

**Aristolochic Acids**  
**Known to be a human carcinogen**  
First listed in the *12th Report on Carcinogens*

**Carcinogenicity**

Aristolochic acids are *known to be human carcinogens* based on sufficient evidence from studies in humans and supporting mechanistic data.

*Human Studies*

There is sufficient evidence for the carcinogenicity of aristolochic acids in humans based on studies of humans who consumed botanical products containing aristolochic acids and on mechanistic studies indicating that aristolochic acids are the cancer-causing agents in those products. Urothelial tumors of the upper urinary tract (tumors of the lining of the ureter and renal pelvis) were found at high rates in individuals with kidney disease (nephropathy) caused by exposure to aristolochic acids.

The strongest evidence for the carcinogenicity of aristolochic acids comes from studies among Belgian patients with nephropathy (progressive interstitial renal fibrosis) related to the consumption of herbal medicines. The patients had consumed Chinese herbal medicines that were inadvertently contaminated with plant species of the genus *Aristolochia*. Aristolochic acids were considered to be the cause of the nephropathy (now referred to as “aristolochic acid nephropathy,” or AAN) because (1) the nephropathy developed immediately after ingestion of the herbs, (2) in most cases, the patients had not been exposed to other agents known to be risk factors for nephropathy, (3) aristolochic acids were identified in the herbal products, and (4) aristolochic acid metabolites bound to DNA (AA-DNA adducts) were found in tissues (usually kidney or urothelial tissue) from some of the patients. (Arlt *et al.* 2002, NTP 2008). Over 100 cases of AAN have been reported in Belgium and over 170 cases in other locations, including the United States, Great Britain, Japan, Taiwan, and China (Arlt *et al.* 2002, NTP 2008).

Two prevalence studies in Belgium (at Cliniques Universitaires St.-Luc and Hospital Erasme) reported high rates of urothelial cancer (40% to 46%), mainly of the upper urinary tract, among female AAN patients who received kidney transplants (Cosyns *et al.* 1999, Nortier *et al.* 2000, Nortier and Vanherweghem 2002). Neither study had an unexposed comparison group. Both studies identified aristolochic acids in the botanical products consumed by the patients, and both studies detected AA-DNA adducts in kidney tissue from the patients, demonstrating that the patients had been exposed to aristolochic acids. In the study at Hospital Erasme, the rate of urothelial cancer was significantly higher among AAN patients who had consumed a high dose of the plant *Aristolochia fangchi* than among patients who had consumed a lower dose. Furthermore, AAN patients with and without urothelial cancer did not differ significantly with respect to other risk factors for urothelial cancer, such as smoking or the use of analgesics or nonsteroidal anti-inflammatory drugs. A 15-year follow-up study of AAN patients from Hospital Erasme found a rate of upper-urinary-tract urothelial cancer similar to that previously reported by Nortier and colleagues (Lemy *et al.* 2008). In addition, AAN

patients with upper-urinary-tract urothelial cancer had an unusually high incidence of urinary-bladder urothelial cancer.

Additional case reports and clinical investigations of urothelial cancer in AAN patients outside of Belgium support the conclusion that aristolochic acids are carcinogenic (NTP 2008). The clinical studies found significantly increased risks of transitional-cell (urothelial) carcinoma of the urinary bladder and upper urinary tract among Chinese renal-transplant or dialysis patients who had consumed Chinese herbs or drugs containing aristolochic acids using non-exposed patients as the reference population (Li *et al.* 2005, 2008). Molecular studies suggest that exposure to aristolochic acids is also a risk factor for Balkan endemic nephropathy (BEN) and upper-urinary-tract urothelial cancer associated with BEN (Grollman *et al.* 2007). BEN is a chronic tubulointerstitial disease of the kidney endemic to Serbia, Bosnia, Croatia, Bulgaria, and Romania that has morphology and clinical features similar to those of AAN. It has been suggested that exposure to aristolochic acids results from consumption of wheat contaminated with seeds of *Aristolochia clematitis* (Hranjec *et al.* 2005, Ivic 1970, NTP 2008). AA-DNA adducts were found in kidney tissue from BEN patients and in urothelial and kidney (renal cortical) tissues from BEN patients who had upper-urinary-tract urothelial cancer. Furthermore, A:T → T:A transversion mutations in the *p53* tumor-suppressor gene were found in urothelial tumors from BEN patients (Grollman *et al.* 2007).

The available studies are limited in their ability to formally address confounding by other factors that could increase the risk of cancer, and the case-series studies did not include unexposed controls; however, a causal association between exposure to aristolochic acids and human cancer is evidenced by the strength of the association, consistency across studies, dose-response effects, detection of AA-DNA adducts in exposed patients, timing of the exposure and disease, and specific mutations in the *p53* gene similar to the A:T → T:A transversions seen in rodents and rodent cell cultures exposed to aristolochic acids. The finding of urothelial cancer among patients who consumed a variety of botanical products from different plant species known to contain aristolochic acids provides additional support for the role of aristolochic acids as the cancer-causing agent in the botanical products. The International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence for the carcinogenicity of herbal remedies containing plant species of the genus *Aristolochia* in humans (IARC 2002).

#### *Studies in Experimental Animals*

There is sufficient evidence for the carcinogenicity of aristolochic acids in experimental animals based on studies demonstrating that aristolochic acids induced tumors at multiple tissue sites and by multiple exposure routes in rodents and rabbits. The studies in which aristolochic acids were administered orally or by subcutaneous (s.c.) or intraperitoneal (i.p.) injection, were typically small and of short duration. However, despite these limitations, the studies showed clear evidence of carcinogenicity. Nearly all of the studies reported the induction of urothelial tumors, consistent with the tumors reported in humans.

The predominant tumor types observed after oral administration of aristolochic acids were forestomach and urinary-tract tumors, and after administration by injection, urinary-tract tumors and connective-tissue tumors (sarcoma) at the injection site were found (NTP 2008). In a study in female mice, oral administration caused tumors of the forestomach, stomach, kidney, lung, and uterus and malignant lymphoma (cancer of lymph nodes or other lymphoid tissue) (Mengs 1988). In several studies in rats, oral administration of aristolochic acids caused tumors of the forestomach, kidney, renal pelvis, urinary bladder, ear duct, thymus, small intestine, and pancreas. Single instances also were reported of tumors of the hematopoietic system (organs and tissues involved in the production of blood), heart, lung, mammary gland, pituitary gland, and peritoneum (the membrane lining the abdominal cavity and surrounding the internal organs) (NTP 2008). Male Wistar rats receiving daily s.c. injections of aristolochic acids developed urothelial carcinoma of the renal pelvis and malignant fibrohistiocytic sarcoma at the injection site (Debelle *et al.* 2002). A single i.p. injection of aristolochic acids initiated liver carcinogenesis in male F344 rats that had also received treatment to stimulate proliferation of liver cells (Rossiello *et al.* 1993). Aristolochic acids administered by i.p. injection to female New Zealand White rabbits induced kidney tumors, a urinary-tract tumor, and mesothelioma of the peritoneal cavity (Cosyns *et al.* 2001).

Three studies investigated the carcinogenicity of extracts of *Aristolochia* species (one study each for *A. manshuriensis*, *A. clematitis*, and *A. contorta*) when administered to rats orally or by injection. Tumors of the forestomach and kidney were the most prevalent findings following oral administration (Hwang *et al.* 2006), but one study reported tumors of the mammary gland, thyroid gland, and skin (Qiu *et al.* 2000), and injection-site polymorphocellular sarcoma also was reported in one study (Ivic 1970). One study exposed rats of both sexes to a weight-loss regimen of herbal ingredients that contained aristolochic acids; the males developed forestomach papilloma and squamous-cell carcinoma (Cosyns *et al.* 1998).

### **Additional Information Relevant to Carcinogenicity**

Aristolochic acids I (AA I) and II (AA II) are the most widely studied aristolochic acids. Aristolochic acids are metabolized to aristolactams, which are further metabolized to a cyclic *N*-acylnitrenium ion, a reactive intermediate that forms adducts (dA-AAI, dG-AAI, dA-AAII, and dG-AAII) with purine bases (adenine and guanine) in DNA. A number of cytosolic and microsomal enzymes (CYP1A1, CYP1A2, NADPH:CYP reductase, prostaglandin H synthase, DT-diaphorase, xanthine oxidase, COX, and NAD(P)H:quinone oxidoreductase) are capable of bioactivating aristolochic acids to the reactive form (NTP 2008).

DNA adducts have been detected *in vitro* in experimental animals exposed to aristolochic acids and in human tissue from individuals exposed to aristolochic acids, including individuals with AAN, BEN, or urothelial cancer associated with AAN or BEN (NTP 2008, Grollman *et al.* 2007). The predominant adduct, dA-AAI, persists for a lifetime in rats and at least 89 months in humans and appears to be responsible for most of the mutagenic and carcinogenic properties of aristolochic acids (NTP 2008).

Aristolochic acids (purified I or II or mixtures) have been shown to be mutagenic in bacteria, cultured cells, and rodents exposed *in vivo*. Aristolochic acid I has been tested the most extensively. In *in vitro* assays, purified aristolochic acids induced mutations in the bacterium *Salmonella typhimurium* and in cultured mammalian cells including (1) *hprt* mutations in rat fibroblast-like cells and Chinese hamster ovary cells, (2) forward mutations in mouse lymphoma cells, and (3) mutations in the *p53* DNA-binding domain in two studies with fibroblast cell cultures from human *p53* knock-in (Hupki) mice (mice carrying a humanized *p53* gene sequence) (NTP 2008). Mutations were identified in the *p53* DNA-binding domain in a third (6 of 18) to half (5 of 10) of the established Hupki mouse fibroblast cultures; A:T → T:A transversions were predominant, occurring in at least 80% of the cell lines with mutations (Liu *et al.* 2004). Aristolochic acid mixtures or plant extracts caused mutations in *S. typhimurium* and sex-linked recessive lethal mutations in the fruitfly *Drosophila melanogaster* (NTP 2008). In studies with rodents exposed *in vivo*, exposure to aristolochic acid mixtures or plant extracts caused (1) mutations in subcutaneous granulation tissue from Sprague-Dawley rats (Maier *et al.* 1985), (2) mutations of the *lacZ* transgene in forestomach, kidney, and colon tissue from transgenic Muta mice (Kohara *et al.* 2002), and (3) mutations of the *cII* transgene in liver and kidney tissue from transgenic Big Blue rats (Chen *et al.* 2006, Mei *et al.* 2006). A:T → T:A transversions were the predominant mutation type in the Muta mice and Big Blue rats. Exposure to aristolochic acid I also caused mutations in granulation tissue from Sprague-Dawley rats (Maier *et al.* 1987).

Aristolochic acids have been shown to bind to adenine in codon 61 in the H-*ras* mouse oncogene and to purines in the human *p53* gene. Mutations identified in tumors of rodents exposed to aristolochic acids include A:T → T:A transversions in codon 61 of the c-Ha-*ras* gene in forestomach tumors (from rats and mice), lung tumors (from rats and mice), and ear-duct tumors (from rats). No mutations were identified in tissues from rats with chronic renal failure that had not been exposed to aristolochic acids (Schmeiser *et al.* 1990, 1991). Similar findings have been reported in humans. A:T → T:A transversion mutations of the *p53* gene were identified in a urothelial tumor from an AAN patient (Lord *et al.* 2004) and at a high frequency (78%) in BEN patients with upper-urinary-tract urothelial cancer. The frequency of A:T → T:A transversions of *p53* mutations in bladder and ureter tumors not caused by aristolochic acid exposure was approximately 5% (Grollman *et al.* 2007). Moreover there was concordance between the location of the *p53* A → T transversions and mutations identified in fibroblast cell cultures from human *p53* knock-in (Hupki) mice treated with aristolochic acid I (Nedelko *et al.* 2008).

Aristolochic acids also caused other types of genetic damage. Aristolochic acids I and II and mixtures caused DNA damage in the SOS chromotest in the bacterium *Escherichia coli*, and aristolochic acid mixtures caused sex-chromosome loss and somatic recombination in *D. melanogaster*. In mammalian cells exposed *in vitro*, aristolochic acid mixtures caused chromosomal aberrations (changes in chromosome structure or number), sister chromatid exchange (an indicator of DNA damage and repair), and micronucleus formation (a sign of chromosome damage or loss) in human lymphocytes (white blood cells). Aristolochic acid I also caused chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells. Neither aristolochic acid I nor II induced DNA

strand breaks in rat liver cells, but aristolochic acids caused DNA damage in a pig kidney cell line (proximal tubular epithelial cells) and human hepatoma (primary liver tumor) cells. In mammalian *in vivo* studies, aristolochic acids (composition not specified) did not induce unscheduled DNA synthesis (a DNA repair response) in the pyloric mucosa (stomach lining) of male rats. DNA damage was reported in kidney cells isolated from male Sprague-Dawley rats administered a single oral dose of an aristolochic acid mixture. One study reported that intravenous injection of aristolochic acid mixtures increased micronucleus formation in polychromatic erythrocytes (immature red blood cells) in bone marrow from NMRI male and female mice, but another study found no increase in micronucleus formation in peripheral blood reticulocytes (circulating young red blood cells) from male Muta mice exposed orally to a mixture of aristolochic acids I and II (NTP 2008).

Together, these findings strongly suggest that exposure to aristolochic acids causes urothelial tumors in humans through formation of DNA adducts (specifically, through binding of the reactive metabolite with adenine) and the resulting transversion mutations in oncogenes.

### Properties

Aristolochic acids are a family of nitrophenanthrene carboxylic acids that occur naturally in plants in the Aristolochiaceae family. The aristolochic acid content of plants or botanical preparations varies depending on the plant species, where it was grown, the time of year, and other factors. However, aristolochic acid I (also called aristolochic acid A) and its demethoxylated derivative, aristolochic acid II (also called aristolochic acid B) are the predominant forms. Aristolochic acid I is a crystalline solid. The molar extinction coefficient ( $\epsilon$ ) for aristolochic acid I in ethanol is 6,500 at 390 nm, 12,000 at 318 nm, and 27,000 at 250 nm (O'Neil *et al.* 2006). Other selected physical and chemical properties of aristolochic acid I are summarized in the table below. No information was located on the physical or chemical properties of aristolochic acid II other than its molecular weight of 311.3 (IARC 2002).

Property	Information for AAI
Molecular weight	341.3
Melting point	281°C to 286°C
Octanol-water partition coefficient (log $K_{ow}$ )	3.48
Water solubility	slightly soluble

Source: IARC 2002.

### Use

*Aristolochia* plants have been used since ancient times in traditional herbal medicines in many parts of the world, and aristolochic acids have been reported to have antibacterial, antiviral, antifungal, and antitumor effects (Kupchan and Doskotch 1962, Zhang *et al.* 2004). The name *Aristolochia* (meaning the best delivery or birth) is thought to be of

ancient Greek origin and reflects centuries of use in obstetrics. Other traditional uses include treatment for snakebite, scorpion stings, fever, infection, diarrhea, and inflammation (Arlt *et al.* 2002, Jiménez-Ferrer *et al.* 2005). In contemporary medicine, *Aristolochia* plant extracts have been used in therapies for arthritis, gout, rheumatism, and festering wounds, but these uses were discontinued in Germany and other countries after the carcinogenic and mutagenic properties of aristolochic acids were first reported in the early 1980s (Arlt *et al.* 2002). Other uses of *Aristolochia* plants include cultivation as ornamental plants. Aristolochic acids also have been used in studies of toxicity and carcinogenicity and in biochemical studies as relatively selective inhibitors of the enzyme phospholipase A2 (NTP 2008).

### Occurrence and Production

Aristolochic acids have been detected only in species belonging to the family Aristolochiaceae, primarily of the genera *Aristolochia* and *Asarum*. More than 30 *Aristolochia* species are native to the United States, and they are present in most states (USDA 2005). The most widely distributed native species include *A. serpentaria* (Virginia snakeroot), *A. tomentosa* (wooly Dutchman's pipe), *A. macrophylla* (pipevine), and *A. clematitidis* (birthwort). In addition, some non-native species are grown as ornamentals or have escaped cultivation and become naturalized. Worldwide, there are an estimated 200 to 350 *Aristolochia* species, and virtually all of them contain aristolochic acids (NTP 2008). *Asarum* species (wild gingers) are also widely distributed in the United States. *Hexastylis* (a genus of Aristolochiaceae), a group of rare plants endemic to the southeastern United States, has been reported to have "unexpectedly high levels" of aristolochic acids (Schaneberg *et al.* 2002)

A number of studies have reported concentrations of aristolochic acids I and II in medicinal plants, including several species of plants used in traditional Chinese medicine. The levels ranged from 3 to 12,980 ppm for aristolochic acid I and from not detected to 6,325 ppm for aristolochic acid II. In *Asarum* species, levels reported for aristolochic acid I and aristolochic acid II have ranged from trace levels to 3,377 ppm. Other studies have detected aristolochic acid IVa (ranging from 79 to 3,360 ppm of crude drug), aristolactam I (ranging from 6 to 358 ppm), and aristolactam II (ranging from 14 to 91 ppm) (NTP 2008). Hong *et al.* (1994) identified 11 aristolochic acid derivatives, including aristolactams and other compounds, in extracts from *Aristolochia cinnabarina* roots, and Wu *et al.* (1994) identified 14 aristolochic acid derivatives in extracts from stems and roots of *Aristolochia kankauensis*.

Aristolochic acids are produced commercially as reference standards and as research chemicals (IARC 2002). No data were found on producers or production volume, but Chemical Sources International (2006) identified nine U.S. suppliers of aristolochic acid A (aristolochic acid I): one supplier each for aristolochic acids B and D (aristolochic acids II and IV), three suppliers for aristolochic acid C (aristolochic acid IIIa), and three suppliers for aristolochic acid, sodium salt.

No specific data on U.S. production, imports, or sales of botanical products that might contain aristolochic acids were identified; however, there are many U.S. suppliers of products that could contain aristolochic acids. Gold and Slone (2003) identified 112

botanical products that could contain aristolochic acids and were available for purchase over the Internet.

## Exposure

Exposure to aristolochic acids may occur through ingestion as a result of either intentional or inadvertent use of herbal and botanical products that contain *Aristolochia* or *Asarum* species. Herbal preparations are available in several forms (e.g., capsules, extracts, teas, or dried herbs). Exposure also could potentially occur through direct contact with the plants, either in their natural habitats or as cultivated ornamentals. Direct contact with the leaves of *Asarum canadense* (Canadian snakeroot or wild ginger) has been reported to cause dermatitis (PFAF 2005).

Schaneberg and Khan (2004) purchased from Internet Web sites 25 herbal products suspected of containing aristolochic acids, of which nine were manufactured in the United States and the rest in China. Aristolochic acids I and II were detected in six of the products, each of which contained six or more types of plants. The U.S. Food and Drug Administration has reported recalls of products containing aristolochic acids beginning in 2000 and continuing with the report of a recall of two products in 2008 (Tou Tong San [Headache Formula] and Du Huo Ji Sheng Tang [Du Huo Joint Relief]) (FDA 2008).

Two herbal remedies prepared from *Aristolochia debilis* or *A. contorta* appear in the official 2005 Chinese pharmacopeia, and three additional entries for drugs derived from *A. debilis*, *A. fangchi*, and *A. manshuriensis* were cancelled in 2003 and 2004 because the content of aristolochic acid in the drugs was high enough to cause AAN (Zhang *et al.* 2006).

In addition to the intentional uses of aristolochic acid-containing plants, herbal preparations can pose a number of quality-related problems, which can lead to inadvertent exposures. These include contamination with prohibited or restricted substances, substitution of ingredients, contamination with toxic substances, and differences between the labeled and actual product contents (MCA 2002).

The complexity of herbal nomenclature systems used in traditional medicines (particularly traditional Chinese medicines) can lead to confusion and increased risk of inadvertent exposure to aristolochic acids (Flurer *et al.* 2001), which was reported for cases in Hong Kong (Liang *et al.* 2006), Belgium (Vanherweghem 1998), and Singapore (Koh *et al.* 2006). Substitutions arising because of name confusion also have been reported between botanicals used in Japanese herbal medicines and botanicals with similar names used in Chinese herbal medicines (EMEA 2000, Tanaka *et al.* 2001). The most extensive exposure resulting from name confusion occurred in the early 1990s in Belgium, where *A. fangchi* was inadvertently substituted for *Stephania tetrandra* to prepare diet pills. The Chinese name for *S. tetrandra* is “fang ji,” which is similar to the name for aristolochic acid-containing *A. fangchi* (“guang fang ji”). Vanherweghem estimated that between 1,500 and 2,000 individuals were exposed to the *Stephania*-labeled powders that contained aristolochic acids ranging from below the detection limit (< 0.02 mg/g) to 2.9 mg/g (2,900 ppm).

For botanical products, high concentrations or intake of aristolochic acids have been reported in studies from China (700 ppm AA I, with estimated aristolochic acid intake of

110 mg), Taiwan (up to 19.97 nmol/g AA I and up to 3.95 nmol/g AA II), Hong Kong (intake of herb from 100 mg to 800 g), Japan (up to 15.1 ppm total aristolochic acids), Australia (up to 40 ppm AA I and up to 210 ppm AA II), and Switzerland (up to 440 ppm AA I) (NTP 2008).

No estimates were found of the number of people in the United States who are exposed to aristolochic acids in herbal medicines, but two U.S. cases of renal failure resulting from ingestion of herbal products containing aristolochic acids have been reported (CR 2004, Grollman *et al.* 2007, Meyer *et al.* 2000). The use of all complementary and alternative medicines increased in the 1990s and 2000s (Barnes *et al.* 2004, Bent and Ko 2004). The Centers for Disease Control and Prevention reported that 10% of adults in the United States ingested herbal medicines in 1999 (Straus 2002), and the total spent on herbs and other botanical remedies in 2001 was \$4.2 billion (Marcus and Grollman 2002).

The possibility also exists for exposure to aristolochic acids in food. Extracts from *Asarum canadense* and *Aristolochia serpentaria* are permitted for use in the United States as flavoring substances in foods or beverages (CFR 2003); however, no information was identified on the use of either in any specific food or beverage products. It has been suggested that contamination of wheat flour by *Aristolochia* species growing as weeds adjacent to wheat fields might be responsible for some cases of BEN (Hranjec *et al.* 2005, Ivic 1970).

Although occupational exposure to aristolochic acids has not been documented, herbalists potentially are exposed while gathering plants and while preparing or applying botanical products. Gardeners, landscapers, or nursery workers who handle or transplant *Aristolochia* or *Asarum* plants could potentially be exposed to aristolochic acids.

## Regulations

### U.S. Food and Drug Administration (FDA)

#### *Dietary Supplement Health and Education Act (DSHEA) of 1994*

Manufacturers and distributors must record adverse events and report to the FDA serious adverse events reported to them about their products.

Label requirements for dietary supplements have been established.

Manufacturers must establish and meet specifications for identity, purity, strength, and composition and for limits on contamination of dietary supplements.

## Warnings and Alerts

### FDA

Warnings issued in 2000 and 2001 (FDA 2000, 2001a, 2001b) covered botanical products that contain aristolochic acids:

Practitioners who prescribe botanical remedies urged to discard those products containing aristolochic acids.

Manufacturers and distributors urged to ensure that botanical products are free of aristolochic acids.

Consumers urged to immediately discontinue use of botanical products that contain or likely contain aristolochic acids.

An import alert issued in 2000 provided for the detention of products labeled as *Aristolochia* or any that could be confused with it unless analytical evidence shows no aristolochic acids.

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