]	AAI
Rat, Sprague-Dawley (F) 6 mo Cui et al. (2005)	Oral 50 mg/kg bw daily for 3 d 14; 10 controls	Kidney (tumours): 4/14 Breast (mammary duct carcinomas): 1/14 Controls—0/10 for each tumour site	[NS] ^a [NS]	AAI
Rat, BD-6 (M) 46 wk Hadjiolov et al. (1993)	Oral 10 mg/kg bw twice weekly for 12 wk 20	Forestomach (squamous cell carcinomas): 9/20 Urinary bladder (carcinomas): 1/20 Thymus (thymomas): 2/20 Controls—0/10 for each tumour site	[NS] ^a [NS]	Mixture NR No control group
Rat, Wistar (M, F) 6 mo Cosyns et al. (1998)	Oral 10 mg/kg bw daily for 3 mo 8/sex; 6/sex (controls)	Forestomach (squamous cell carcinomas): M—3/6 Kidney (malignant tumours): F—2/6 Bladder (carcinomas): M—1/6 Controls (for each tumour site)— M 0/6 F 0/6	[NS] ^a [NS] [NS]	Mixture (44% AAI, 56% AAI) no fibrosis detected Small number of animals

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (M) 105 d Debelle et al. (2002)	s.c. 1 or 10 mg/kg bw daily for 35 d 24; 18 (controls)	Urothelial carcinomas: high-dose-3/24 Fibrohistiocytic sarcomas at the injection site: high-dose-7/11 low-dose-2/6		Mixture (40% AAI, 60% AAI) together with single i.p. injection of furosemide and were remained on a low-salt diet Controls: tumour incidence NR
Rat, Sprague-Dawley (M, F) 90 d Hwang et al. (2006)	Oral 5 mg/kg bw daily for 90 d 10; 10 (controls)	Forestomach (carcinomas): M-10/10 F-1/10 Forestomach (papillomas): M-9/10 F-9/10 Kidney (carcinomas): M-1/10	[$P < 0.0001$] ^a (M); [NS] (F); [$P < 0.0001$] (M); [$P < 0.0001$] (F)	Mixture (44% AAI, 56% AAI)
Rabbit, New Zealand (F) 21 mo Cosyns et al. (2001)	i.p. 0.1 mg/kg 5 d per wk for 17-21 mo 12; 10 (controls)	Controls-0/10 for each tumour site Kidney (one carcinoma and one adenoma): 2/12 Ureter (carcinomas): 1/12 Peritoneum (mesotheliomas): 1/12 Controls-0/10 for each tumour site	[NS] ^a [NS] [NS]	Mixture (44% AAI, 56% AAI)

^a Fisher Exact test, Working Group analysis

AA, aristolochic acid; bw, body weight; d, day or days; F, female; i.p., intraperitoneal; M, male; mo, month or months; NR, not reported; NS, not significant; s.c., subcutaneous; wk, week or weeks

Oral administration of aristolochic acid to rats caused a dose- and time-dependent tumour response. Exposure to 50 mg/kg body weight aristolochic acid I for 3 days resulted in neoplastic lesions of the kidney after 6 months (Cui *et al.*, 2005). Rats exposed to lower doses by gavage over a longer period (1–10 mg/kg body weight for 3–6 months or 0.1 mg/kg body weight for 12 months) developed a variety of benign or malignant tumours, including those of the forestomach, kidney, renal pelvis, urinary bladder, ear duct, thymus, small intestine, and pancreas. Single cases of haematopoietic system, lung, mammary gland, and peritoneal tumours were also reported (Mengs *et al.*, 1982; Mengs, 1983; Schmeiser *et al.*, 1990; Hadjiolov *et al.*, 1993; Cosyns *et al.*, 1998).

Subcutaneous injection of 10 mg/kg body weight aristolochic acid into rats for 35 days induced a low incidence of urothelial carcinomas and fibrohistiocytic sarcomas at the injection site (Debelle *et al.*, 2002). A single intraperitoneal

injection of aristolochic acid at 10 mg/kg body weight increased the incidence of liver neoplastic nodules in male F344 rats when coupled with the liver tumour promoter orotic acid (Rossiello *et al.*, 1993).

Rabbits given intraperitoneal injections of aristolochic acid at 0.1 mg/kg body weight for 17–21 months developed tumours of the kidney, ureter, and of the peritoneal cavity (Cosyns *et al.*, 2001).

3.2 Extracts from *Aristolochia* species

Decoctions from *A. manshuriensis* and an aqueous extract of *A. fructus*, when administered orally to rats, induced tumours of the forestomach and the kidney (Qiu *et al.*, 2000; Hwang *et al.*, 2006).

See [Table 3.2](#)

Table 3.2 Studies of cancer in experimental animals exposed to extracts from *Aristolochia* species

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (F) 6 mo Qiu et al. (2000)	Oral 20 g/kg bw daily for 15 d 30 or 50 g/kg daily for 7 d 30, 30, 40; 30 (controls)	Kidney (stromal renal tumours): 20 g/kg bw–0% 30 g/kg bw–25% 50 g/kg bw–42.8% Controls–0%	NR	Decoctions of <i>A. manshuriensis</i> with amount of aristolochic acids not determined
Rat, Sprague-Dawley (M, F) 90 d Hwang et al. (2006)	Oral 0 (control) or 2135 mg/kg bw daily for 90 d (equivalent to 5 mg/kg bw aristolochic acid) 10, 10	Forestomach (carcinomas): M–3/10 F–2/10 Forestomach (papillomas): M–7/10 F–18/10 Kidney (carcinomas): M–2/10 Controls–0/10 for each tumour site	[NS] ^a (M, F); [P < 0.001] (F) [NS]	Aqueous extract of <i>A. fructus</i> containing aristolochic acids

^a Fisher Exact test, Working Group analysis

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NS, not significant

Table 3.3 Studies of cancer in experimental animals exposed to a herbal weight-loss regimen containing aristolochic acid

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (M/F) 14 mo Cosyns et al. (1998)	Oral 0 or 70 mg/kg bw herbal powder containing 0.15 mg/kg bw aristolochic acids daily for 3 mo 8, 8	Forestomach (squamous cell carcinomas): M–2/4 Controls– M 0/7	[NS] ^a	Included a mixture of various herbs and other treatments to mimic the weight-loss regimen prescribed at a Belgian clinic in the early 1990s

^a Fisher Exact test, Working Group analysis
bw, body weight; F, female; M, male; mo, month or months; NS, not significant

3.3 Herbal remedy containing aristolochic acids

Squamous cell carcinomas of the forestomach were found in male rats treated with a weight-loss regimen of herbal ingredients that contained aristolochic acids ([Cosyns et al., 1998](#)).

See [Table 3.3](#)

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Aristolochic acid is absorbed from the gastrointestinal tract and distributed unchanged and/or in metabolized form throughout the body. Several structurally defined metabolites (mainly nitroreduction products) were identified following the oral administration of aristolochic acid I and aristolochic acid II to rats and mice ([Krumbiegel et al., 1987](#); [Chan et al. 2006, 2007](#)). Fewer metabolites were observed in beagle dogs, rabbits, guinea-pigs, and humans than in rats or mice ([Krumbiegel et al., 1987](#)). The major metabolites of aristolochic acid are products derived from nitroreduction, O-demethylation, and denitration. In humans, the reduction products

aristolactam I and II are the only metabolites detected in urine ([Krumbiegel et al., 1987](#)), although full metabolic profiles have not been reported. Phase II metabolites of aristolochic acids have been identified in the urine of rats, and include N- and O-glucuronides, and acetate and sulfate esters ([Chan et al., 2007](#)).

4.2 Toxic effects

The toxic effects of aristolochic acids I and II have been inferred from effects seen in patients diagnosed with kidney nephropathy as a result of consuming herbal mixtures containing *Aristolochia* species, leading to rapidly progressive fibrosing interstitial nephritis ([Nortier et al., 2000](#)). In experimental animals, high doses of aristolochic acids administered either orally or intravenously caused severe necrosis of the renal tubules, atrophy of the spleen and thymus, and ulceration of the forestomach, followed by hyperplasia and hyperkeratosis of the squamous epithelium ([IARC, 2002](#); [Cosyns, 2003](#)).

4.3 Genotoxic effects

Aristolochic acids are consistently active in genotoxicity tests *in vivo* and *in vitro* ([Arlt et al., 2002a](#); [IARC, 2002](#)). The major activation

pathway involves reduction of the nitrogroup, and is catalysed by several human cytosolic and microsomal enzymes such as hepatic and renal cytosolic NAD(P)H:quinone oxidoreductase (NQO1), hepatic microsomal cytochrome P450 (CYP)1A2 and renal microsomal NADPH:CYP reductase – NQO1 being the most important (Stiborová *et al.*, 2008). During reductive activation, aristolochic acids form an electrophilic cyclic *N*-acylnitrenium ion that reacts with purine bases to form DNA adducts. These aristolochic-acid-specific DNA adducts have been identified and detected in experimental animals exposed to aristolochic acid or botanical products containing aristolochic acid, and in urothelial tissues from aristolochic acid nephropathy patients (Arlt *et al.*, 2002a, b). In addition, Grollman *et al.* (2007) detected DNA adducts derived from aristolochic acids in formalin-fixed renal cortical tissues embedded in paraffin blocks from four patients with a verified Balkan endemic nephropathy and in tumour tissue from three long-term residents of endemic villages who had upper urinary tract cancer. No such adducts were detected in five control patients with common forms of chronic renal disease or in five control patients with upper urinary tract transitional cell cancers who resided in a non-endemic region in Croatia. In rodent tumours, the major DNA adduct formed by aristolochic acid (7-[deoxyadenosin-*N*⁶-yl]aristolactam I) has been associated with the activation of RAS oncogenes through a specific CAA→CTA transversion mutation in codon 61 (Schmeiser *et al.*, 1990; Cheng *et al.*, 2006). Such A:T→T:A transversions were the predominant mutation type in studies using transgenic MutaTMmice (Kohara *et al.*, 2002), Big Blue transgenic rats (Chen *et al.*, 2006; Mei *et al.*, 2006), and in human TP53 knock-in mouse fibroblasts treated with aristolochic acid (Liu *et al.*, 2004; Feldmeyer *et al.*, 2006). In humans, A:T→T:A transversion mutations in codon 139 of exon 5 of the TP53 gene were identified in an urothelial tumour

from an aristolochic acid nephropathy patient (Lord *et al.*, 2004), and in several patients having Balkan endemic nephropathy, along with aristolochic-acid-specific DNA adducts (Lord *et al.*, 2004; Grollman & Jelaković, 2007; Grollman *et al.*, 2007).

4.4 Synthesis

Key steps in the mechanism by which aristolochic acid causes tumours in experimental animals have been identified (Arlt *et al.* 2002a), and are consistent with events occurring in patients with urothelial cancers associated with aristolochic acid nephropathy and Balkan endemic nephropathy. The same DNA adducts identified in humans are also found in experimental animals (Arlt *et al.* 2002a, b) exposed to the natural mixture or the pure major components. A:T→T:A transversions in the TP53 gene in urothelial tumours of aristolochic acid nephropathy and Balkan endemic nephropathy patients were the predominant mutations found in human TP53 knock-in mouse fibroblasts treated with aristolochic acid (Liu *et al.*, 2004; Feldmeyer *et al.*, 2006; Arlt *et al.*, 2007). Collectively, these data support the strong mechanistic evidence of the carcinogenicity of aristolochic acid – a mixture of aristolochic acids I and II – in humans.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of plants containing aristolochic acid. Plants containing aristolochic acid cause cancer of the renal pelvis, and of the ureter.

There is *sufficient evidence* in experimental animals for the carcinogenicity of extracts of plants containing aristolochic acid.

There is *limited evidence* in humans for the carcinogenicity of aristolochic acid.

There is *sufficient evidence* in experimental animals for the carcinogenicity of aristolochic acid.

Plants containing aristolochic acid are *carcinogenic to humans (Group 1)*.

Aristolochic acid is *carcinogenic to humans (Group 1)*.

In making the overall evaluation of aristolochic acid, the Working Group took into consideration that:

- Aristolochic-acid-specific DNA adducts identified in experimental animals exposed to aristolochic acid or herbal products containing aristolochic acid were found in urothelial tissue of aristolochic acid nephropathy patients, in renal tissue from Balkan endemic nephropathy patients, and in tumour tissue from residents of endemic villages.

- A:T→T:A transversions were found in the *TP53* gene of urothelial tumours from aristolochic acid nephropathy patients and Balkan endemic nephropathy patients. The same type of mutation predominated in human *TP53* knock-in mouse fibroblasts treated with aristolochic acid.

References

- Arlt VM, Alunni-Perret V, Quatrehomme G *et al.* (2004). Aristolochic acid (AA)-DNA adduct as marker of AA exposure and risk factor for AA nephropathy-associated cancer. *Int J Cancer*, 111: 977–980. doi:10.1002/ijc.20316 PMID:15300815
- Arlt VM, Ferluga D, Stiborova M *et al.* (2002b). Is aristolochic acid a risk factor for Balkan endemic nephropathy-associated urothelial cancer? *Int J Cancer*, 101: 500–502. doi:10.1002/ijc.10602 PMID:12216081
- Arlt VM, Stiborova M, Schmeiser HH (2002a). Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. *Mutagenesis*, 17: 265–277. doi:10.1093/mutage/17.4.265 PMID:12110620
- Arlt VM, Stiborová M, vom Brocke J *et al.* (2007). Aristolochic acid mutagenesis: molecular clues to the aetiology of Balkan endemic nephropathy-associated urothelial cancer. *Carcinogenesis*, 28: 2253–2261. doi:10.1093/carcin/bgm082 PMID:17434925
- Buckingham J, editor (2001). *Dictionary of Natural Products on CD-ROM*. Boca Raton, FL: CRC Press, Chapman & Hall/CRC.
- Chan W, Cui L, Xu G, Cai Z (2006). Study of the phase I and phase II metabolism of nephrotoxin aristolochic acid by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*, 20: 1755–1760. doi:10.1002/rcm.2513 PMID:16676316
- Chan W, Luo HB, Zheng Y *et al.* (2007). Investigation of the metabolism and reductive activation of carcinogenic aristolochic acids in rats. *Drug Metab Dispos*, 35: 866–874. doi:10.1124/dmd.106.013979 PMID:17344338
- Chen L, Mei N, Yao L, Chen T (2006). Mutations induced by carcinogenic doses of aristolochic acid in kidney of Big Blue transgenic rats. *Toxicol Lett*, 165: 250–256. doi:10.1016/j.toxlet.2006.04.008 PMID:16764999
- Cheng CL, Chen KJ, Shih PH *et al.* (2006). Chronic renal failure rats are highly sensitive to aristolochic acids, which are nephrotoxic and carcinogenic agents. *Cancer Lett*, 232: 236–242. doi:10.1016/j.canlet.2005.02.021 PMID:16458120
- Commission of the Ministry of Public Health (2000). *Pharmacopoeia (Part I)*. Beijing: Chemical Industry Press, pp. 31, 39, 41, 114, 154.
- Cosyns JP (2003). Aristolochic acid and ‘Chinese herbs nephropathy’: a review of the evidence to date. *Drug Saf*, 26: 33–48. doi:10.2165/00002018-200326010-00004 PMID:12495362
- Cosyns JP, Dehoux JP, Guiot Y *et al.* (2001). Chronic aristolochic acid toxicity in rabbits: a model of Chinese herbs nephropathy? *Kidney Int*, 59: 2164–2173. PMID:11380818
- Cosyns JP, Goebbels RM, Liberton V *et al.* (1998). Chinese herbs nephropathy-associated slimming regimen induces tumours in the forestomach but no interstitial nephropathy in rats. *Arch Toxicol*, 72: 738–743. doi:10.1007/s002040050568 PMID:9879812
- Cosyns JP, Jadoul M, Squifflet JP *et al.* (1994). Urothelial malignancy in nephropathy due to Chinese herbs. [letter] *Lancet*, 344: 188 doi:10.1016/S0140-6736(94)92786-3 PMID:7912776
- Cosyns JP, Jadoul M, Squifflet JP *et al.* (1999). Urothelial lesions in Chinese-herb nephropathy. [see comments] *Am J Kidney Dis*, 33: 1011–1017. doi:10.1016/S0272-6386(99)70136-8 PMID:10352187
- Cui M, Liu ZH, Qiu Q *et al.* (2005). Tumour induction in rats following exposure to short-term high dose aristolochic acid I. *Mutagenesis*, 20: 45–49. doi:10.1093/mutage/gei007 PMID:15644423
- Debelle FD, Nortier JL, De Prez EG *et al.* (2002). Aristolochic acids induce chronic renal failure with interstitial fibrosis in salt-depleted rats. *J Am Soc Nephrol*, 13: 431–436. PMID:11805172
- EMA (2000). *Working Party on Herbal Medicinal Products: Position paper on the risks associated with the use of herbal products containing Aristolochia species*

- (EMEA/HMPWP/23/00). London: European Agency for the Evaluation of Medicinal Products.
- Feldmeyer N, Schmeiser HH, Muehlbauer KR *et al.* (2006). Further studies with a cell immortalization assay to investigate the mutation signature of aristolochic acid in human p53 sequences. *Mutat Res*, 608: 163–168. PMID:16835015
- FDA (2001). *Aristolochic acid: Listing of botanical ingredients of concern*. Available from <http://www.fda.gov/Food/DietarySupplements/Alerts/ucm095283.htm>, accessed September 2010.
- Grollman AP & Jelaković B (2007). Role of environmental toxins in endemic (Balkan) nephropathy. October 2006, Zagreb, Croatia. *J Am Soc Nephrol*, 18: 2817–2823. doi:10.1681/ASN.2007050537 PMID:17942951
- Grollman AP, Shibutani S, Moriya M *et al.* (2007). Aristolochic acid and the etiology of endemic (Balkan) nephropathy. *Proc Natl Acad Sci USA*, 104: 12129–12134. doi:10.1073/pnas.0701248104 PMID:17620607
- Hadjiolov D, Fernando RC, Schmeiser HH *et al.* (1993). Effect of diallyl sulfide on aristolochic acid-induced forestomach carcinogenesis in rats. *Carcinogenesis*, 14: 407–410. doi:10.1093/carcin/14.3.407 PMID:8453716
- Hwang MS, Park MS, Moon JY *et al.* (2006). Subchronic toxicity studies of the aqueous extract of *Aristolochia fructus* in Sprague-Dawley rats. *J Toxicol Environ Health A*, 69: 2157–2165. doi:10.1080/15287390600747965 PMID:17062506
- IARC (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*, 82: 1–556. PMID:12687954
- Kohara A, Suzuki T, Honma M *et al.* (2002). Mutagenicity of aristolochic acid in the lambda/lacZ transgenic mouse (MutaMouse). *Mutat Res*, 515: 63–72. PMID:11909755
- Krumbiegel G, Hallensleben J, Mennicke WH *et al.* (1987). Studies on the metabolism of aristolochic acids I and II. *Xenobiotica*, 17: 981–991. doi:10.3109/00498258709044197 PMID:3673113
- Liu Z, Hergenahn M, Schmeiser HH *et al.* (2004). Human tumor p53 mutations are selected for in mouse embryonic fibroblasts harboring a humanized p53 gene. *Proc Natl Acad Sci USA*, 101: 2963–2968. doi:10.1073/pnas.0308607101 PMID:14976251
- Lord GM, Cook T, Arlt VM *et al.* (2001). Urothelial malignant disease and Chinese herbal nephropathy. *Lancet*, 358: 1515–1516. doi:10.1016/S0140-6736(01)06576-X PMID:11705569
- Lord GM, Hollstein M, Arlt VM *et al.* (2004). DNA adducts and p53 mutations in a patient with aristolochic acid-associated nephropathy. *Am J Kidney Dis*, 43: e11–e17. doi:10.1053/j.ajkd.2003.11.024 PMID:15042566
- Mei N, Arlt VM, Phillips DH *et al.* (2006). DNA adduct formation and mutation induction by aristolochic acid in rat kidney and liver. *Mutat Res*, 602: 83–91. PMID:17010389
- Mengs U (1983). On the histopathogenesis of rat forestomach carcinoma caused by aristolochic acid. *Arch Toxicol*, 52: 209–220. doi:10.1007/BF00333900 PMID:6860143
- Mengs U (1988). Tumour induction in mice following exposure to aristolochic acid. *Arch Toxicol*, 61: 504–505. doi:10.1007/BF00293699 PMID:3190449
- Mengs U, Lang W, Poch JA (1982). The carcinogenic action of aristolochic acid in rats. *Arch Toxicol*, 51: 107–119. doi:10.1007/BF00302751
- Nortier JL, Martinez MC, Schmeiser HH *et al.* (2000). Urothelial carcinoma associated with the use of a Chinese herb (*Aristolochia fangchi*) *N Engl J Med*, 342: 1686–1692. doi:10.1056/NEJM200006083422301 PMID:10841870
- O’Neil MJ, editor (2006). *The Merck Index*, 14th ed. Whitehouse Station, NJ: Merck & Co., Inc., p. 129.
- Qiu Q, Liu ZH, Chen HP *et al.* (2000). Long-term outcome of acute renal injury induced by *Aristolochia manshuriensis* Kom in rats. *Acta Pharmacol Sin*, 21: 1129–1135. PMID:11603288
- Rossiello MR, Laconi E, Rao PM *et al.* (1993). Induction of hepatic nodules in the rat by aristolochic acid. *Cancer Lett*, 71: 83–87. doi:10.1016/0304-3835(93)90101-E PMID:8364902
- Schmeiser HH, Janssen JW, Lyons J *et al.* (1990). Aristolochic acid activates ras genes in rat tumors at deoxyadenosine residues. *Cancer Res*, 50: 5464–5469. PMID:2201437
- Stiborová M, Frei E, Arlt VM, Schmeiser HH (2008). Metabolic activation of carcinogenic aristolochic acid, a risk factor for Balkan endemic nephropathy. *Mutat Res*, 658: 55–67. doi:10.1016/j.mrrev.2007.07.003 PMID:17851120
- Vanherweghem JL, Tielemans C, Simon J, Depierreux M (1995). Chinese herbs nephropathy and renal pelvic carcinoma. *Nephrol Dial Transplant*, 10: 270–273. PMID:7753464
- WHO (1997). *Medicinal Plants in China. A Selection of 150 Commonly Used Species*. Manila: WHO Regional Publications, Western Pacific Series No. 2.
- Yang CS, Lin CH, Chang SH, Hsu HC (2000). Rapidly progressive fibrosing interstitial nephritis associated with Chinese herbal drugs. *Am J Kidney Dis*, 35: 313–318. doi:10.1016/S0272-6386(00)70343-X PMID:10676733

