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## A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

# 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***

| *Aristolochia* species | Part used | Pin Yin Name |
|---|---|---|
| *Aristolochia fangchi* | Root | Guang Fang Ji |
| *Aristolochia manshuriensis* | Stem | Guan Mu Tong |
| *Aristolochia contorta* | Fruit | Ma Dou Ling |
| *Aristolochia debilis* | Fruit | Ma Dou Ling |
| *Aristolochia contorta* | Herb | Tian Xian Teng |
| *Aristolochia debilis* | Herb | Tian Xian Teng |
| *Aristolochia debilis* | 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

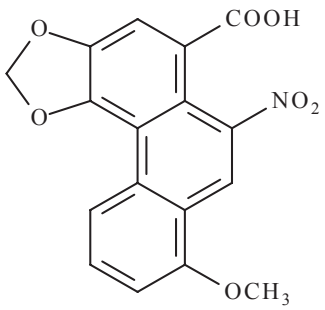
the Chinese literature, 'Mu Tong' is substituted with 'Ma Dou Ling' (EMEA, 2000).

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; Descresept; 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*

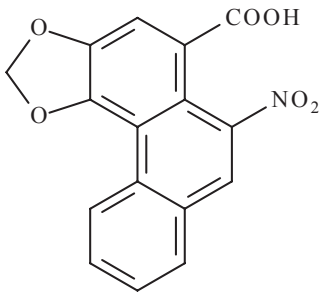



$C_{17}H_{11}NO_7$
Relative molecular mass: 341.27

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

**Table 1.2 (continued)**

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

## Table 1.2 (continued)

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

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 Studies of cancer in experimental animals exposed to aristolochic acid

| Species, strain (sex)<br>Duration<br>Reference | Route<br>Dosing regimen<br>Animals/group at start | Incidence of tumours | Significance | Comments |
|---|---|---|---|---|
| Mouse, NMRI (F)<br>up to 56 wk<br>Mengs (1988) | Oral<br>5 mg/kg bw daily for 3 wk<br>39, 11 (controls) | Forestomach: 25/39 (including 10/39 squamous cell carcinomas)<br>Stomach (adenocarcinomas): 1/39<br>Kidney (adenomas): 11/39<br>Lung (carcinomas): 13/39<br>Uterus (haemangiomas): 3/39<br>Malignant lymphomas: 10/39<br>No tumours observed in controls | [$P < 0.0001$], [NS][a] (carcinomas)<br>[NS]<br>[$P = 0.04$]<br>[$P = 0.02$]<br>[NS]<br>[NS] | Mixture (77.2% AAI, 21.2% AAII)<br>Mice were kept for 56 wk with interim sacrifice at 3, 9, 18, 26, 37, and 48 wk<br>First tumours at 26 wk; by Week 56 all remaining mice had tumours |
| Rat, Wistar (M, F)<br>up to 9 mo<br>Mengs et al. (1982) | Oral<br>0 or 10 mg/kg bw daily for 3 mo<br>30/sex | Forestomach (squamous cell carcinomas):<br>M–13/18 killed at 6 mo<br>F–8/13 killed at 6 mo;<br>F–4/4 killed at 9 mo<br>Controls–<br>M 0/10 killed at 6 mo<br>F 0/10 killed at 6 mo | 6 mo:<br>[$P = 0.0002$][a] (M);<br>[$P = 0.0003$] (F) | Mixture (77.2% AAI, 21.2% AAII)<br>No controls at 9 mo |
| | | Renal pelvis (carcinomas):<br>M–8/18 killed at 6 mo<br>F–2/13 killed at 6 mo<br>Controls–<br>M 0/10 killed at 6 mo<br>F 0/10 killed at 6 mo | [$P = 0.01$] (M);<br>[NS] (F) | |
| | | Urinary bladder (carcinomas):<br>M–3/18 killed at 6 mo<br>F–1/13 killed at 6 mo<br>Controls–<br>M 0/10 killed at 6 mo<br>F 0/10 killed at 6 mo | [NS] (for either sex) | |

**Table 3.1 (continued)**

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

**Table 3.1 (continued)**

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

**Table 3.1 (continued)**

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

[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

**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 *A.manshuriensis* 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 | [NS][a] (M, F); [$P < 0.001$] (F) | Aqueous extract of *A.fructus* containing aristolochic acids |
| | | Kidney (carcinomas): M–2/10 Controls–0/10 for each tumour site | [NS] | |

[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

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 3.3 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 Muta™mice (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 *TP*53 gene of urothelial tumours from aristolochic acid nephropathy patients and Balkan endemic nephropathy patients. The same type of mutation predominated in human *TP*53 knock-in mouse fibroblasts treated with aristolochic acid.

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